available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Region-specific mechanisms for testosterone-induced Fos in hamster brain

Anita Nagypál[1], *Ruth I. Wood**

Neuroscience Program, Department of Cell and Neurobiology, Keck School of Medicine of the University of Southern California, 1333 San Pablo St., BMT 401, Los Angeles, CA 90033, USA

ARTICLE INFO

Article history:
Accepted 6 January 2007
Available online 12 January 2007

Keywords:
Hamster
Testosterone
Anabolic agent
Immediate–early gene product
Reinforcement
Bed nucleus of the stria terminalis

ABSTRACT

Hamsters self-administer androgens. Previously, we determined that testosterone (T) activates select steroid- and opiate-sensitive brain regions. Is T-stimulated neuronal activation androgenic? Thirty-five castrated males with physiologic T replacement ($n=7$/group) were pre-treated with the androgen antagonist flutamide (15 mg/kg sc) or ethanol (0.25 ml) and infused into the lateral ventricle (ICV) for 4 h with 40 μg T (TF and TE, respectively) or 40 μl vehicle (VF and VE). To determine if androgens and opiates activate overlapping brain areas, 7 additional males received 20 μg morphine sulfate ICV following ethanol injection (ME). Immediately after ICV infusion, animals were perfused. Sixty-micrometer coronal brain slices were stained for Fos. Fos-positive neurons were counted in a 0.3-mm^2 area from 5 regions previously shown to express T-induced Fos: the posteromedial bed nucleus of the stria terminalis (BSTPM), posteromedial amygdala (MeP), lateral habenula (LHb), ventral tegmental area, and lateral pontine nucleus. T induced Fos in all areas reported previously (TE vs. VE, $p<0.05$), except LHb ($p>0.05$). Morphine induced Fos in all 5 brain regions (ME vs. VE, $p<0.05$), indicating that androgens and opiates activate overlapping brain regions. Flutamide alone did not induce Fos (VF vs. VE, $p>0.05$). Moreover, flutamide treatment blocked T-induced Fos expression only in the steroid-sensitive BSTPM, suggesting that androgens mediate neuronal activation in this area (mean ± SEM: TF: 68.4 ± 13.2 vs. TE: 137.9 ± 17.6, $p<0.05$). The absence of flutamide effects on T-induced Fos in the steroid-sensitive MeP (TE: 210.6 ± 50.0 vs. TF: 215.3 ± 28.2, $p>0.05$) suggests that distinct mechanisms activate Fos in individual androgen-responsive nuclei.

1. Introduction

Androgenic–anabolic steroids (AAS) are drugs of abuse (Brower, 2002; Yesalis et al., 1993). Animal studies have demonstrated that androgens are reinforcing via actions in the brain, suggesting the potential for dependence (Packard et al., 1997; Wood et al., 2004). In particular, a recent study from our laboratory using hamsters indicated that testosterone induces neuronal activation in brain areas activated by other commonly abused drugs, including the ventral tegmental area (VTA, DiMeo and Wood, 2006a). In addition, testosterone activates select steroid- and opiate-sensitive brain regions.

* *Corresponding author*. Fax: +1 323 442 3466.
E-mail address: riw@usc.edu (R.I. Wood).
[1] Present address: Department of Gerontology, Leonard Davis School of Gerontology, University of Southern California, LA 90033, USA.

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.01.022

The present study investigated the role of androgen receptors in brain activation, and the potential overlap in neuronal activation after morphine and testosterone exposure.

In another study from our laboratory, the competitive non-steroidal androgen antagonist, flutamide, blocked testosterone self-administration, indicating that androgens *per se* are essential for testosterone reinforcement (Peters and Wood, 2004a,b). This is significant because many effects of testosterone on brain are mediated by aromatization to estrogen. In the present study, we applied a similar approach to determine if flutamide can block testosterone-induced Fos activation. The aim was to identify brain regions that respond to the androgenic effects of acute intracerebroventricular (ICV) testosterone infusion, thereby linking form and function in the neural control of androgen reinforcement. Fos-immunoreactive neurons were counted in brain regions previously shown to express testosterone-induced Fos (DiMeo and Wood, 2006a): the posteromedial bed nucleus of the stria terminalis (BSTPM), posteromedial amygdala (MeP), lateral habenula (LHb), VTA, and lateral pontine nucleus (Pn). Interestingly, all of these areas have been shown to be responsive to opiate agonists. In particular, the BSTPM plays an important role in opiate reinforcement (Walker et al., 2000). Furthermore, we previously showed that the opiate antagonist naltrexone also blocks testosterone self-administration (Peters and Wood, 2004a,b). This suggests that opioids may also play role in testosterone reinforcement. Thus, to determine if androgens activate opiate-responsive brain regions, we compared morphine- and testosterone-induced neuronal activation.

2. Results

2.1. *Flutamide pre-treatment and testosterone-induced neuronal activation*

Hamsters ($n=7$/group) were pre-treated with flutamide or ethanol vehicle sc 30 min prior to ICV infusion of 40 μg testosterone (TF and TE, respectively) or 40 μl aqueous vehicle (VF and VE). Photomicrographs in Fig. 1 illustrate Fos expression in the MeP from representative males after TE, TF, VE, and VF treatment. As in our previous study (DiMeo and Wood, 2006a), a 4-h infusion of testosterone ICV induced Fos in MeP. Cell counts are presented as the mean±SEM of Fos-positive neurons in a 0.3-mm^2 region of brain. The number of Fos neurons in the MeP of TE males was nearly twice that of VE controls (TE: 210.6±50.0 vs. VE: 113.8±23.1, $p<0.05$). Flutamide treatment alone did not induce Fos expression above control (VF: 118.6±17.3 vs. VE: 113.8±23.1, $p>0.05$). Furthermore, flutamide pre-treatment failed to block testosterone-induced neuronal activation (TE: 210.6±50.0 vs. TF: 215.3±28.2, $p>0.05$). A similar pattern of Fos expression was observed in the VTA and lateral Pn.

Photomicrographs in Fig. 2 illustrate Fos expression in the BSTPM from representative males after TE, TF, VE, and VF treatment. As for MeP, testosterone infusion in BSTPM nearly doubled Fos expression above vehicle controls (TE: 137.9±17.6 vs. VE: 77.5±16.2, $p<0.05$), and flutamide alone did not induce Fos (VF: 66.1±10.7 vs. VE: 77.5±16.2, $p>0.05$). However, flutamide pre-treatment abolished testosterone-induced neuronal activation (TF: 68.4±13.2 vs. TE: 137.9±17.6, $p<0.05$).

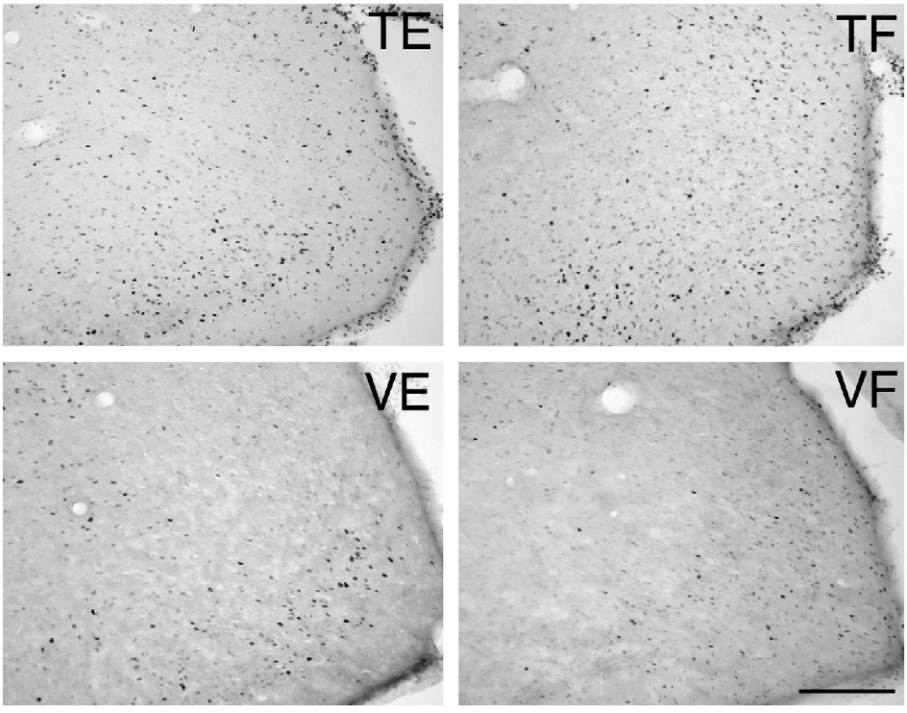

Fig. 1 – Photomicrographs of the posterior medial amygdala showing Fos protein expression in representative male hamsters after 4 h of 40 μg ICV testosterone (1.0 μg/μl, top) or 40 μl vehicle (bottom) with pre-treatment of ethanol (left) or flutamide (right). Abbreviations: TE: testosterone+ethanol; TF: testosterone+flutamide; VE: vehicle+ethanol; VF: vehicle+flutamide. Scale bar=200 um.