Hormones and Behavior 60 (2011) 380–388

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Male rats with the testicular feminization mutation of the androgen receptor display elevated anxiety-related behavior and corticosterone response to mild stress

Damian G. Zuloaga *, Jessica E. Poort, Cynthia L. Jordan, S. Marc Breedlove

Department of Psychology and Program in Neuroscience, 108 Giltner Hall, Michigan State University, East Lansing, MI 48824-1101, USA

A R T I C L E I N F O

Article history:
Received 9 April 2011
Revised 30 June 2011
Accepted 7 July 2011
Available online 23 July 2011

Keywords:
Androgen receptor
Anxiety
Corticosterone
Sensorimotor gating
Testicular feminization mutation

A B S T R A C T

Testosterone influences the hypothalamic–pituitary–adrenal axis, anxiety-related behavior, and sensorimotor gating in rodents, but little is known about the role of the androgen receptor (AR) in mediating these influences. We compared levels of the stress hormone corticosterone at baseline and following exposure to a novel object in an open field in wild type (wt) male and female rats, and male rats with the testicular feminization mutation (Tfm) of the *AR*, which disables its function. Basal corticosterone was equivalent in all groups, but exposure to a novel object in an open field elicited a greater increase in corticosterone in Tfm males and wt females than in wt males. Tfm males also showed increased behavioral indices of anxiety compared to wt males and females in the test. Analysis of the immediate early gene c-Fos expression after exposure to a novel object revealed greater activation in Tfm males than wt males in some regions (medial preoptic area) and lesser activation in others (dentate gyrus, posterodorsal medial amygdala). No differences were found in a measure of sensorimotor gating (prepulse inhibition of the acoustic startle response), although Tfm males had an increased acoustic startle response compared to wt males and females. These findings demonstrate that ARs play a role in regulating anxiety-related behaviors, as well as corticosterone responses and neural activation following exposure to a mild stressor in rats.

© 2011 Elsevier Inc. All rights reserved.

Introduction

In humans, gonadal hormones influence mood disorders including anxiety and depression. Women are diagnosed with anxiety disorders and depression more often than are men, and these disorders often coincide with a decline in levels of estrogen during menopause (Arpels, 1996). Furthermore, estrogen replacement therapy has been reported to decrease anxiety in postmenopausal women (Yazici et al., 2003). In men, a similar but less abrupt decline in androgen levels with age is also often accompanied by symptoms of anxiety and depression (Kaminetsky, 2005; Lund et al., 1999; Eskelinen et al., 2007; Cooper and Ritchie, 2000). Androgen treatment of aging men, or of younger men with decreased testicular production of testosterone (T), ameliorates some of these symptoms (Amore et al., 2009; Genazzani et al., 2004; Seidman et al., 2009; Kaminetsky, 2005; Cooper and Ritchie, 2000; Kumano, 2007). Similarly, boys and girls with low T levels show greater indices of depression and anxiety than those with high T (Granger et al., 2003).

Gonadal hormones also appear to influence anxiety and depression-related behaviors in rodents. In rats, females often show fewer anxiety-related behaviors than males (Archer, 1975; Masur et al., 1980; Slob et al., 1981; Seliger, 1977; Lucion et al., 1996). Furthermore, administration of either estrogens or androgens generally results in decreased indices of anxiety and depression-related behaviors in rodents (Frye and Lacey, 2001; Walf and Frye, 2005; Lund et al., 2005; Frye et al., 2008; Bing et al., 1998; Bitran et al., 1993). Evidence suggests that anxiolytic actions of estrogens are largely mediated through activation of the estrogen receptor (ER) isoform ERβ (Lund et al., 2005; Imwalle et al., 2005). On the other hand, androgen action can be mediated through several mechanisms including androgen receptors (ARs), GABA-A receptors (Edinger and Frye, 2006; Lund et al., 2005; Edinger and Frye, 2005), and ERs.

Sex hormones also modulate hypothalamic–pituitary–adrenal (HPA) axis activity (Handa et al., 1994a; Lund et al., 2005). These hormones may in turn affect behavior in rodents exposed to an aversive situation, since hyperactivity of the HPA axis is a symptom of rats that display trait anxiety (Jakovcevski et al., 2008; Landgraf et al., 1999). In particular, T treatment decreases, while estrogen treatment increases, the release of stress hormones adrenocorticotropic hormone (ACTH) from the pituitary gland, and corticosterone from the adrenal cortex. Specific activation of sex hormone receptors ERα and ERβ increases and decreases HPA axis activity respectively, while AR activation also appears to decrease HPA activity, although its role is less clear (Handa et al., 1994b; Lund et al., 2004, 2005, 2006).

There are also sex differences in sensorimotor gating (men> women; Swerdlow et al., 1993) as assessed by an experimental model of sensorimotor gating, prepulse inhibition of the acoustic startle response (PPI; Swerdlow et al., 1996). Furthermore, PPI varies in

* Corresponding author at: Basic Medical Sciences University of Arizona College of Medicine-Phoenix, USA. Fax: +1 602 827 2130.
E-mail address: d.zuloaga@arizona.edu (D.G. Zuloaga).

0018-506X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2011.07.008

women according to the stage of the menstrual cycle, suggesting that variations in sex hormones can influence PPI (Swerdlow et al., 1997; Jovanovic et al., 2004). Sex differences in PPI have also been reported in Wistar rats and in mice (males > females; Lehmann et al., 1999; Ralph et al., 2001; Ison and Allen, 2007), and PPI varies across the estrus cycle in rats (Koch, 1998). Administration of estrogens and androgens can facilitate PPI (van den Buuse and Eikelis, 2001; Gogos and Van den Buuse, 2003), although little is known about the role of specific hormone receptors, including the AR, that may mediate these changes.

Use of pharmacological agents to probe which steroid receptors mediate a response to T are useful, but interpretation of these experiments can be complicated. DHT, a non-aromatizable androgen, can also be metabolized to 3α-androstanediol (3α-diol) which has a high affinity for GABA-A receptors. Moreover, the 3β-diol metabolite of DHT is an estrogenic compound, with greater affinity for ERβ than ERα (Lund et al., 2004). Another approach to ascertain the receptor(s) mediating T effects on stress hormone responses and behavior is to examine animals with a dysfunctional AR allele, the testicular feminization mutation (Tfm; Allison, 1965; Yarbrough et al., 1990; Zuloaga et al., 2008a). If XY males carrying this allele behave identically to wildtype (wt) males, then AR is not necessary for the behavior. Alternatively, if Tfm males differ from wt males for any given behavior, then AR normally influences that behavior.

We previously investigated corticosterone responses and anxiety-related behaviors in response to a mild stressor in wt male and Tfm male mice and found that a dysfunctional AR led to increases in both (Zuloaga et al., 2008b). We chose to examine these same responses to stress in the Tfm rat model to determine whether the apparent role of AR in the HPA axis is idiosyncratic to mice or more broadly relevant to other mammals. Another reason to examine Tfm male rats stems from a drawback of the Tfm mutation in mice; namely, Tfm male mice secrete virtually no T in adulthood, so behavioral differences between wild-type and Tfm male mice could be due either to differences in AR function or to differences in circulating T, either during or before the time of testing. While androgen can be pharmacologically replaced in Tfm mice, such paradigms are necessarily imperfect. Furthermore, because both Tfm rats and humans with complete androgen insensitivity secrete levels of T that are *higher* than in normal males (Roselli et al., 1987; Vague, 1983), Tfm rats may be a better model for the human condition than Tfm mice. In Tfm rats, as in CAIS humans, reduced AR-mediated negative feedback causes increased gonadotropin release and therefore greater androgen secretion (Yarbrough et al., 1990). While the Tfm mutation in rats does not completely render the AR nonfunctional, as it does in the mouse, the decreases in function by 85–90% (Yarbrough et al., 1990) are sufficient to produce an entirely feminine external phenotype (Allison, 1965). As a result, the Tfm rat may also be a better model for the human partial androgen insensitivity syndrome. In the present study, we investigated the role of ARs in the regulation of the HPA axis, anxiety-related behavior, and sensorimotor gating in the Tfm rat model. Our results suggest that in rats, as in mice, AR normally dampens HPA axis activation and anxiety-related behaviors. Furthermore, we present evidence of three brain regions that may mediate these effects of AR.

Materials and methods

Animals

Long Evans rats carrying the *Tfm* allele, which were bred with commercially purchased Long Evans (Charles River) sires for over 10 generations, were group housed in our colony at Michigan State University with a 12/12 L/D cycle, lights on at 0600. These rats are descendents of the original King–Holtzman strain of Tfm rats (Allison, 1965). Upon weaning at 21 days of age, ear punches were obtained to determine genotype using a modified polymerase chain reaction (PCR) to detect Tfm versus wt alleles for AR, and the presence or absence of the Sry gene found only on the Y chromosome (Fernandez et al., 2003). Wt males, wt females that were not carrying the Tfm allele, and Tfm males were used. All animals received care that meets standards of the National Institutes of Health and all experiments were approved by the Michigan State University IACUC.

Experiment 1: the role of ARs in HPA response to mild stress

Plasma corticosterone was collected from 120 to 180 day old rats at baseline (wt male: N = 10; wt female: N = 10; Tfm male: N = 10) or 20 min after initial exposure to an open field with a novel object (wt male: N = 10; wt female: N = 10; Tfm male: N = 10).

Experiment 2: the role of ARs in HPA axis recovery

To assess whether the time course of HPA axis recovery differed in wt and Tfm males following exposure to a mild stressor, 120–180 day old rats were assayed for corticosterone at baseline (wt male: N = 7; Tfm male: N = 6), or at 20 (wt male: N = 7; Tfm male: N = 7), 40 (wt male: N = 6; Tfm male: N = 6), 60 (wt male: N = 6; Tfm male: N = 6), or 120 (wt male: N = 6; Tfm male: N = 6) minutes after exposure to an open field with a novel object. This experiment focused only on wt and Tfm males to further probe the role of ARs in the male stress response, as sex differences in rodent corticosterone recovery from stress are already well documented (Handa et al., 1994a; Kudielka and Kirschbaum, 2005).

Experiment 3: the role of ARs in anxiety-related behavior

One hundred twenty–one hundred fifty day old wt male (N = 8), wt female (N = 5), and Tfm male rats (N = 11) were tested for anxiety-related behaviors in the open field/novel object test as described below.

Experiment 4: the role of ARs in immediate early gene activation

To investigate how specific brain areas may be activated differently due to the presence or absence of functional ARs in males, we immunostained for the immediate early gene c-Fos in 120–150 day old Tfm and wt male rats at baseline (wt male: N = 7; Tfm male: N = 7) and after exposure to an open field with a novel object (wt male: N = 7; Tfm male: N = 7).

Experiment 5: the role of ARs in sensorimotor gating

One hundred twenty–one hundred fifty day old wt male (N = 9), wt female (N = 11), and Tfm male rats (N = 10) were tested for PPI and acoustic startle response (ASR) as described below.

Open field/novel object test

Open field/novel object testing was conducted between 1000 and 1400 in a 122 cm × 122 cm white plastic box illuminated from directly above by a 60 W light, a meter above the floor of the box. A grid was drawn in the box (individual grids were 20.3 cm × 20.3 cm) to demarcate entries into the center area and activity (grid crossings). For corticosterone and immediate early gene analysis, rats were placed into the open field containing a novel object (a 4″ diameter × 8″ high cylindrical metal oxygen tank cap) for 10 min. For behavioral analysis, rats were first placed into a corner of the empty open field and behavior was recorded via an overhead video camera for 5 min. After 5 min rats were removed, the box was cleaned with 70% ethanol, and a novel object was placed in the center of the chamber. Three minutes after removal from the open field, rats were replaced into the chamber now containing the novel object and behavior was recorded for another 5 min. We recorded the amount of time spent grooming, number of entries into the center area, time spent in the center area, visits to the novel object, and time spent visiting the novel object, which have previously been reported to reflect anxiety in rodents (Delini-Stula and Hunn, 1988). The number of grid crossings and rearings were assessed as measures of activity and all behaviors were

Download English Version:

https://daneshyari.com/en/article/10300701

Download Persian Version:

https://daneshyari.com/article/10300701

Daneshyari.com