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ANDROGENS AND OPIATES: TESTOSTERONE INTERACTION WITH MORPHINE SELF-ADMINISTRATION IN MALE RATS

Sarah E. Cooper¹ and Ruth I. Wood

Department of Cell and Neurobiology, Keck School of Medicine of the University of Southern California, Los Angeles, CA 90033

Abstract

Abuse of anabolic-androgenic steroids (AAS) and opioids intersect in athletics. Evidence from humans and animals suggests that AAS may act in the brain via opioidergic mechanisms, and may potentiate effects of opioids. To determine if AAS enhance motivation for opioid intake, this study treated male rats chronically for 6 weeks with high levels of testosterone (7.5 mg/kg) or vehicle s.c. and tested them for morphine self-administration under fixed (FR) and progressive ratio (PR) schedules. Initially, rats received chronic morphine infusion (16.8–50 mg/kg/day) over 7 days. Subsequently, rats were tested for morphine self-administration (3.2 mg/kg) 6h/day for 3 days under an FR1 schedule, and for 7 days under a PR 9-4 schedule. With FR1, controls self-administered more morphine (95.9±8.5 mg/kg), compared with testosterone-treated rats (63.2±7.2 mg/kg, $p<0.05$). Under PR, there was no effect of testosterone on morphine intake or operant responding (26.7±5.7 responses vs 30.9±5.9 responses for vehicle; n.s.). To determine if testosterone enhances morphine sedation, additional rats were treated with testosterone or vehicle and evaluated for locomotor behavior and rearing activity over 30 min in response to saline or 10 mg/kg morphine. Morphine inhibited locomotor activity and rearing; testosterone selectively reduced rearing behavior, but did not alter locomotor behavior. These results suggest that testosterone does not increase motivation for morphine.

Keywords

anabolic agents; operant conditioning; opioid analgesics; progressive ratio

INTRODUCTION

Although anabolic-androgenic steroids (AAS) are controlled substances, AAS users downplay similarities with street drugs, arguing that “Unlike, say, crack addicts, the men who take steroids are basically healthy, clean-living people” [1]. However, emerging evidence suggests that AAS users may abuse other drugs, particularly opioids [2–6]. Kashkin and Kleber [7] hypothesized that AAS dependence might involve opioidergic

Address all reprint requests and correspondence to: Ruth I. Wood, Department of Cell and Neurobiology, Keck School of Medicine of the University of Southern California, 1333 San Pablo St., BMT 408, Los Angeles, CA 90033. Telephone (323) 442-1980, Fax: (323) 442-2411, ; Email: riw@usc.edu

¹Present address: Neuroscience Graduate Program, Michigan State University, East Lansing, MI.

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mechanisms. Arvary and Pope [2] suggested that AAS could act as a gateway drug to opioid dependence. In 223 men entering drug treatment, AAS use was considerably higher (25%) among opioid users, compared with men using other drugs (5%) [3]. A recent study from New Zealand found that 50% of dependent AAS users also met DSM-IV criteria for a lifetime history of opioid abuse or dependence, compared to 19% of nondependent AAS users and 7% of nonusers [8]. In the clinical literature, naloxone has been used to treat AAS withdrawal in a bodybuilder [4], and AAS appear to interact with heroin in accidental drug overdose [5].

Animal studies also support links between AAS and opioids. At physiologic levels, testosterone enhances the response to opioids [see 9]. At pharmacologic levels, testosterone self-administration causes autonomic depression similar to opioid overdose, which is blocked by the opioid antagonist naltrexone [10]. In other studies, AAS enhance morphine-induced hypothermia [11], even as they reduce analgesic effects of morphine [12], and attenuate tolerance to morphine's antinociceptive action [11]. This is consistent with AAS-induced opioid receptor binding in brain [13].

The present study determined if AAS increase motivation for morphine. The approach was to compare operant responding for morphine under both fixed- (FR) and progressive-ratio (PR) schedules of reinforcement in morphine-dependent male rats exposed chronically to high-dose testosterone or vehicle. PR reinforcement estimates relative reinforcing efficacy, where the animal is required to make increasing numbers of responses for each successive reinforcement [14]. If testosterone enhances motivation for morphine, testosterone-treated males should complete more ratios than vehicle-treated males. We also determined if testosterone enhances the sedative effects of morphine on locomotor activity.

METHODS

Animals—Male Long-Evans rats (Charles River Laboratories, MA) were individually-housed under a reversed 14L:10D photoperiod (lights off at 9 am) and stable ambient temperature (24°C). This long-day photoperiod is equivalent to summer daylengths in Los Angeles. Food and water were available *ad libitum*, except during training for food reward. Rats remained gonad-intact to approximate AAS use in humans. Care was supplied by the USC Vivaria. Experimental procedures were approved by USC's Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, 8th Ed (National Research Council, National Academies Press, Washington DC; 2011).

AAS treatment—Beginning at 7 weeks of age, rats received testosterone (7.5 mg/kg; Steraloids, RI) or vehicle [3% ethanol, 13% cyclodextrin (RBI, MA) in water] by daily sc injection (n=8/group). Compared with oil, the aqueous vehicle improves steroid bioavailability. This approximates a dose of 450 mg/week in humans adjusted to body surface area according to US Federal Drug Administration guidelines, and has been used to demonstrate AAS effects on behavior in rats [15, 16].

Morphine self-administration

Operant chambers—Training and testing were conducted in operant chambers (Med Associates, St. Albans, VT) connected to a PC running WMPC software (Med Associates). Each chamber was equipped with a house light, computer-controlled syringe pump connected via lever arm with fluid swivel, food pellet dispenser, and 2 levers each with stimulus light.

Behavior was tested during the start of the dark phase with the house light illuminated. Initially, rats were trained to respond for 45-mg sucrose pellets (Bioserv, Frenchtown, NJ) under a FR2 schedule in daily 20-min sessions. A stimulus light facilitated responding on the correct lever. During training, rats were maintained on a slow rate of growth (1–2 g/day). Once rats achieved 50 pellets/session, food was available ad libitum, and each rat was implanted with a chronic Silastic jugular catheter (Dow Corning, MI), connected to a 22-gauge cannula assembly on the dorsal skin. Catheters were flushed daily with 25 IU/ml heparin and 0.125 ug/ml gentamycin sulfate in saline.

The study followed established methods for morphine dependence and self-administration under FR and PR schedules [14]. Initially, rats received continuous morphine infusion for 7 days. The dose increased by 20% each day from 16.8 to 50.0 mg/kg/day. Next, rats were tested 6h/day for 3 days with self-administration of 3.2 mg/kg morphine on an FR1 schedule, where each response on the lever delivered a morphine infusion. A non-contingent injection was delivered at the start of each session, and rats were limited to 44 injections/session. During FR and PR sessions, the stimulus light over the active lever was illuminated, and only responses on the active lever were reinforced. When the active lever was pressed (FR) or a ratio was completed (PR), morphine was delivered via syringe pump, and both the stimulus and house lights were extinguished for 5 seconds. Responses on the active lever during this time-out were recorded, but not reinforced.

Once rats received >20 morphine infusions for 2 consecutive FR1 sessions, they were tested on a PR 9-4 schedule for 1 week. For PR, the response requirement is initially 1 response per morphine infusion, but increases with successive infusions. The breakpoint, when the rat ceases responding, provides an estimate of relative reinforcing efficacy. Under the PR 9-4 schedule (Fig. 1), the rat must complete 9 ratios before the response requirement equals 4 lever presses. Compared with PR schedules for cocaine self-administration (e.g. PR 3–4), the PR 9-4 schedule supports operant responding for morphine [14]. Responses on the active lever during time-out were not counted towards the next ratio.

Morphine intoxication

To determine if chronic high-dose testosterone increases sedative effects of morphine, separate groups of rats were treated for at least 6 weeks with testosterone or vehicle, as described above. Daily injection of testosterone or vehicle continued throughout open field testing. These males were videotaped for 30 min in an open field (0.8 × 0.8 m, grid lines every 20 cm) following daily treatment (testosterone or vehicle) and injection of saline ip. Videotaping was repeated 1 week later after daily treatment followed by injection of 10

mg/kg morphine ip. Videos were analyzed for line crossing and rearing by an observer blinded to treatment groups.

Statistical analysis

Morphine intake and operant responses on both levers during FR1, and during the final 3 sessions of PR were averaged for each rat. Group means from testosterone- and vehicle-treated males were compared by Student's t-test. Locomotor responses to saline and morphine in vehicle- and testosterone-treated rats across the 30-min test were analyzed by repeated-measures ANOVA (RM-ANOVA). Statistical significance was $p < 0.05$.

RESULTS

Vehicle- and testosterone-treated rats required the same amount of time to meet criteria for FR2 food training (n.s.; Fig 1). Body weight also did not differ significantly between the 2 groups. Under FR1, morphine intake was substantially greater, compared with the PR 9-4 schedule of reinforcement. While vehicle- and testosterone-treated males consumed similar amounts of morphine under the PR schedule (n.s.), self-administration under FR1 was significantly lower in testosterone-treated rats (63.2 ± 7.2 mg/kg/d), compared with vehicle controls (95.9 ± 8.5 mg/kg/d; $p < 0.05$).

Under an FR1 schedule, vehicle-treated rats responded more vigorously for 3.2 mg/kg morphine, averaging 30.0 ± 2.7 responses/6h, vs 19.7 ± 2.3 responses in testosterone-treated rats (Fig 2, $p < 0.05$). The number of unreinforced responses on the active lever not different between the two groups (n.s.), as was the number of responses on the inactive lever (n.s.).

Under a PR 9-4 schedule of reinforcement, there was no effect of testosterone on responding for 3.2 mg/kg morphine, as shown in Figure 3. On average, vehicle-treated rats completed 10.6 ± 1.2 ratios in 6h (range 6.0–15.0), with a maximum requirement of 5.0 ± 0.6 responses to receive a morphine infusion (30.9 ± 5.9 responses/6h). This is comparable to previous studies [14]. Testosterone-treated rats completed 9.6 ± 1.1 ratios/6h (range 5.5–13.0), for a maximum response requirement of 4.6 ± 0.6 responses (26.7 ± 5.7 responses/6h; n.s.). Both groups of rats made few unreinforced responses on the active lever, but responded vigorously on the inactive lever (n.s.).

When tested for locomotor activity (Fig 4), there was a significant effect of time [$F_{(3, 18)} = 13.845$, $p < 0.05$] by RM-ANOVA. Rats displayed initially high levels of exploratory behavior, which declined throughout both 30-min tests. This decrease in locomotor activity was especially marked after 10 mg/kg morphine, with a significant interaction of morphine x time [$F_{(3, 18)} = 2.906$, $p < 0.05$]. However, testosterone did not alter locomotor activity, and there was no interaction of testosterone with morphine or time (n.s.). For rearing behavior, there was a significant decrease in rearing over time [$F_{(3, 18)} = 14.611$, $p < 0.05$], and a significant effect of morphine [$F_{(1, 26)} = 99.731$, $p < 0.05$]. Testosterone-treated rats displayed significantly less rearing than vehicle-treated controls [$F_{(1, 26)} = 4.646$, $p < 0.05$], although there were no significant interactions between morphine, time, and testosterone (n.s.).

DISCUSSION

The present study exposed male rats chronically to high levels of testosterone and tested for morphine self-administration to determine if AAS increase the rewarding effects of opioids. Rats received non-contingent morphine infusion, and were tested under FR1 and PR schedules of reinforcement. Under FR1, a single lever press delivered an infusion of morphine iv. To measure motivation for morphine, the PR schedule increases the responses for each infusion. Testosterone failed to increase morphine intake. Indeed, during FR1 self-administration, testosterone-treated rats consumed less morphine than vehicle controls. This suggested the possibility that AAS might enhance sedative effects of opioids. Accordingly, we compared locomotor activity and rearing in separate groups of testosterone- and vehicle-treated rats in response to saline or morphine. Morphine suppressed both rearing and locomotion. Testosterone selectively decreased rearing without altering locomotor activity. Although previous studies in animals and humans have demonstrated interactions of AAS with opioids, both for behavior and neurochemistry [13], it appears that testosterone does not substantially increase motivation for morphine self-administration.

The incidence for non-medical use of AAS or opioids in the United States is surprisingly high. According to the 2012 Monitoring the Future survey, 12.2% of American 12th grade students have engaged in non-medical use of opiates ('narcotics other than heroin'), and 1.8% have used AAS [17]. Moreover, it is likely that these data underestimate the prevalence of AAS use, since most users start in their early 20's [3]. Surveys of opioid users in treatment show relatively high rates of concurrent AAS use [2]. As posited by Arvary and Pope [2], AAS may serve as a gateway drug for opioids. The present study was designed to address this question in animals. Animal studies can examine the reinforcing effects of AAS unencumbered by the motivation to increase muscle mass and athletic performance. The present study used testosterone and morphine because these are the prototypic steroid and narcotic, respectively. All AAS are derived from testosterone, have a similar 19-carbon structure, and bind to the androgen receptor (AR). Morphine is the most abundant opiate from the opium poppy. It is a potent analgesic in clinical use, but also has potential for abuse. Rewarding and analgesic effects are mediated via the mu opioid receptor (MOR). Reinforcing effects of morphine are mediated via the ventral tegmental area (VTA) and nucleus accumbens (Acb), while negative aspects of morphine withdrawal involve actions in the bed nucleus of the stria terminalis (BST), periaqueductal gray (PAG), and preoptic area (POA) [18]. While VTA and Acb have relatively few AR-positive neurons, AR are abundant in BST, PAG and POA [see 16]. These brain areas may represent neuroanatomical substrates for interaction of AAS and opioids.

Already, animal studies have suggested the potential for interactions of AAS with opioidergic systems involving the MOR and its ligands. When male hamsters self-administer testosterone icv, they demonstrate symptoms reminiscent of opioid overdose: hypothermia, low respiration and locomotor activity [10]. These can be reversed with the preferential mu-opioid receptor antagonist naloxone, and naloxone pretreatment blocks testosterone self-administration. Interactions of AAS and opioids are also evident when administered together: nandrolone reduces morphine-induced conditioned place preference in mice, and potentiates symptoms of naloxone-induced morphine withdrawal [11]. Based on the

observations from humans and animal studies, we hypothesized that testosterone might increase operant responding for intravenous morphine self-administration.

However, using an established PR regime for morphine self-administration [14], the present study did not find evidence of greater reinforcing efficacy in male rats chronically exposed to high-dose testosterone. Although these findings did not support our hypothesis, they are consistent with subjective reports from human users. For many athletes, AAS and opioids are used for their anabolic and analgesic effects, respectively. Intoxication and euphoria are not primary objectives [8]. Therefore, synergistic effects on reward and reinforcement are not necessarily essential for co-abuse of AAS and opioids in humans. In view of the presence of AR in brain regions that contribute to aversive aspects of morphine withdrawal (e.g. BST, PAG, and POA), it is tempting to speculate that AAS may modify symptoms of opioid withdrawal. This remains to be tested

Of course, AAS and opioids may interact in the brain to modify other behavioral responses. Human users believe that AAS reduce pain and speed recovery from overtraining injury. Studies in rats and mice have found no antinociceptive effect of AAS on acute thermal or inflammatory pain [19]. Instead, steroids appear to modify the response to opioids: they reduce morphine-induced analgesia [12] and attenuate tolerance to antinociceptive effects of morphine [11]. In a similar manner, nandrolone increases the hypothermic effects of morphine in mice [11]. These findings are consistent with a study of AAS use in deaths from heroin overdose. Co-abuse of AAS appears to reduce the lethal threshold for heroin overdose [5], where death is due to hypothermia and respiratory depression. There is also potential for AAS to enhance the sedative effects of opioids, including suppression of locomotor responses [11, 20]. Increased sedation is consistent with the lower intake of morphine on an FR1 schedule of reinforcement in testosterone-treated rats from the present study. Interactions of AAS and opiates to reduce activity might seem surprising, since AAS are used most often in conjunction with intensive exercise. By itself, testosterone has minimal effects on locomotor activity [11]. The present study observed similar results with open-field locomotion. However, testosterone decreased rearing behavior compared to vehicle controls. Rearing may represent exploratory behavior [20], but may also reflect anxiety [21], and is a symptom of opioid withdrawal in rodents [22].

A number of studies have investigated neurobiologic interactions of AAS and brain opioid systems [13]. AAS reduce MOR in cultured neuroblastoma cells [23]. In brain, AAS modify endogenous opioids and opioid receptors in a region-specific manner. AAS reduce b-endorphins in Acb [13] and the hypothalamic arcuate nucleus [24]. Elsewhere, b-endorphins are increased by AAS in paraventricular thalamus [25] and hypothalamus, BST [24], and VTA [13]. Furthermore, icv infusion of testosterone acutely stimulates Fos in VTA [16], even as systemic AAS reduces Fos response to morphine in the caudate putamen [25]. Together, these results suggest that AAS may modify activity of the b-endorphin/MOR system through a combination of classical AR-mediated mechanisms, as well as via non-genomic actions. Further studies of AAS modification of MOR will help reveal mechanisms for AAS action in the brain.

Acknowledgments

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Figure 1.

Left: Schedule for reinforcement under 9-4 progressive ratio (PR 9-4, *shaded area*), including the response requirement to obtain reinforcement at each step. Right: Operant training for food, body weight, and morphine intake (3.2 mg/kg) under fixed-ratio and progressive-ratio schedules of reinforcement in vehicle- (V, open bars) and testosterone- (T, closed bars) treated male rats. Data are presented as mean ± SEM. *Asterisk* indicates significant difference between groups.

Figure 2.

Operant responses (mean $\pm$ SEM) for morphine (3.2 mg/kg) under a continuous reinforcement schedule in vehicle- (V, open bars) and testosterone- (T, closed bars) treated male rats, including reinforced responses on the active lever (active lever, top) and unreinforced responses on the active (unreinforced, middle) or inactive lever (bottom).

Asterisk indicates significant difference between groups.

Figure 3.

Operant responses (mean $\pm$ SEM) for morphine (3.2 mg/kg) under a progressive ratio reinforcement schedule in vehicle- (V, open bars) and testosterone- (T, closed bars) treated male rats, including the number of completed ratios (upper left), reinforced responses on the active lever (active lever, upper right) and unreinforced responses on the active (unreinforced, lower left) or inactive lever (lever right).

Figure 4.

Locomotor activity (top) and rearing (bottom) over 30 minutes in an open field in vehicle- (open symbols) and testosterone- (testo, closed symbols) treated male rats. Immediately before testing, rats received an injection of saline (left) or 10mg/kg morphine (right). Data are presented as mean $\pm$ SEM.