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Behavioral sexual dimorphism in models of anxiety and depression due to changes in HPA axis activity

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ABSTRACT

Anxiety and depression are considered as stress-related disorders, which present considerable sex differentiation. In animal models of anxiety and depression sex differences have been described and linked to the sexually dimorphic hypothalamus–pituitary–adrenals (HPA) axis. The present study aimed to adjust corticosterone, the main HPA axis stress hormone, in male and female adrenalectomized rats with oral (25 μg/ml) corticosterone replacement (ADXR). Subsequently we investigated the behavioral performance of ADXR rats in the open field, light/dark and forced swim test (FST). Male ADXR rats showed less anxiety-like behavior when compared to sham-operated controls, despite adequate corticosterone replacement. They further showed increased swimming and reduced climbing behavior in the FST, while immobility duration did not differ from sham-operated males. On the contrary, adrenalectomy and corticosterone replacement did not have significant effects on the female behavioral response. Females were generally more active and presented less anxiety-like behavior than males, while they exhibited higher depressive-like symptomatology in the FST. ADXR affected behavioral responses predominantly in males, which in turn modified sex differences in the behavioral profile. Females in proestrous and estrous did not differ from females in diestrous and methestrous in any measured behavioral response. Present results suggest that the male and not the female behavioral responses in models of anxiety and depression were mainly affected by ADXR. These findings may play a significant role in explaining the differential coping strategy of the two sexes in response to stressful experiences.

This article is part of a Special Issue entitled 'Anxiety and Depression'.

1. Introduction

It is now well-established that stress is crucially related to the pathophysiology of affective disorders (Goel and Bale, 2009; Oitzl et al., 2010; Young and Korszun, 2010), while the sex differentiation in stress response attracts particular attention since women are more vulnerable to anxiety and depression (Alonso et al., 2004; Kessler et al., 1994; Young and Korszun, 2010). The physiological stress response in both sexes involves a complex neurocircuitry which ultimately results into the hypothalamus–pituitary–adrenals (HPA) axis releasing glucocorticoids to the systemic blood stream (Riedemann et al., 2010). Numerous studies have established that in females the HPA axis shows a higher baseline tone and during the stress response, release of glucocorticoids is more rapid and intense, while de-escalation of the HPA axis drive is slower (Galea et al., 1997; Kitay, 1961). Earlier observations in humans discovered the link between glucocorticoids and affective states because patients with elevated glucocorticoids develop depressive-like symptomatology and inversely, depressed patients show impairments in HPA axis function (Holsboer and Ising, 2010; Ising et al., 2005). Furthermore, previous human studies have shown that glucocorticoids levels also correlate well with anxiety disorders (Vreeburg et al., 2010), that depression correlates more with glucocorticoids when co-morbid anxiety is present (Vreeburg et al., 2009) and that glucocorticoid levels are influenced by gonadal hormones (Lederbogen et al., 2010). In addition, it was previously shown that in depression the HPA axis dysregulation differs in men and women (Young and Ribeiro, 2006) and antidepressant treatment affects the HPA axis activity and stress

Abbreviations: ADXR, Adrenalectomy with Corticosterone Replacement; ANOVA, Analysis of Variance; CRH, Corticotrophin releasing hormone; D, Diestrous; E, Estrous; FST, Forced Swim Test; HPA, Hypothalamus–pituitary–adrenals; M, Methestrous; P, Proestrous.

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response in a sex-dependent manner (Binder et al., 2009; Stewart and Roper, 2008).

Animal models are widely used to study the neurobiology of depression and anxiety as well as the mechanism of action of candidate and established treatments (Cryan et al., 2002; Dalla et al, 2011; Palanza, 2001). The open field and the light/dark box have been extensively used to assess anxiety, based on the aversion of rodents for open and illuminated spaces (Belzung and Griebel, 2001; Merlo Pich and Samanin, 1989). On the other hand, the Forced Swim Test (FST) has been extensively used to assess antidepressant potential and it can successfully differentiate serotonergic and noradrenergic behavioral responses (Cryan et al., 2002, 2005). However most research is performed on male animals and relatively less effort has been devoted into validating and investigating models of anxiety and depression for females (Becker et al., 2007; Dalla et al., 2011; Palanza, 2001). Indeed, our recent studies showed an increased female vulnerability to the detrimental effect of stress on mood- and anxiety-related behaviors along with serotonergic, dopaminergic and glutamatergic differentiation at baseline and following antidepressant treatment (Dalla et al., 2005, 2008; Drossopoulou et al., 2004; Kokras et al., 2009b; Pitychoutis et al., 2009).

Moreover, we recently showed that antidepressant treatment can result in convergent behavioral responses from both sexes; however the magnitude of such behavioral response depends on the sex of the animal (Dalla et al., 2010; Kokras et al., 2009a, 2011, in press). The human analogy in anxiety and depression would be of women presenting a differential loading of symptoms than men (Marcus et al., 2008), and although treatment generally alleviates the disease in both sexes, the therapeutical response often appears of different efficacy between the two sexes (Kornstein et al., 2000). Besides other contributing factors, involving pharmacodynamics and pharmacokinetics (Kokras et al., 2011), it is also possible that the apparent difference in antidepressant response is due to the reported differences in baseline anxiety and depressive symptoms between men and women (Frank et al., 1988; Marcus et al., 2008, 2005). Finally, it should be noted that sex hormones are essentially involved in the female stress and antidepressant response (Estrada-Camarena et al., 2010; Walf and Frye, 2006), as well as in the presentation of affective disorders in women (Kornstein et al., 2010; Young et al., 2007).

Therefore in order to better estimate the magnitude of a sex-differentiated response, the baseline sex differences should be taken into account. Based on the above mentioned rationale, the present study aimed to investigate the sex differences in tests of anxiety and depression in the presence or absence of manipulated peripheral corticosterone levels. In particular, we artificially adjusted in males and females the peripheral corticosterone levels using adrenalectomy and corticosterone replacement, and compared the resulting sex-differentiated behavioral profile in the spontaneous open field activity, the light/dark paradigm and the forced swim test. Despite the well-established sex differences in peripheral glucocorticoids at baseline and following stress, we hypothesized that an abolishment of such peripheral differentiation would still permit the appearance of behavioral sex differences, although affected by our manipulation.

2. Methods

2.1. Animals

Forty-eight adult male and female Wistar rats, aged 12 weeks at the beginning of the experiment, were used. Sham-operated male and female rats weighed 304 ± 46 and 212 ± 19 g at the beginning of the experiment and 352 ± 39 and 228 ± 18 g at the end of the experiment. Adrenalectomized male and female rats with corticosterone replacement (ADXR) weighed 302 ± 32 and 201 ± 20 at the beginning of the experiment and 338 ± 38 and 221 ± 17 at the end of the experiment. Animals were group-housed under controlled 12:12 light/dark cycles (lights on at 07:00 a.m.) and temperature (22 ± 2 °C), with free access to food and either tap water pre-operatively or an aqueous solution post-operatively, as described in Section 2.3. Behavioral testing took place during the morning, between 0900 and 1200 h. A timeline of the experiment is depicted in Fig. 1. All efforts were made to minimize animal suffering and to reduce the number of animals used. All animal experiments were carried out in accordance with the EEC directive 86/609.

2.2. Estrous cycle

In the case of females, a semi-random process controlled for disparities regarding the phases of the estrous cycle. Specifically, female rats were selected from a larger pool of experimental animals on the basis of a regular 4 day cycle and assigned to surgery groups (Sham-operated or ADXR). The equal distribution of estrous cycle phases was then daily monitored by vaginal smears for one week before commencement and during behavioral testing, as described elsewhere (Becker et al., 2005). Although previous studies have shown that stress (Paredes et al., 1998) and adrenalectomy alone without corticosterone replacement (Galvez et al., 1999) may affect ovarian function, in our ADXR rats we did not observe any disruption in the estrous cycle. For the purpose of increasing statistical power, female rats in proestrous and estrous phase of the cycle were grouped together (females P + E) and similarly females in methestrous and diestrous phase of the cycle were also grouped together (females M + D), under the understanding that P + E females are generally under the influence of higher estrogens levels than M + D female rats (Consoli et al., 2005; Salomon et al., 2011).

2.3. Adrenalectomy & corticosterone replacement

Assignment to sham-operated or adrenalectomy with corticosterone replacement (ADXR) groups was random for male rats and semi-random for females, as described in Section 2.2. The bilateral adrenalectomy was aseptically performed via the dorsal approach with animals under anesthesia with 100 mg/kg ketamine, 10 mg/kg xylazine and 0.5 mg/kg atropine intraperitoneally. Tissue materials removed from all animals were immediately inspected to ensure the complete removal of the adrenal glands. In sham-operated animals the adrenal glands were reached and gently manipulated but not removed. Immediately after surgery and continuously throughout the experiment all rats were offered ad libidum a drinking solution consisting of 0.9% NaCl and 0.2% ethanol dissolved in tap water. ADXR rats were also receiving in their drinking solution corticosterone (C2505, Sigma Aldrich, St. Louis, MO) to a final concentration of 25 μg/ml. This method of corticosterone replacement has been shown to mimic the circadian secretion of endogenous corticosterone and this particular dose was suggested to produce basal levels of corticosterone and

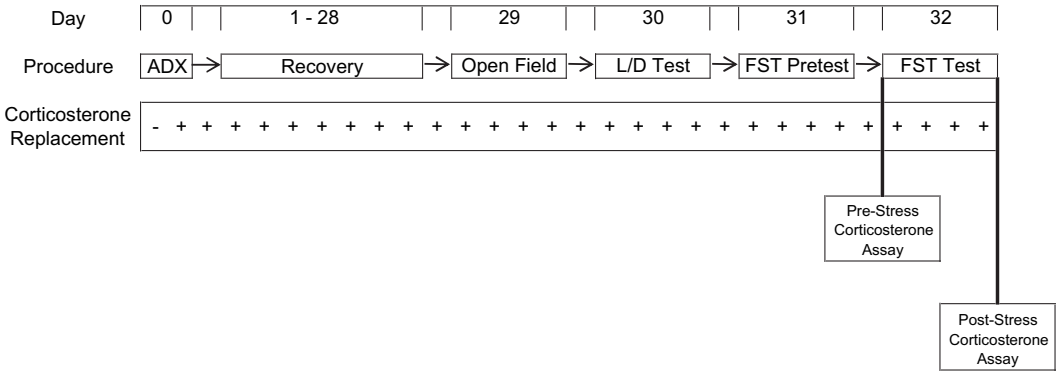

Fig. 1. Timeline of the experiment.