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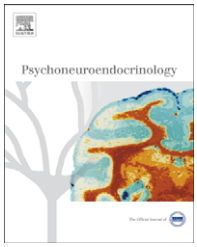

Testosterone and nucleus accumbens dopamine in the male Syrian hamster

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Summary
Most drugs of abuse increase dopamine (DA) in nucleus accumbens (Acb). However, the effects of anabolic androgenic steroids (AAS) on Acb DA have not been examined. We determined the effects of subcutaneous (sc) testosterone (T) on Acb DA in male hamsters. The effects of sc amphetamine were also examined for comparison. In addition, Acb DA was evaluated during intracerebroventricular (ICV) T infusion, designed to mimic T intake during ICV T self-administration in drug-naïve and drug-preexposed animals. Acb DA was measured using *in vivo* microdialysis and HPLC-EC. T (7.5 or 37.5 mg/kg), amphetamine (1 or 5 mg/kg), or vehicle was injected sc and Acb DA monitored for 4 h. In the ICV experiment, T (1 or 2 μg/infusion) or vehicle was infused ICV every 6 min for 4 h and Acb DA monitored. ICV T preexposure was accomplished by repeating the same ICV T infusion (1 μg/infusion) daily for 14 days, and T infusion was accompanied by microdialysis on 15th day. Neither sc nor ICV T administration increased Acb DA. At high dose (2 μg/infusion), ICV T decreased Acb DA. Likewise, daily ICV infusion of T for 15 days did not alter Acb DA. In contrast, sc amphetamine significantly increased Acb DA at both doses. Therefore, unlike many drugs of abuse, AAS does not increase Acb DA levels. The reduction in DA at high T doses is likely due to autonomic depressant effects of AAS. We suggest that AAS act via mechanism distinct from those of stimulants, but may share neural substrates with other drugs of abuse.

1. Introduction

Anabolic androgenic steroid (AAS) use is widespread among athletes and non-athletes (Yesalis et al., 1993). Physical (Leshner, 2000) and psychological (Brower, 2002; Pope and Katz, 1994) effects of AAS use are of significant concern from a public health perspective. Brower (2002) has recently suggested that most AAS users initiate use for the anabolic properties, but many subsequently develop physical and psychological dependence. However, the addictive potential of AAS has received little attention so far. The results of studies using animal models of drug abuse indicate that AAS

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are reinforcing. For example, AAS induces conditioned place preference (CPP) in rats (Packard et al., 1997) and mice (Arnedo et al., 2000). In addition, AAS are self-administered through various routes in rats (Sato et al., 2006; Wood et al., 2004) and hamsters (Ballard and Wood, 2005; DiMeo and Wood, 2006b; Frye et al., 2007; Johnson and Wood, 2001; Peters and Wood, 2005; Wood, 2002). However, it is not known how AAS affect neural circuitry underlying the reinforcing effects of other drugs of abuse.

The mesolimbic dopamine (DA) system, the DAergic projection from the ventral tegmental area (VTA) to the nucleus accumbens (Acb), is a major substrate for drugs of abuse (Berridge and Robinson, 1998; Koob and Nestler, 1997). Selective lesion of DAergic fibers disrupts self-administration of stimulants (Roberts et al., 1977). DA antagonists also have been shown to attenuate the reinforcing effects of cocaine during self-administration (Caine and Koob, 1994). In addition, most commonly abused drugs are known to increase DA levels in Acb, including stimulants (Di Chiara and Imperato, 1988), opiates (Di Chiara and Imperato, 1988), ethanol (EtOH, Di Chiara and Imperato, 1985), and nicotine (Imperato et al., 1986).

There is some evidence implicating a role for the mesolimbic DA system in AAS abuse. For example, AAS induces CPP when injected into Acb (Packard et al., 1997), an effect blocked by the DA antagonist α-flupenthixol (Packard et al., 1998). Furthermore, acute intracerebroventricular (ICV) administration of AAS induces c-Fos expression in the VTA (DiMeo and Wood, 2006a). Based on these data, we have hypothesized that the reinforcing effects of AAS are mediated by the mesolimbic DA system. If AAS utilize the same neural substrates as other drugs of abuse, then AAS should also increase DA in the Acb. The current study was designed to examine Acb DA release in response to AAS administration. Acb DA release was measured in hamsters, using *in vivo* microdialysis with high-performance liquid chromatography with electrochemical detection (HPLC–EC). We examined the effects of acute systemic administration of testosterone (T) on Acb DA release. As a control, we also examined the effects of acute amphetamine administration on Acb DA. Furthermore, we examined the effects of ICV T infusions designed to mimic drug intake during self-administration. Finally, we tested the effects of ICV T following repeated (15-day) ICV T administration, in order to control for possible interference from the autonomic depressant effects of T.

2. Materials and methods

2.1. Animals

Adult male Syrian hamsters (120–160 g BW) were obtained from Charles River Laboratories (Wilmington, MA, USA). Hamsters were housed individually under a reversed long-day photoperiod (14L:10D) with lights off at 9 AM. Food and water were available *ad libitum*. All tests were conducted during the dark phase of their light cycle.

2.2. Experimental design

In the first experiment, we examined the effects of an acute injection of systemic T on Acb DA release. Following baseline sample collection, hamsters received a subcutaneous (sc) injection of 7.5 mg/kg T ($n = 5$), 37.5 mg/kg T ($n = 5$), or vehicle ($n = 5$). Acb DA levels were monitored for 4 h following the injection. The lower dose (7.5 mg/kg) has been used previously in rats and mice to test the effects of androgens on seizure activity (Frye and Reed, 1998), anxiety (Rojas-Ortiz et al., 2006), aggression (Martinez-Sanchis et al., 1998), and social behavior (Barreto-Estrada et al., 2004). Furthermore, 7.5 mg/kg T is comparable to doses tested in human volunteers. According to the National Center for Health Statistics (NHANES, 2007), the average American man weighs 86 kg. Thus, a typical human dose of 600 mg T (Bhasin et al., 1996; Kouri et al., 1995; Tricker et al., 1996) is equivalent to 7 mg/kg.

The Acb DA responses to drugs of abuse have not been previously examined in Syrian hamsters. Therefore, we examined Acb DA response to amphetamine in hamsters as a control. Hamsters were subcutaneously injected with either 1 or 5 mg/kg amphetamine, and Acb DA was monitored for 4 h. These doses of amphetamine are known to induce robust increases in Acb DA in rats (Birgner et al., 2007; Di Chiara and Imperato, 1988).

In the second set of experiments, Acb DA levels were examined using a drug infusion paradigm similar to ICV self-administration. Following baseline sample collection, T was infused through a modified microdialysis probe inserted into the lateral ventricle. Drug-naïve animals received vehicle ($n = 5$), 1 μg/infusion T (40 μg total; $n = 6$), or 2 μg/infusion T (80 μg total; $n = 4$) every 6 min over 4 h. Solutions were delivered as 1 μl infusions every 6 min (40 μl/4 h), using a programmable syringe pump (BS-8000, Braintree Scientific, Braintree, MA, USA). Acb DA was monitored throughout the 4 h infusion. Forty micrograms (1 μg/infusion) T was designed to approximate a heavy dose of T during ICV T self-administration, while 80 μg (2 μg/infusion) T is a maximal dose. We have previously used this method to examine ICV T-induced c-Fos expression (DiMeo and Wood, 2006a) and physiologic effects of T (Peters and Wood, 2005).

As we have previously demonstrated, T exerts autonomic depressant effects when administered ICV (Peters and Wood, 2005). It is possible that any influence of T on Acb DA may be masked by its autonomic depressant effects. In order to control for this possibility, we infused 1 μg/infusion T ($n = 5$) ICV daily for 14 days as described above. This duration is sufficient for hamsters to develop tolerance to autonomic depressant effects of T (Peters and Wood, 2005). On 15th day, T infusion was accompanied by microdialysis sampling.

2.3. Surgery

Surgical procedures were carried out under aseptic conditions according to "Principles of laboratory animal care" (NIH publication no. 86-23, revised 1985). Hamsters were anesthetized with sodium pentobarbital (80 mg/kg) and secured in a Kopf stereotaxic apparatus with lambda and bregma in the same horizontal plane. A microdialysis guide cannula (CMA/12, CMA, N. Chelmsford, MA) was lowered to 1 mm above Acb. Stereotaxic coordinates were: AP: +3.3 mm, ML: +1.1 mm, DV: −6.0 mm from bregma (Figure 1a, Morin and Wood, 2001). For ICV infusion, another