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SEX-SPECIFIC EFFECTS OF CHRONIC ANABOLIC ANDROGENIC STEROID TREATMENT ON $GABA_A$ RECEPTOR EXPRESSION AND FUNCTION IN ADOLESCENT MICE

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Abstract—Anabolic androgenic steroids are synthetic derivatives of testosterone designed for therapeutic uses, but now taken as drugs of abuse. Potential health risks associated with anabolic androgenic steroid abuse are believed to be higher in adolescents than in adults, but few studies have tested anabolic androgenic steroid effects in adolescent subjects or determined if effects of these steroids differ between females and males. We have studied $GABA_A$ receptor expression and function in the medial preoptic nucleus of mice chronically treated during adolescence with the anabolic androgenic steroid, 17α-methyltestosterone. Three-week treatment did not elicit significant differences the expression of α_1, α_2 or α_5 subunit mRNAs in animals of either sex, although there was a trend toward decreases in all three subunit mRNAs in female mice, which was augmented and attained significance for the α_2 subunit mRNA in females treated for six weeks. Immunocytochemical analysis revealed that treatment with 17α-methyltestosterone for 6 weeks also elicited a significant decrease in the number of α_2-immunopositive neurons in female subjects. To test if anabolic androgenic steroid treatment also promoted changes in $GABA_A$ receptor function, spontaneous inhibitory synaptic currents were analyzed in adolescent animals treated for 3–4 weeks. This treatment regimen promoted a significant decrease in spontaneous inhibitory synaptic current frequency in female, but not male mice. Finally, anabolic androgenic steroid treatment was found to have no effect on the numbers of interneurons within the medial preoptic nucleus, as assessed by immunoreactivity for calcium binding proteins, suggesting that the decrease in the frequency of spontaneous inhibitory synaptic currents in female mice does not arise from an anabolic androgenic steroid-induced loss of interneurons. Taken together, our results indicate that chronic exposure to 17α-methyltestosterone elicits significant changes in GABAergic transmission in the medial preoptic nucleus of female, but not male, mice effectively enhancing the sexually dimorphic nature of GABAergic transmission in a forebrain region crucial for the expression of aggression and sexual behaviors.

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Abbreviations: AAS, anabolic androgenic steroids; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BSA, bovine serum albumin; C_T, threshold cycle; DAPI, 4′,6-diamidino-2-phenylindole; $GABA_A$, GABA type A receptor; I_{peak}, peak current amplitude; MPN, medial preoptic nucleus; mPOA, medial preoptic area; PBS, phosphate-buffered saline; PN, postnatal day; RT-PCR, reverse transcription coupled with polymerase chain reaction; sIPSC, spontaneous inhibitory postsynaptic current; τ, time constant of decay; τ_w, weighted time constant; 17α-MeT, 17α-methyltestosterone.

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Anabolic androgenic steroids (AAS) are testosterone derivatives originally designed to enhance muscular mass and for the treatment of many clinical conditions (for review, Basaria et al., 2001; Shahidi, 2001), but are now taken predominantly as drugs of abuse (for review, Lukas, 1996). AAS abuse in the human population is characterized by supraphysiological concentrations of steroids, estimated to be 10–1000 times the levels of endogenous androgens or those resulting from therapeutic administration (for review, Lukas, 1993; Wu, 1997; Daly et al., 2001). AAS use has steadily increased over the past few decades, and the profile of those who self-administer these steroids has been changing. Whereas historically misuse of AAS has been restricted to elite athletes, recent estimations indicate an increasing use by adolescents, especially adolescent girls (Bahrke et al., 1998; Irving et al., 2002; Monitoring the Future Study, 2004).

In addition to the well-known consequences of AAS use on peripheral organ systems, AAS are also known to have a wide range of behavioral effects encompassing both positive mood symptoms, including euphoria, hypomania, increased libido, and negative symptoms, including increased anxiety, irritability, elevated levels of aggression and decreased libido (Su et al., 1993; Franke and Berendonk, 1997; Gruber and Pope, 2000; Pope et al., 2000; Perry et al., 2003). The complexity of these behavioral manifestations is further enhanced by reports indicating that long-term risks associated with AAS use are higher in women than in men (Franke and Berendonk, 1997) and that adolescents may be more sensitive than adults (O'Connor and Cicero, 1993). While human studies of these abused drugs are made difficult by their illicit nature, many of the AAS-induced changes in behavior are recapitulated in animals, which therefore provide useful models for steroid abuse (for review, Clark and Henderson, 2003).

Neural transmission mediated by GABA type A ($GABA_A$) receptors in the medial preoptic area (mPOA) is implicated in the expression of sexual behaviors (for review, McCarthy, 1995; Blaustein and Erskine, 2002; Hull et al., 2002), aggression (for review, Kruk, 1991), and anxiety (for review, Wilson, 1996; McNaughton and Corr, 2004), suggesting that GABAergic circuits in the mPOA may provide an important substrate for central AAS effects. In rats, the central region of the medial preoptic nucleus (MPN) is one of the most steroid-sensitive and sexually dimorphic

regions of the brain (for review, Simerly, 2002), suggesting that this region may also be an important site for AAS action and that effects of these synthetic steroids may not be equivalent in this region in males and females. Prior studies from our laboratory demonstrating that the ability of a chronic (4 week) treatment of C57Bl/6 mice with the AAS, 17α-methyltestosterone (17α-MeT) to elicit changes in $GABA_A$ receptor subunit mRNAs in the basal forebrain depends not only on the dose of the steroid, but also on the age and the sex of the subjects (McIntyre et al., 2002), support this supposition.

Here, we have determined whether the AAS, 17α-MeT, alters $GABA_A$ receptor subunit mRNAs in the MPN of mice treated in adolescence or treated in adolescence continuing into adulthood. We have further assessed whether AAS-dependent changes in mRNA are reflected in changes in $GABA_A$ receptor subunit proteins and in $GABA_A$ receptor-mediated synaptic transmission in the MPN. Finally, we have investigated whether there are sex-specific differences in the ability of this AAS to alter $GABA_A$ receptor function and expression within this critical forebrain region.

EXPERIMENTAL PROCEDURES

Animal care and use

Gonadally-intact male and female C57Bl/6J mice (Jackson Laboratories; Bar Harbor, ME, USA) were housed in a temperature-controlled and 12-h light/dark cycle facility with lights on starting at 07:00 h. Beginning on postnatal day 21 (PN21), treated subjects were administered 7.5 mg/kg/day 17α-MeT in sesame oil intraperitoneally for a period of three or six weeks. Control subjects were administered the same volume (10–60 μl based on body weight) of sesame oil alone. This dose is comparable to high doses in human abusers (Lukas, 1996), and is one that is known to alter the onset of puberty and to inhibit reproductive behaviors in both male and female rats (for review, Clark and Henderson, 2003). All animal care procedures were approved by the Institutional Animal Care and Use Committee at Dartmouth and are in agreement with the guidelines and recommendations of the National Institutes of Health and the American Veterinary Medical Association. All experiments were performed to minimize the numbers of animals used and their suffering.

Tissue analysis

All electrophysiological and immunocytochemical analyses were made from the central region of the MPN corresponding to the dorsal aspect of the MPN-medial and encompassing the MPN-central, as defined by Franklin and Paxinos (1997: Figs. 29–32) (Fig. 1). In contrast to the rat, there is not a marked sexual dimorphism in the volume of this brain region in the mouse (Young, 1982), and the equivalent region in both sexes was assayed in all analyses performed. For mRNA analyses, dissections were made from a region corresponding to the entire MPN.

RNA extraction and real time reverse transcription coupled with polymerase chain reaction (RT-PCR)

Coronal slices were prepared as described above. Data from animals examined at three weeks were obtained from three separate experiments ($n=17$ control and 20 treated females; $n=23$ control and 33 treated males). Data from animals examined at 6 weeks were obtained from three separate experiments ($n=12$ control and 11 treated females; $n=4$ control and 4 treated males). Tissue was dissected and stored in the RNA stabilization solution, RNAlater® (Ambion Inc., Austin, TX, USA) at −20 °C. Total RNA was extracted according to manufacturer's protocol for RNAqueous-4PCR kit (Ambion Inc.). Briefly, tissue was added to 200 μl lysis/binding buffer and homogenized. An equal volume of 64% EtOH was added and the sample vortexed. The lysate/ethanol mix was applied to an RNAqueous filter (supplied in the kit) and centrifuged for 1 min at 12,000 r.p.m. The flow through was discarded, the cartridge washed several times and RNA eluted with 50 μl hot elution buffer. The RNA was treated with two units DNase1 (supplied in the kit) for 30 min at 37 °C to remove any contaminating genomic DNA. DNase was inactivated by the addition of 0.1 volume DNase Inactivation Reagent, incubated at room temperature 2 min, centrifuged, and the DNase-free RNA supernatant collected. The concentration of the RNA was deter-

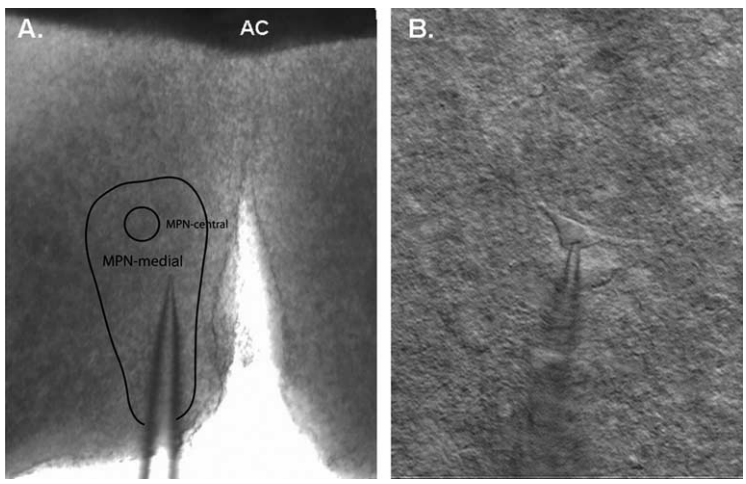

Fig. 1. Regions analyzed for electrophysiological, molecular biological and immunocytochemical assays. Representative micrographs of (A) an acutely isolated slice at the level of the MPN and a patch electrode. For both patch clamp recording and immunocytochemical analyses, tissue in the dorsal portion of the medial MPN that included the central-MPN was analyzed. Tissue isolated for mRNA analysis included a region approximating the extent of the MPN. (B) Representative higher power micrograph taken during the course of recording from an MPN neuron. AC, anterior commissure.