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Sex hormones affect acute and chronic stress responses in sexually dimorphic patterns: Consequences for depression models

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ABSTRACT

Background: Alterations in peripheral sex hormones may play an important role in sex differences in terms of stress responses and mood disorders. It is not yet known whether and how stress-related brain systems and brain sex steroid levels fluctuate in relation to changes in peripheral sex hormone levels, or whether the different sexes show different patterns. We aimed to investigate systematically, in male and female rats, the effect of decreased circulating sex hormone levels following gonadectomy on acute and chronic stress responses, manifested as changes in plasma and hypothalamic sex steroids and hypothalamic stress-related molecules.

Method: Experiment (Exp)-1: Rats (14 males, 14 females) were gonadectomized or sham-operated (intact); Exp-2: gonadectomized and intact rats (28 males, 28 females) were exposed to acute foot shock or no stressor; and Exp-3: gonadectomized and intact rats (32 males, 32 females) were exposed to chronic unpredictable mild stress (CUMS) or no stressor. For all rats, plasma and hypothalamic testosterone (T), estradiol (E2), and the expression of stress-related molecules were determined, including corticotropin-releasing hormone, vasopressin, oxytocin, aromatase, and the receptors for estrogens, androgens, glucocorticoids, and mineralocorticoids.

Results: *Surprisingly,* no significant correlation was observed in terms of plasma sex hormones, brain sex steroids, and hypothalamic stress-related molecule mRNAs ($p \geq 0.113$) in intact or gonadectomized, male or female, rats. Male and female rats, either intact or gonadectomized and exposed to acute or chronic stress, showed different patterns of stress-related molecule changes.

Conclusion: Diminished peripheral sex hormone levels lead to different peripheral and central patterns of change in the stress response systems in male and female rats. This has implications for the choice of models for the study of the different types of mood disorders which also show sex differences.

1. Introduction

A multitude of different genetic and developmental causes may lead to alterations in a network mediated by stress- and reward-related neurotransmitter and neuromodulator systems, which - in different ways - cause individuals to be vulnerable for depression facing the stressful life events (Bao et al., 2012). The hypothalamo-pituitary-adrenal (HPA) axis is a hub for integrating the neuroendocrine responses to stress (Ramot et al., 2017) and for the pathogenesis of mood disorders (Bao et al., 2005). It is well-accepted that, although the stress response is necessary to maintain homeostasis, long-term activation of the stress system brings detrimental or even fatal effects, and increases the risk of depression (Selye, 1998). Abnormalities in the 'stress system' or a hyperactive HPA axis has been observed in different types of depressive disorders (Belvederi Murri et al., 2016; Lu et al., 2018; Wessa et al., 2006; Zaconeta et al., 2015).

The neuroendocrine response to stress and the prevalence of depression show clear sex differences (Bao and Swaab, 2011). There is a wealth of evidence that female rats show a much larger magnitude of HPA activation to stress than male rats (for review, see (Goel et al.

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(2014)). Sex steroids have a major influence on the HPA activity and can impact gene expression, protein synthesis and cellular excitability through their actions on androgen receptors (AR) and estrogen receptors (ER) (Goel et al., 2014). We and other groups have observed that sex steroids may differentially regulate gene expression of corticotrophin-releasing hormone (CRH), the central driving force of HPA activity. Estrogen stimulates CRH gene expression (Vamvakopoulos and Chrousos, 1994), while testosterone (T) inhibits it (Bao et al., 2006). This explains at least some of the sex differences in stress response and depression.

It has been noticed that during the reproductive age, the prevalence of depression in women is double that of men (Goodwin and Gotlib, 2004). This time in a woman's life is characterized by fluctuations in the peripheral sex hormone levels (Schmidt et al., 2015). Our previous study found that female major depression patients did indeed have significantly higher amplitudes of diurnal estradiol rhythms than controls (Bao et al., 2004). Also, elderly men with lower plasma T levels are more frequently depressed (Barrett-Connor et al., 1999), and severely depressed men show lower plasma T levels (Heuser, 2002). However, these findings lack data on a possible causal role of peripheral sex hormone changes. Based upon the role that sex steroids play in the regulation of brain stress-related molecules, such as CRH (see above), it would be interesting to know more about the relation – if there is one - between the fluctuation of peripheral sex hormone levels and brain stress-related molecules.

Sex steroids are not only produced in the gonads and adrenal gland (Stocco, 2012), but also in the brain, by neurons and glia cells (Baulieu, 1998). It is generally presumed – although to our knowledge this presumption has not been proven – that, as small lipophilic molecules, sex steroid levels may diffuse freely over the blood-brain-barrier, and brain sex steroid levels may fluctuate simultaneously with peripheral levels and have feedback effects on the brain. However, there is our observation that the mRNA levels of stress-related compounds, e.g. CRH, ERα, ERβ, and AR, were the same in proestrus and diestrus stages in the rat hypothalamus, but that the plasma estradiol (E2) and T levels differed (Lu et al., 2015). In the present study, this unexpected observation is followed up with larger plasma level changes following gonadectomy. In addition, in stress-related mood disorders, changes have been reported in peripheral sex hormone levels (Barrett-Connor et al., 1999; Goodwin and Gotlib, 2004; Heuser, 2002), which makes this topic also clinically relevant.

Although there is - fragmented - information that peripheral sex hormones are involved in the stress response (Jacobs et al., 2015), a systematic analysis of the central and peripheral stress response in male and female rats undergoing the same acute or chronic stress under the same experimental conditions is still lacking. The working hypothesis of the present study was that diminished peripheral sex hormone levels would lead to different patterns of change in the peripheral and central stress response systems in male and female rats. The implications will be discussed for the choice of model for the study of the different types of mood disorders, which show sex differences, e.g. in prevalence.

We therefore investigate - separately for each sex - the effect of diminished peripheral sex steroids, caused by gonadectomy, on the patterns of the central and peripheral stress response, following acute or chronic stress. It should be noted that due to the limited availability of hypothalamic tissue for measurement of sex steroid and stress-related molecule-mRNA expression from the same rat, we were able to measure only brain T in male rats and brain E2 in female rats. In addition, since the gonadectomy removes different hormones in male and female rats and are different procedures, direct parameter comparisons between male and female rats would be unsuitable. Therefore, after gonadectomy, we monitored and discussed the patterns of change in male/female rats separately, with or without acute or chronic stress. We paid special attention to the effect of removing peripheral sex hormones on stress systems which include hypothalamic sex steroid and stress-related molecule expression, since sex steroids play significant roles in regulating HPA activity, and thus for the vulnerability for depression (see above). The discussion section shows our findings in relation to the question whether the different paradigms deliver suitable models for mood disorder studies.

2. Material and methods

2.1. Animals

Adult male (250–300 g) and female (200–250 g) SD rats (aged 70–80 days) were kept on a 12 h–12 h light-dark cycle (lights on at 0700) with *ad libitum* access to food and water. Animals were acclimated to the environment for 7 days before the study. The estrous cycle of female rats was monitored by daily vaginal smears, and only those that showed cycles of 4–5 days were used in this study. Rats were randomly assigned to the different groups.

All the animal care procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996, USA) and approved by the National Committee for the Use of Experimental Animals for Medical Purposes, China, Zhejiang Branch. Every effort was made to minimize animal suffering and to use as few animals as possible.

2.2. Experimental design

2.2.1. Experiment (Exp)-1: Effect of gonadectomy on stress systems

Rats were gonadectomized, i.e. castrated (CAS, n = 7) or ovariectomized (OVX, n = 7), or sham-operated (intact, 7 male and 7 female rats) under chloral hydrate (10% i.p.) anesthesia. During the following 3 weeks of recovery, daily vaginal smears were taken from female rats between 0800 and 0900. OVX rats showed the characteristic diestrous smear and female intact rats were sacrificed in proestrous, since proestrous rats showed the highest plasma E2 levels (Lu et al., 2015).

Rats were sacrificed by decapitation between 0900 and 1100. Trunk blood was collected in EDTA-containing tubes. Plasma was frozen (−80 °C) until assayed for CORT, T and E2.

The hypothalamus, frontal cortex and hippocampus were dissected; brain samples were weighed and frozen at −80 °C until assayed for sex steroids and mRNA expression of hypothalamic stress-related molecules. The brain areas were dissected along the following borders: i) *hypothalamus*: anterior border of the optic chiasm, posterior border of the mamillary bodies, lateral hypothalamic nuclei and a dorsal cut at approximately 3 mm from the bottom; ii) *frontal cortex*: after removal of the olfactory lobe, a frontal cut was made at bregma 1.7 mm (Paxinos, 2004); iii) *hippocampus*: following exposure of its medial surface the closed tip of a curved forceps was used to remove the diencephalon and to detach the hippocampus from the cortex (Hill et al., 2014). Brain tissues were weighed and frozen at −80 °C until assayed for sex steroids and mRNA expression of hypothalamic stress-related molecules.

2.2.2. Exp-2: Effect of gonadectomy on acute stress response

The protocol of acute electric foot shock (FS) was: FS 0.8 mA, 5 s duration, 15 s blank, and a total period of 150 s (Bali and Jaggi, 2015). The rats were grouped as follows: male control-intact, control-CAS, male FS-intact, FS-CAS; female control-intact, control-OVX, female FS-intact, and FS-OVX. After post-operative recovery, the groups were exposed to either FS or no stress as control. The rats were sacrificed 5 min after the beginning of FS as in Exp-1, except that only the hypothalamus was taken to measure sex steroid levels and mRNA levels of stress-related molecules.

2.2.3. Exp-3: Effect of gonadectomy on chronic stress response

The chronic unpredictable mild stress (CUMS) protocol is often used as an animal model for depression (Mao et al., 2009). CUMS rats were single-housed and subjected to 2 different stressors per day (white