Psychoneuroendocrinology (2010) 35, 1473—1485

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Chronic anabolic androgenic steroid exposure alters corticotropin releasing factor expression and anxiety-like behaviors in the female mouse

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Received 6 January 2010; received in revised form 5 April 2010; accepted 27 April 2010

KEYWORDS
Anabolic;
Steroid;
CRF;
Anxiety;
Extended amygdala;
Startle;
Mouse

Summary In the past several decades, the therapeutic use of anabolic androgenic steroids (AAS) has been overshadowed by illicit use of these drugs by elite athletes and a growing number of adolescents to enhance performance and body image. As with adults, AAS use by adolescents is associated with a range of behavioral effects, including increased anxiety and altered responses to stress. It has been suggested that adolescents, especially adolescent females, may be particularly susceptible to the effects of these steroids, but few experiments in animal models have been performed to test this assertion. Here we show that chronic exposure of adolescent female mice to a mixture of three commonly abused AAS (testosterone cypionate, nandrolone decanoate and methandrostenolone; 7.5 mg/kg/day for 5 days) significantly enhanced anxiety-like behavior as assessed by the acoustic startle response (ASR), but did not augment the fear-potentiated startle response (FPS) or alter sensorimotor gating as assessed by prepulse inhibition of the acoustic startle response (PPI). AAS treatment also significantly increased the levels of corticotropin releasing factor (CRF) mRNA and somal-associated CRF immunoreactivity in the central nucleus of the amygdala (CeA), as well as neuropil-associated immunoreactivity in the dorsal aspect of the anterolateral division of the bed nucleus of the stria terminalis (dBnST). AAS treatment did not alter CRF receptor 1 or 2 mRNA in either the CeA or the dBnST; CRF immunoreactivity in the ventral BnST, the paraventricular nucleus (PVN) or the median eminence (ME); or peripheral levels of corticosterone. These results suggest that chronic AAS treatment of adolescent female mice may enhance generalized anxiety, but not sensorimotor gating or learned fear, via a mechanism that involves increased CRF-mediated signaling from CeA neurons projecting to the dBnST.

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doi:10.1016/j.psyneuen.2010.04.015

1. Introduction

Anabolic androgenic steroids comprise a large class of synthetic androgens developed for therapeutic purposes (for review, Basaria et al., 2001), but whose predominant use is now illicit self-administration to enhance athletic performance or body image (for review, Trenton and Currier, 2005; Kanayama et al., 2008). Adult men are reported to self-administer AAS at concentrations that reflect 10–100× therapeutic doses of testosterone, which are 10–40 mg/day (Wu, 1997; Daly et al., 2001; Trenton and Currier, 2005) and are prescribed to restore circulating testosterone to normal adult levels of 10–35 nmol/l (Perry et al., 2003; Matsumoto and Bremner, 2004). Girls and women are reported to take AAS at levels equivalent to or even exceeding those administered by men (Franke and Berendonk, 1997); thus the same self-administered doses that produce 10–100-fold higher than replacement levels in males may be expected to yield circulating levels of androgens that are orders of magnitude higher still than normal physiological levels of androgens in women and girls (<2 ng/l; Wu, 1997).

Laboratory experiments that assess the actions of a single AAS have provided excellent mechanistic insight into the actions of a given individual steroid (for review, Clark and Henderson, 2003; Clark et al., 2006). However, humans who use AAS nearly always do so in intricate and complex patterns that involve concurrent administration of combinations of different classes and types of AAS in a process called stacking (for review, Kutscher et al., 2002; Llewellyn, 2007). The rationale offered by athletes for combining AAS is that stacking activates multiple signaling pathways resulting in "synergistic actions" that augment the ratio of anabolic response to unwanted side effects (Sturmi and Diorio, 1998). Experimental data support the existence of such interactions. For example, recent data suggest that inclusion of 17α-alkylated AAS may inhibit endogenous aromatase, therefore altering the metabolic fate of 19-nortestosterone or testosterone ester AAS derivatives (Penatti et al., 2009b) that are substrates for aromatization (Ryan, 1959; Winters, 1990).

Approximately 0.5% of adolescent girls and ~2% of adolescent boys are estimated to abuse AAS (Bahrke et al., 2000; Miller et al., 2005; Johnston et al., 2009), engendering concern over the effects of exposure to high levels of synthetic steroids at a time when the regions of the brain important in affective behaviors are still developing and highly hormone-sensitive (Sato et al., 2008). Long-term risks associated with AAS use are reported to be higher in women than in men (Franke and Berendonk, 1997), and adolescents may be more sensitive than adults (O'Connor and Cicero, 1993). Sex-specific differences in sensitivity to AAS may also be compounded by sex-specific differences in susceptibility to some behavioral disorders, such as anxiety, which are more prevalent in women (for review, Zender and Olshansky, 2009). Despite the potential for greater untoward effects, few studies have focused on the effects of AAS use in females, and female adolescent subjects are particularly underrepresented (for review, Henderson et al., 2006).

While taken for performance- and image-enhancing actions, AAS use is also associated with multiple behavioral effects, including mania, hypomania, somatization, increased anxiety, irritability, extreme mood swings, abnormal levels of aggression, and paranoia (Pagonis et al., 2006; for review, Gruber and Pope, 2000; Trenton and Currier, 2005). Due to ethical constraints, there have been few randomized clinical trials, however, in those that have been performed, using moderate doses of a single AAS in adult male subjects, increased levels of hostility and anxiety were reported (Su et al., 1993; Pope et al., 2000). The extended amygdala, comprising the CeA and adjoining structures including the BnST, is fundamental to the expression of anxious states (for review, Davis and Whalen, 2001; Davis et al., 2010). Prior studies in rodents suggest that the CeA is paramount in the acquisition and expression of short-duration threat responses that reflect fear while the BnST is key in responses reflecting generalized (Walker et al., 2009a,b) and social (Lee et al., 2008) anxiety. Both changes to specific threats and generalized anxiety are noted in steroid abusers, but the latter may be particularly important with respect to the expression of inappropriate reactions (aggression) and increased negative perception of self and others (Pagonis et al., 2006). The ASR is an involuntary, highly reproducible contraction of facial and skeletal muscles in response to sudden acoustic stimuli that is conserved across all mammalian species and is highly sensitive to changes in perceptual or emotional state (for review, Koch, 1999). Unconditioned startle responses provide an assay of generalized anxiety, while pairing of an aversive stimulus with an auditory or visual cue (FPS) provides an assay of conditioned fear (for review, Davis, 2006).

The CRF family of peptides acts to integrate sensory, endocrine and autonomic information and formulate an appropriate response to stress (for review, Korosi and Baram, 2008). Elevated levels of central CRF are implicated in anxiety and stress disorders in humans (for review, Reul and Holsboer, 2002; Holsboer and Ising, 2008) and central infusion of CRF in rodents stimulates anxiety-like behaviors (for review, Bale and Vale, 2004). The lateral portion of the CeA is the major extrahypothalamic site of CRF mRNA expression (Day et al., 1999; Asan et al., 2005). Neurons from the CeA send a strong projection to the anterolateral BnST (Sun and Cassell, 1993) where ~60% of the CRF immunolabeling in the BnST is associated with axonal profiles (Jaferi et al., 2009). CRF receptor 1 (CRF-R1) mRNA is expressed throughout the BnST, and CRF receptor 2 (CRF-R2) mRNA is expressed in the posterior, but not anterior, divisions of this nucleus (van Pett et al., 2000). CRF, acting primarily through CRF-R1, augments the ASR, but not FPS, and the BnST is the critical site for CRF-dependent ASR enhancement (Swerdlow et al., 1986, 1989; Liang et al., 1992; Risbrough et al., 2003, 2004; Risbrough and Geyer, 2005; Walker et al., 2009a,b).

Few studies have assessed AAS effects on stress hormones or their receptors (Ahima and Harlan, 1992; Schlussman et al., 2000). The goal of the present study was to determine if chronic exposure of adolescent female mice to a mixture of commonly abused AAS would promote changes in either learned fear or generalized anxiety and whether such behavioral actions could be correlated with AAS-dependent alterations in the expression of CRF and its receptors in the CeA and/or BnST.