Hormones and Behavior 106 (2018) 10–18

Contents lists available at ScienceDirect

Hormones and Behavior

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

Androgen receptor is a negative regulator of contextual fear memory in male mice

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ARTICLE INFO

Keywords:
Fear conditioning
Androgen
Testosterone
Flutamide
Gonadectomy
Gene expression
Hippocampus

ABSTRACT

Although sex-hormones have a well-documented role in memory formation, most literature has focused on estrogens, whereas the role of androgens and their receptor (the androgen receptor; AR) in fear memory is relatively unexplored. To address this gap, we used a transgenic mouse model of AR overexpression (CMV-AR) to determine if AR regulates fear memory, and if this effect can be reversed either by the removal of circulating androgens via gonadectomy, or by antagonising AR activity with flutamide. We found that AR overexpression results in reduced freezing in response to foot shock, and that this difference is reversed with both gonadectomy and flutamide treatment. Differences between genotypes were reinstated by testosterone replacement in gonadectomized mice, suggesting that reduced fear memory in mutants results from AR activation by testosterone and is not secondary to group differences in circulating testosterone. Potential transcriptional mechanisms by which CMV-AR exerts its effects on fear memory were assessed by quantitating the expression of memory-related genes in area CA1 of the hippocampus. Several genes that are altered with AR inhibition and activation, including genes that encode for the histone variant H2A.Z, cholinergic receptors, glutamate receptors, and brain-derived neurotrophic factor. Overall, our findings suggest that AR is a negative regulator of fear memory and identify potential gene targets through which AR may mediate this effect.

1. Introduction

Hormones powerfully regulate emotional and cognitive systems that are involved in establishing both normal and pathological forms of associative fear memory, which manifest in conditions such as post-traumatic stress disorder (PTSD) at different rates in men and women (Aikey et al., 2002; Dalla and Shors, 2009; Edinger and Frye, 2004, 2005; Jovanovic et al., 2015; Kranz et al., 2015; McHenry et al., 2014; Morgan and Pfaff, 2001; Suzuki et al., 2013). For example, PTSD is characterized by excessive fear and intrusive memories that disproportionately affect women compared to men who are exposed to trauma (Breslau et al., 1998; Holbrook et al., 2002), suggesting that testosterone is protective against PTSD, as it is against other types of affective disorders (Kaminetsky, 2005; Veras and Nardi, 2010). Despite evidence that androgens impact cognition and emotional memory, the nature of their influence is unclear (Burkitt et al., 2007; Galea et al., 2008; Gouchie and Kimura, 1991; Janowsky, 2006; Moffat and Hampson, 1996). Indeed, the majority of research on the role of sex hormones has focused primarily on estrogenic mechanisms (Frick et al., 2017; Galea et al., 2008, 2017; Sherwin, 1988, 2005), whereas the role of the androgen receptor (AR) in hippocampus-dependent fear memory remains unclear.

AR is expressed in brain regions that regulate contextual fear memory, most notably in areas CA1 and CA3 of the hippocampus (Kerr et al., 1995; Raskin et al., 2009). Studies have reported mixed effects of AR and its ligands, testosterone and dihydrotestosterone (DHT), on fear memory. For example, some studies report no effects of gonadectomy on fear memory in male rodents (Anagnostaras et al., 1998), whereas others report distinct effects of androgenic manipulations on contextual and cued fear memory (Chen et al., 2014; Edinger et al., 2004; Frye et al., 2008; MacLusky et al., 2005; Rubin, 2011; Zhang et al., 2014). Moreover, some evidence shows that gonadectomy reduces fear memory (Edinger et al., 2004; McDermott et al., 2012) and that this deficit is rescued by treatment with either testosterone or 17β estradiol, but not with DHT (Edinger et al., 2004). Given that DHT is a direct AR agonist, whereas testosterone can be further aromatized into 17β estradiol to activate estrogen receptors (Hojo et al., 2004; Mukai et al., 2010; Ooishi et al., 2012), these data suggest that testosterone may

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https://doi.org/10.1016/j.yhbeh.2018.08.012
Received 13 May 2018; Received in revised form 21 August 2018; Accepted 29 August 2018

influence fear memory through AR-independent actions. In contrast, both DHT and testosterone improved performance on the inhibitory avoidance test, in which mice learn to avoid an environment associated with shock (Edinger et al., 2004), supporting a direct role of AR in regulating fear-based tasks.

Androgenic regulation of morphological and functional plasticity in the hippocampus is also complex, with dissociable effects of androgen manipulations on excitability, spine density and neurotrophin production, and evidence for both androgenic and estrogenic mechanisms of gonadal testosterone (see Atwi et al., 2016 for review). For example, neural-specific AR deletion in mice reduces LTP induced by high-frequency stimulation, without impacting LTP induced by theta-burst stimulation (Picot et al., 2016), indicating that AR has selective effects on different types of hippocampal plasticity. Spine density is reduced in the medial prefrontal cortex (mPFC) of *Tfm* rats (Hajszan et al., 2007), and either testosterone or DHT maintain CA1 hippocampal spine synapse density in castrated male rats (Leranth et al., 2003). Androgens also increase hippocampal neurogenesis through modulation of survival of new neurons (see Galea et al., 2013 for review). Long-term testosterone/DHT replacement consistently ameliorates the impaired survival of new neurons in castrated males (Hamson et al., 2013; Spritzer et al., 2011b; Wainwright et al., 2011), while short-term androgen replacement shows mixed results (Carrier and Kabbaj, 2012; Spritzer et al., 2011a, 2011b; Wainwright et al., 2016). Further, survival is also impaired upon AR blocking with flutamide (Hamson et al., 2013).

Given the complexity of androgenic compound actions, it can be difficult to distinguish between androgenic compounds that act through the AR compared to alternative modes of action. That is, testosterone can activate AR directly, or through its metabolite, DHT. In addition, DHT can be further metabolized to 3α-androstanediol to act on the GABA-A receptors, and testosterone can also be aromatized to estradiol to activate the estrogen receptor, which typically potentiates fear memory (Maeng and Milad, 2015). Thus, to specifically address the role of AR in fear memory, we utilized an AR-overexpressing mouse line (Swift-Gallant et al., 2016b), which we previously used to reveal novel insights into AR function in several behavioural processes, including the resident-intruder paradigm, sexual and aggressive behaviors, and partner preference (Swift-Gallant et al., 2016a, 2016b). Whereas loss-of-function mutations are invaluable in determining the necessity of AR, AR overexpressing mice take advantage of increased androgen response as a result of increased receptor expression to elucidate phenotypes that emerge at the high range of androgenic signaling. This approach has revealed functions of high AR levels in disease and sexual differentiation that do not always correlate with predictions made from loss-of-function mutant studies (Coome et al., 2017; Ramzan et al., 2015; Swift-Gallant et al., 2016b), demonstrating a unique utility of this mouse line for studying AR function in fear memory.

In the present study, our goal was to investigate the role of AR in fear memory by addressing four questions: 1) Does increased AR density affect fear memory; 2) Is this effect mediated by testosterone; 3) Can effects of AR density on fear memory be reversed by blocking AR receptors, either indirectly (through gonadectomy) or directly (using the AR antagonist, flutamide); and 4) Given that AR is a ligand-activated transcription factor, does increased AR density alter the expression of memory-related genes in area CA1 of the hippocampus?

2. Methods

2.1. Animals

Male C57Bl/6 mice bred in our colony were pair housed after weaning and were assigned to testing groups at 60–90 days of age. Mice were housed in 7.5 × 11.5 × 5 in cages and maintained on a 12 h light cycle, with ad libitum access to standard mouse chow (Harlan Teklad, Madison, WI) and water. Transgenic mice containing a CMV-stop-AR transgene were crossed with a CMV-*Cre* line to obtain tissue-wide AR overexpression, as previously described (Swift-Gallant et al., 2016b). Briefly, CMV-stop-AR mice contain a cytomegalovirus (CMV) promoter coupled to the human androgen receptor (hAR) gene, separated by a floxed stop sequence (Fig. 1a), which is excised when mice are crossed with the CMV-*Cre* line to allow for hAR transcription, beginning ~ embryonic day 8.5, when CMV expression occurs (Baskar et al., 1996). Details on mouse generation can be found in (Swift-Gallant et al., 2016b). All of the procedures were approved by the University of Toronto Mississauga animal care committee and complied with institutional guidelines and the Canadian Council on Animal Care.

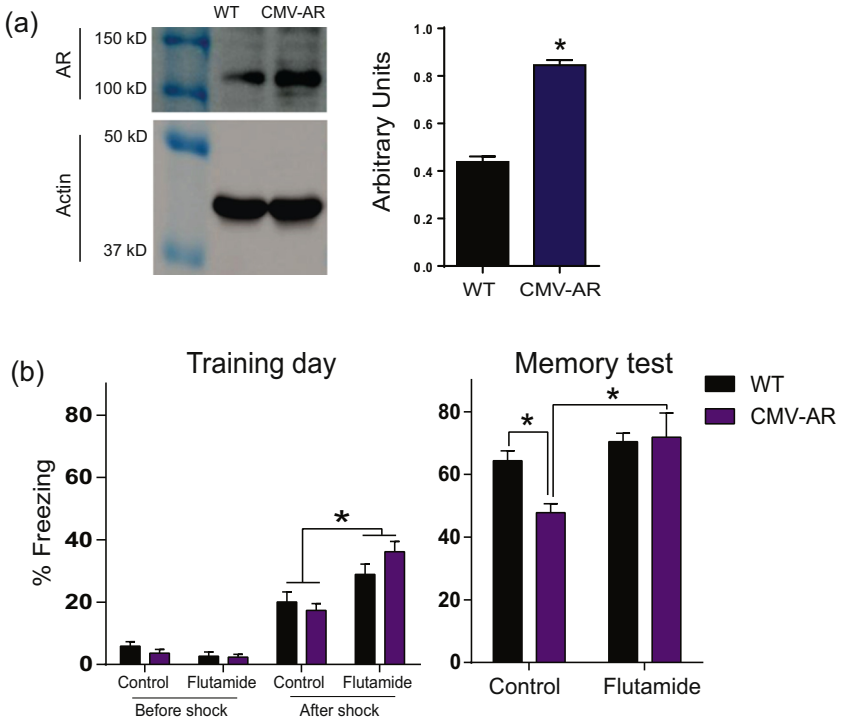

Fig. 1. AR activation impairs fear memory in CMV-AR mice. (a) Representative immunoblot demonstrating that CMV-AR have higher AR protein levels in the hippocampus compared to WT controls. Quantification of the western blot (N = 2/group) is shown on the right. (b) Freezing behaviour during the training session (left) and during the memory test (right), conducted 24 h after training. N = 13/group for WT and CMV-AR in the control condition and 5/group for WT and CMV-AR in the flutamide condition. *p ≤ 0.05.