

## ANDROGEN DEPENDENCE IN HAMSTERS: OVERDOSE, TOLERANCE, AND POTENTIAL OPIOIDERGIC MECHANISMS

K. D. PETERS AND R. I. WOOD\*

Department of Cell and Neurobiology, Keck School of Medicine at the University of Southern California, 1333 San Pablo Street, BMT 401, Los Angeles, CA 90033, USA

**Abstract**—Anabolic steroids are drugs of abuse. However, the potential for steroid reward and addiction remains largely unexplored. This study used i.c.v. testosterone self-administration and controlled infusions of testosterone or vehicle in hamsters to explore central mechanisms of androgen overdose. Forty-two hamsters used nose-pokes to self-administer 1  $\mu\text{g}/\mu\text{l}$  testosterone i.c.v. 4 h/day in an operant chamber. During 1–56 days of androgen self-administration, 10 (24%) hamsters died. Deaths correlated with peak daily intake of testosterone. Of the hamsters that self-administered a peak intake of  $<20 \mu\text{g}/\text{day}$ , there was 100% survival (10/10). Survival decreased to 86% (19/22) when daily testosterone intake peaked at 20–60  $\mu\text{g}/\text{day}$ . Only 30% (three of 10) survived when daily testosterone intake exceeded 60  $\mu\text{g}/\text{day}$ . Deaths are not due to volume or vehicle because i.c.v. infusions of 80  $\mu\text{l}$  vehicle had no effect. Testosterone overdose resembles opiate intoxication. When male hamsters received infusions of 40  $\mu\text{g}$  testosterone, locomotion ( $25.1 \pm 18.8$  grid-crossings/10 min), respiration ( $72.7 \pm 5.4$  breaths/min) and body temperature ( $33.5 \pm 0.4$  °C) were significantly reduced, compared with males receiving vehicle infusions ( $186.1 \pm 8.1$  crossings/10 min,  $117.6 \pm 1.0$  breaths/min,  $35.9 \pm 0.1$  °C,  $P < 0.05$ ). However, males developed tolerance to continued daily testosterone infusion. After 15 days, locomotion ( $170.2 \pm 6.3$  crossings), respiration ( $118.4 \pm 1.3$  breaths/min), and body temperature ( $35.3 \pm 0.3$  °C) in testosterone-infused males were equivalent to that in vehicle controls ( $P > 0.05$ ). The depressive effects of testosterone infusion are blocked by the opioid antagonist, naltrexone. With naltrexone pretreatment (10 mg/kg s.c.), locomotion ( $183.7 \pm 1.8$  crossings/10 min), respiration ( $116.9 \pm 0.3$  breaths/min), and body temperature ( $36.1 \pm 0.4$  °C) during testosterone infusion were equivalent to vehicle controls. Likewise, naltrexone prevents the reinforcing effects of i.c.v. testosterone self-administration. These results indicate that testosterone at high doses causes central autonomic depression, which may be a factor in deaths during self-administration. As well, the depressive effects of large quantities of testosterone may be mediated, at least in part, by an opioidergic mechanism. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

**Key words:** hamsters, tolerance, opioids, naloxone, self-administration, anabolic steroids.

Anabolic–androgenic steroid (AAS) abuse has been on the rise despite increased reports of adverse medical and psychiatric effects (Johnston et al., 2003; Koch, 2002;

NIDA Research Report Series, 2002). In addition to the negative societal impact of trafficking in illegal steroids (crime from buying and selling steroids, impure steroids, needle sharing), AAS use in quantities beyond the normal physiologic range of circulating androgen concentrations can produce serious health consequences including myocardial infarction, cardiomyopathy, stroke, and hepatic tumors and lesions (Brower, 2002; see also Ishak and Zimmerman, 1987; Creagh et al., 1988; Brower et al., 1991b; Sullivan et al., 1998; Fineschi et al., 2001; Parssinen and Seppala, 2002). According to Brower (2002), anabolic effects of AAS on muscle growth account for the initial stage of steroid use. However, with chronic exposure, users can develop physical and psychological dependence on AAS, as defined by Diagnostic and Statistical Manual of Mental Disorders III revised (DSM-III-R) (Brower et al., 1991a). When steroid use is discontinued, many AAS users experience withdrawal symptoms characterized by a hyperadrenergic state resembling opioid withdrawal (Kashkin and Kleber, 1989). Other studies suggest that AAS may possess euphorogenic effects (Cicero and O'Connor, 1990; Brower et al., 1991b; see also Kashkin and Kleber, 1989; Galloway, 1997; Leshner, 2000; Do-weiko, 2002). While these data suggest that AAS are rewarding independent of their anabolic effects, defining the potential for AAS addiction in humans has been difficult.

Research in laboratory animals is useful to explore androgen reinforcement and potential addiction. Previous studies have demonstrated conditioned place preference for testosterone in rats (Alexander et al., 1994; Packard et al., 1997) and mice (Arnedo et al., 2000). In addition, our laboratory has shown that hamsters will voluntarily consume testosterone by oral (Johnson and Wood, 2001), i.v., and i.c.v. self-administration (Wood et al., 2004). Together, these data suggest that testosterone is rewarding in an experimental context where athletic performance is irrelevant. Is testosterone addictive in animals? One of the hallmarks of drug addiction is continued use despite harm (Institute of Medicine, 1996). Physical dependence, including tolerance, may also accompany addiction. In the present study, we report deaths associated with voluntary i.c.v. testosterone self-administration in hamsters. Although human users ingest AAS orally, transdermally, and by i.m. injection, i.c.v. self-administration was used in the present study to focus on central effects of androgens. To investigate the cause of death with i.c.v. testosterone, our subsequent studies used controlled infusions of testosterone or vehicle. Because excess intake of androgen produces symptoms resembling opiate overdose, we further

\*Corresponding author. Tel: +1-323-442-1980; fax: +1-323-442-3158. E-mail address: riw@usc.edu (R. I. Wood).

Abbreviations: AAS, anabolic–androgenic steroids.

tested the effects of opioid antagonists during controlled infusion or self-administration of testosterone. It appears that excessive androgen intake depresses behavior and central autonomic function. However, hamsters develop tolerance, both behavioral (locomotion) and physiologic (respiration, body temperature), to repeated infusions of testosterone. Tolerance and fatal overdose suggest the potential for androgen addiction. Moreover, both the reinforcing (measured as operant responses) and depressive effects (measured as locomotion, respiration, and body temperature) of exogenous androgens can be blocked by opioid antagonists.

## EXPERIMENTAL PROCEDURES

### Animals

Adult male and female Syrian hamsters (*Mesocricetus auratus*, 130–150 g; Charles River Laboratories, Wilmington, MA, USA) were used. They were individually housed under a long-day photoperiod (14:10 LD) and stable ambient temperature (24 °C). Hamsters remained gonad-intact to more closely approximate AAS use in humans. All hamsters were tested under dim illumination during the first 4 h of the dark phase when activity peaks. Water and food were available at all times, except during daily 4 h i.c.v. infusions. All experimental procedures were in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council 1996) and were approved by the University of Southern California Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

### Surgery

One week before testing in the operant chambers, each hamster was implanted with a 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA, USA) into the lateral ventricle under stereotaxic guidance, as described previously (Wood et al., 2004). Hamsters were anesthetized with sodium pentobarbital (100 mg/kg). The cannula assembly was anchored to the skull with stainless steel screws and dental acrylic. Coordinates for the lateral ventricle were AP: +1.0 mm, ML: +1.0 mm, DV: –3.0–5.0 mm relative to bregma according to the hamster brain atlas of Morin and Wood (2001). Testosterone or vehicle (1  $\mu$ l/injection) were delivered (0.2  $\mu$ l/s) for 5 s via a 28 gauge infusion cannula introduced into the guide cannula at the start of each test session. At other times, the guide cannula was protected by a dummy cannula with dust cap.

### Apparatus

Each operant conditioning chamber (Med Associates, St. Albans, VT, USA) was enclosed in a sound-attenuating cubicle with a fan for ventilation. Operant conditioning chambers were equipped with a house light, two nose-poke holes, and a computer-controlled syringe pump (Med Associates) with balance arm and fluid swivel (Instech, Plymouth Meeting, PA, USA). Nose-poke holes were located 6 cm above the cage floor on the left wall of the chamber, below the house light. Aqueous solutions of testosterone or vehicle from a 100  $\mu$ l glass syringe (Hamilton Co., Reno, NV, USA) were delivered to the i.c.v. cannula via Tygon tubing protected by a metal spring. Hamsters were tested daily (5 $\times$ /week) in the operant chambers during 4 h sessions for testosterone self-administration or controlled infusions of testosterone or vehicle.

### Drugs

To enable i.c.v. delivery of an aqueous androgen solution, testosterone (Steraloids, Newport, RI, USA) for i.c.v. self-administration was dissolved in an aqueous vehicle of 2-hydroxypropyl- $\beta$ -cyclodextrin (RBI, Natick, MA, USA). Each 1  $\mu$ l injection contained 1  $\mu$ g testosterone in 13%  $\beta$ -cyclodextrin. Vehicle infusions consisted of the 13%  $\beta$ -cyclodextrin solution alone. Solutions were filtered through a syringe-mounted Millex-HA filter (Millipore, Bedford, MA, USA) immediately before use. Glass was used for preparation and delivery of testosterone and vehicle solutions since steroids readily adsorb to plastic (Bruning et al., 1981).

### Experiment 1: testosterone overdose (n=42)

For testosterone self-administration, each response on the active nose-poke delivered 1  $\mu$ g testosterone in 1  $\mu$ l vehicle. The location of the active nose-poke (to the front or rear of the left wall of the chamber) was balanced to control for side preferences. Hamsters received no prior exposure or training in the operant chambers before testing for testosterone self-administration. Based on our previous studies of i.c.v. testosterone self-administration in male and female hamsters, hamsters acquire a consistent preference for the active nose-poke (first of 4 consecutive days in which responses on the active nose-poke > responses on the inactive nose-poke) after only 4–5 days of exposure (Wood et al., 2004; Triemstra and Wood, 2004). To aid in discrimination of the active nose-poke, the house light was extinguished and the stimulus light was illuminated during the 5-s infusion interval. Further operation of the active nose-poke during this 5-s time-out period was recorded, but not reinforced with drug delivery. Likewise, operation of the inactive nose-poke was recorded, but produced no injection or stimulus light. Data from both nose-pokes were collected by a PC running Windows-compatible software (WMPC; Med Associates).

To determine the potential for fatal testosterone overdose, we evaluated testosterone intake in all hamsters tested for i.c.v. testosterone self-administration in our laboratory to date. These included 25 adult males and 17 adult females that self-administered testosterone for 1–56 days (mean: 19.1 $\pm$ 2.2 days). For each hamster, the average daily testosterone intake and the maximum daily dose of testosterone were determined. Testosterone intake of animals that died during self-administration was compared with that of unaffected animals. Testosterone intake was correlated with percent survival by test of logistic regression using Statview 5.1 (SAS Institute Inc., Cary, NC, USA).

### Experiment 2: controlled infusion (n=29)

There are many possible reasons for deaths with high-dose androgen intake. In particular, self-administration of large quantities of androgen also necessarily involves injection of large quantities of fluid i.c.v., and it is possible that deaths result from fluid volume or the cyclodextrin vehicle. Using self-administration, it is not possible to compare the effects of large volumes of testosterone and vehicle because hamsters do not voluntarily self-administer equivalent volumes of vehicle i.c.v. Moreover, with testosterone self-administration, there is considerable variability in androgen intake within and between animals. Therefore, to explore mechanisms for fatal androgen overdose under more controlled conditions, additional groups of male hamsters were tested with controlled i.c.v. infusions of testosterone (n=16) or vehicle (n=13). Although there were no sex differences in testosterone self-administration or overdose in experiment 1, subsequent studies were conducted in males to eliminate potentially confounding effects of sex differences in endogenous hormone levels. For controlled infusion of testosterone, hamsters (n=9) received 1  $\mu$ l infusions i.c.v. on a fixed schedule (every 6 min) to deliver testosterone at levels subthreshold for overdose (40  $\mu$ g/4 h). A separate

group of hamsters ( $n=7$ ) received the same volume of vehicle i.c.v. on the same fixed schedule (every 6 min). Controlled infusions continued for 15 days. During controlled infusion, operation of the nose-pokes was not recorded, and produced no response. Two animals receiving testosterone infusions died before the end of the testing period (after days 12 and 13 of infusion), and there was no evidence of hydrocephalus or infarction found at necropsy. To determine physiologic and behavioral effects of i.c.v. infusion, locomotor activity, body temperature, and respiration were recorded immediately after each infusion session (see below). In addition, locomotor activity was re-tested after a challenge with the opiate antagonist, naloxone. Behavioral and physiologic responses in males receiving 40  $\mu\text{l}$  testosterone or vehicle were compared on the first and last day of the infusion period by Student's *t*-test with Bonferroni's correction for multiple comparisons. After the final infusion session, males were weighed and killed under deep pentobarbital anesthesia (180 mg/kg). Testes, seminal vesicles, and epididymal fat pads were weighed to estimate systemic androgen effects of central testosterone infusion. Organ weights in the two groups of males receiving 40 infusions/4 h were compared by MANOVA.

A third group of males ( $n=6$ ) received controlled i.c.v. infusions of 80  $\mu\text{l}$  vehicle (1  $\mu\text{l}$  every 3 min for 4 h) daily for 5 days; 80  $\mu\text{l}$  is well within the lethal range for testosterone self-administration. These males serve as a further control for deaths due to testosterone overdose in experiment 1. As with the 40  $\mu\text{l}$  controlled infusions, males were tested for locomotion, respiration and body temperature immediately after 80  $\mu\text{l}$  vehicle infusions (see below). Behavioral and physiologic responses in all testosterone- and vehicle-treated males were compared on the first 5 days of the infusion period by factorial ANOVA.

To determine if testosterone produces a biphasic locomotor response (inhibition, followed by activation) similar to that induced by morphine (Schnur et al., 1983), a fourth group of males ( $n=7$ ) received controlled i.c.v. infusions of 40  $\mu\text{g}$  testosterone and 40  $\mu\text{l}$  vehicle on alternating days for 8 days. Locomotion was tested 30, 90, 120, and 150 min following the end of each infusion session. Between locomotion tests, animals remained in their home cages. Mean locomotor responses following vehicle or testosterone infusion were compared over time by repeated measures ANOVA.

Locomotion was measured in a circular arena (95 cm diam) marked with a 15 $\times$ 15 cm grid. A male was placed at the center of the arena, and the number of grid crossings was recorded for 10 min. Initially, animals were habituated to the arena on two occasions. Baseline activity was recorded in a third test conducted before the first controlled infusion. Thereafter, locomotor behavior was measured immediately after each 4 h infusion.

To measure body temperature and respiration, males were briefly anesthetized with ketamine (100 mg/kg). This dose is the minimum required for temporary (ca. 5 min) immobility. Rectal temperature was recorded with a digital thermometer (Becton-Dickinson, Franklin Lakes, NJ, USA). Respiration (breaths/min) was determined from the average of three 15-s measurements. As with locomotor activity, baseline recordings for both were obtained before the first infusion session. Thereafter, body temperature and respiration were measured after each 4 h infusion.

Following temperature and respiration measures, hamsters were allowed 20 min to recover from ketamine sedation. Each male then received a naloxone challenge. To acutely block opioid receptors, hamsters received naloxone (2.5 mg/kg in saline; Sigma, St. Louis, MO, USA) i.p. This dose is based on previous studies of morphine withdrawal in hamsters using naloxone at 1–4 mg/kg (Avis and Peeke, 1975). Ten minutes following the naloxone injection, locomotor activity was recorded a second time, using the methods above.

### Experiment 3: opioid blockade during controlled infusion ( $n=13$ )

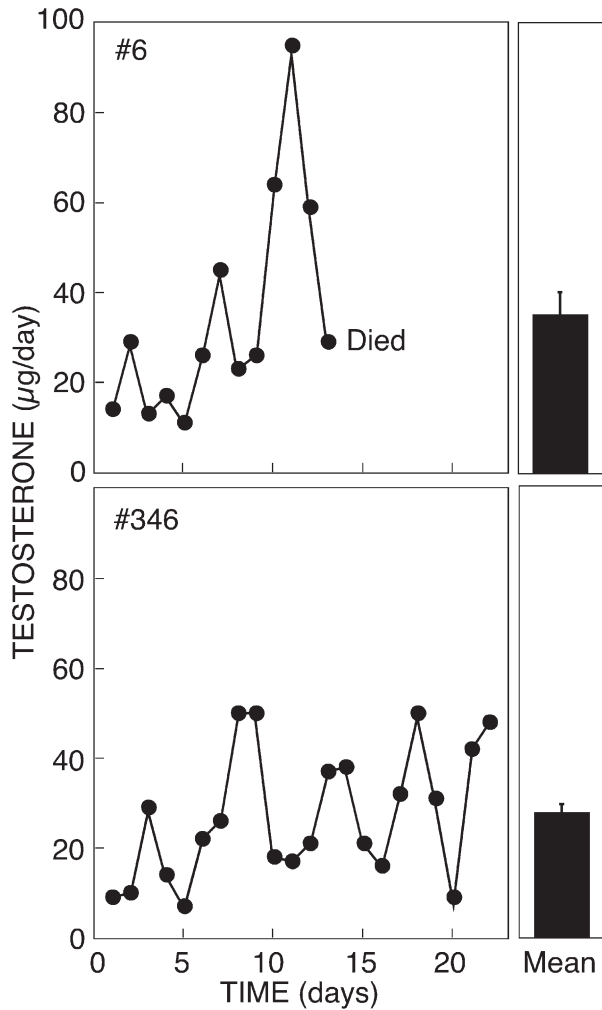
To determine if blocking opioid receptors prevents the sedative effects of testosterone, eight male hamsters were pre-treated with the long-acting opioid antagonist naltrexone before receiving controlled infusions of testosterone according to the methods for "Experiment 2: controlled infusion," above. For long-acting opioid receptor blockade, hamsters received naltrexone (10 mg/kg in saline) s.c. At 10 mg/kg, naltrexone reduces stimulated drinking in hamsters, but does not affect feeding or spontaneous locomotion (Lowy and Yim, 1982; Lowy et al., 1985). Briefly, 30 min before infusion, each male received 5.0 mg/kg naltrexone s.c. Males received a second injection of naltrexone (5.0 mg/kg, s.c.) halfway through the infusion session. Locomotion, body temperature, and respiration were measured following each infusion session, as described above. Males received controlled infusions of testosterone with naltrexone pretreatment for 10 days, followed by an average of  $11.7 \pm 2.2$  days of controlled infusion with vehicle (range: 9–13 days). Behavioral and physiologic responses were compared on the first and last days of naltrexone and vehicle treatment by paired *t*-test with Bonferroni's correction for multiple comparisons.

To determine the amount of naltrexone required to block the sedative effects of testosterone, an additional group of five males was pretreated with naltrexone at 1 mg/kg. At 1 mg/kg, naltrexone partially blocks the sedative effects of 15 mg/kg morphine in hamsters (Schnur and Barela, 1984). After baseline testing for locomotion, respiration and body temperature, males were pretreated with 1 mg/kg naltrexone (two injections of 0.5 mg/kg) and received controlled infusion of testosterone for 5 days. Locomotion, body temperature, and respiration were measured following each infusion session. Mean responses during the 5 days of infusion were compared with baseline values in each male by paired *t*-test.

### Experiment 4: opioid blockade during testosterone self-administration ( $n=13$ )

To determine if blocking opioid receptors prevents the reinforcing effects of testosterone, eight male hamsters were pre-treated with naltrexone before testing for testosterone self-administration according to the methods for "Experiment 1: testosterone overdose," above. Naltrexone pretreatment was the same two-dose regimen as used in "Experiment 3: opioid blockade during controlled infusion," above. Each male was tested for testosterone self-administration with naltrexone pretreatment for 10 days, followed by 20 days of testosterone self-administration without naltrexone. Responses on the active and inactive holes after 10 days of naloxone or vehicle pretreatment were compared by paired *t*-test with Bonferroni's correction for multiple comparison. Additionally, the number of days to express a consistent preference for the active nose-poke hole was calculated for each animal.

To determine the amount of naltrexone required to block the reinforcing effects of testosterone, five males were tested for testosterone self-administration following pretreatment with naltrexone at 1 mg/kg. These males had previously received 5 days of controlled testosterone infusion with 1 mg/kg naltrexone pretreatment to test sedative effects of testosterone (see experiment 3, above). Each male was tested for 1  $\mu\text{g}/\mu\text{l}$  testosterone self-administration following 1 mg/kg naltrexone pretreatment (two injections of 0.5 mg/kg) for 10 days. Mean operant responses on the active and inactive nose-pokes were compared.



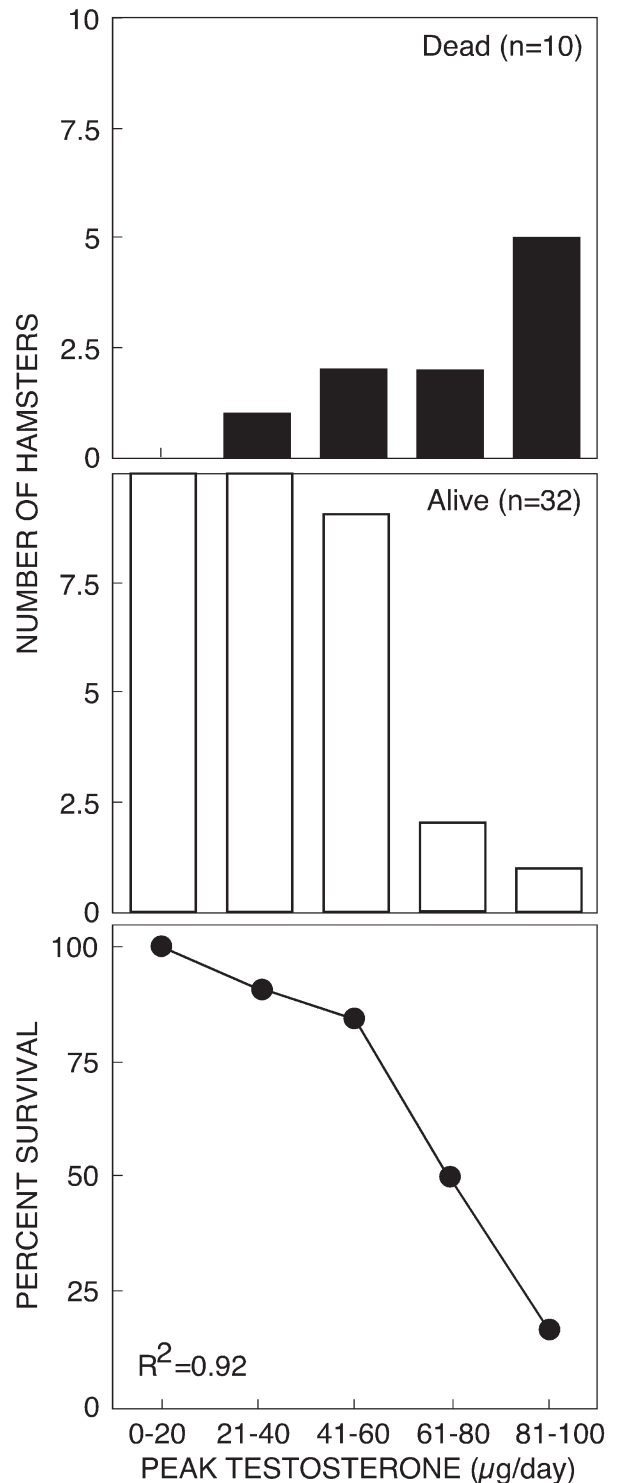
**Fig. 1.** Left: Daily i.c.v. testosterone self-administration in two representative male hamsters. Male no. six (top) died shortly after consuming 99 µg testosterone on day 11, while male no. 346 (bottom) continued to self-administer testosterone at more consistent levels. Right: mean daily androgen intake was not significantly different ( $P>0.05$ ).

**RESULTS**

**Experiment 1: testosterone overdose**

A total of 42 hamsters have been tested for i.c.v. testosterone self-administration in our laboratory. Of these, 10 (24%) died during testing. None of the deaths occurred in the operant chambers. Instead, hamsters often died during the night, several hours after removal from the operant chamber. Eight animals were autopsied to determine if i.c.v. testosterone infusion caused acute brain injury. No evidence of hydrocephalus or brain infarction was found.

Deaths during testosterone self-administration were more closely related to peak testosterone intake rather than average daily self-administration. Fig. 1 presents operant responses for two representative males during testosterone self-administration. Although average daily androgen intake in the two males was similar (no. 6:  $34.7 \pm 6.8$  µg; no. 346:  $27.1 \pm 3.1$  µg,  $P>0.05$ ), peak intake in no. 6 (95 µg on day 11) was substantially greater than in



**Fig. 2.** Deaths during i.c.v. testosterone self-administration in male hamsters relative to peak daily androgen intake (0–20, 21–40, 41–60, 61–80, 81–100 µg testosterone). Top: number of animals that died at each testosterone dose. Middle: number of animals that survived at each testosterone dose. Bottom: percent survival. Survival decreased significantly above 60 µg testosterone/day ( $P<0.05$ ).

no. 346 (50 µg on days 8, 9, and 18). After reaching a peak on day 11, male no. 6 showed a progressive decline in

androgen intake on days 12 and 13, and died on day 14. By contrast, no. 346 continued to self-administer testosterone at more modest levels for 21 days with no evident impairment.

Fig. 2 compares percent survival versus peak testosterone intake in all hamsters self-administering testosterone i.c.v.. There was a significant correlation between peak testosterone and percent survival ( $R^2=0.92$ ,  $P<0.05$ ). In 10 hamsters whose peak daily testosterone intake did not exceed 20  $\mu\text{g}$ , there were no deaths. With peak androgen intake between 20 and 60  $\mu\text{g}$ , survival was 86% (19 of 22 hamsters). At peak doses above 60  $\mu\text{g}/4\text{ h}$  (60–100  $\mu\text{g}$ ) only 30% survived (three of 10 hamsters). Animals that died during the study reached their highest testosterone intake after  $15.4\pm 3.8$  days of self-administration. Death occurred  $2.3\pm 1.5$  days after peak testosterone intake, and was preceded by profound autonomic depression. There were no sex differences in peak testosterone intake (males:  $47.1\pm 5.6\ \mu\text{g}$ , females:  $40.0\pm 6.4\ \mu\text{g}$ ), and no sex differences in survival (19/25 males, 76%; 13/17 females, 76.5%).

### Experiment 2: controlled infusion

Fig. 3 illustrates the effects of controlled infusions on locomotion, respiration and body temperature. Before the start of controlled infusion, there were no differences in baseline locomotor activity between males receiving testosterone or vehicle. At baseline, males averaged  $185.2\pm 6.2$  grid-crossings/10 min. Thereafter in males receiving controlled infusions of vehicle at 40 or 80  $\mu\text{l}/4\text{ h}$ , locomotor behavior remained at baseline levels throughout the experiment ( $188.7\pm 3.3$  grid-crossings/10 min,  $181.7\pm 3.0$  grid-crossings/10 min, respectively). By contrast, controlled infusion of 40  $\mu\text{g}$  testosterone/4 h significantly depressed locomotor activity. For testosterone-treated animals, locomotion averaged  $25.1\pm 18.8$  grid-crossings/10 min on the first day of treatment. However, locomotor activity gradually increased over the next 10 days. By the last day of the study, grid-crossings after testosterone infusion ( $169.0\pm 5.5/10\text{ min}$ ) were comparable to those in males with vehicle infusion ( $184.6\pm 7.0/10\text{ min}$ ,  $P>0.05$ ). Unlike morphine (Schnur et al., 1983), testosterone does not produce a biphasic effect on locomotor activity. Instead, during the first 4 days of testosterone infusion, locomotor activity was reduced below baseline from 30 min ( $10.3\pm 0.6$  grid-crossings/10 min) through 150 min ( $9.2\pm 0.8$  grid-crossings/10 min) following testosterone infusion ( $P<0.05$ ; Table 1). By contrast, motor behavior after vehicle infusion in the same males was unaffected (30 min:  $183.8\pm 2.7$  grid-crossings/10 min vs 150 min:  $186.4\pm 3.0$  grid-crossings/10 min,  $P>0.05$ ).

The effects of controlled infusions on respiration and body temperature were similar. Vehicle infusions of 40  $\mu\text{l}$  or 80  $\mu\text{l}$  did not alter resting respiration ( $116.5\pm 0.3$  breaths/min,  $116.8\pm 0.6$  breaths/min, respectively) or body temperature ( $36.2\pm 0.1\ ^\circ\text{C}$ ;  $36.3\pm 0.1\ ^\circ\text{C}$ ) compared with baseline measurements ( $117.0\pm 0.5$  breaths/min,  $35.8\pm 0.4\ ^\circ\text{C}$ ). By contrast, on the first day of testing controlled infusion of testosterone significantly reduced respiration to  $72.7\pm 5.4$  breaths/min and body temperature to

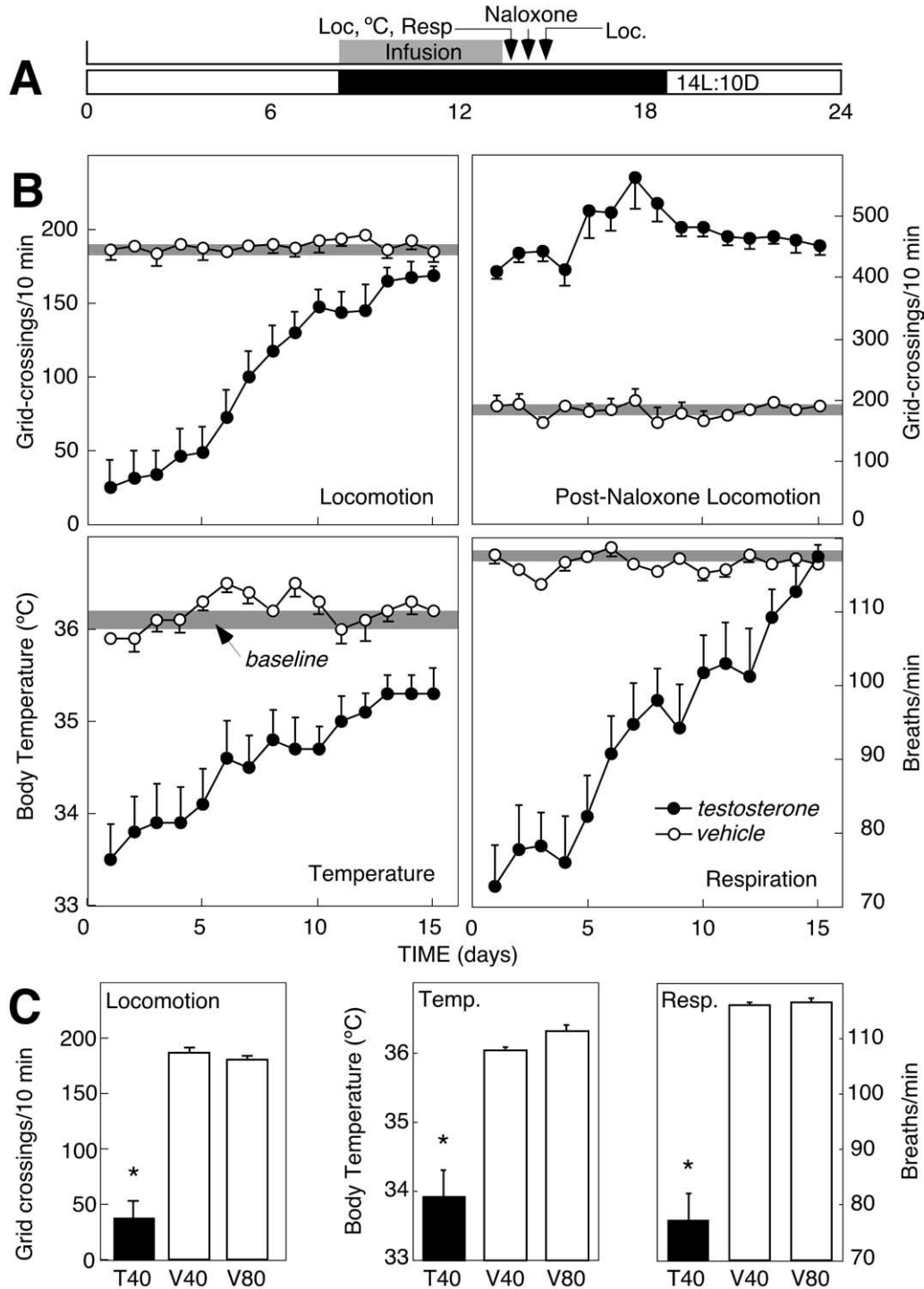
$33.5\pm 0.4\ ^\circ\text{C}$  ( $P<0.05$ ) compared with baseline control values. However, by the last day of infusion, respiration ( $117.5\pm 1.4$  breaths/min) had returned to baseline values, and body temperature ( $35.3\pm 0.3\ ^\circ\text{C}$ ) was not significantly different from vehicle controls ( $P>0.05$ ).

Following vehicle infusion, the opiate antagonist naloxone did not alter locomotor activity ( $183\pm 3.0$  grid-crossings/10 min,  $P>0.05$ ). However, naloxone produced a striking effect on males with testosterone infusion. Ten minutes after naloxone injection, locomotor activity in testosterone-infused males increased to  $472.6\pm 10.5$  grid-crossings/10 min, exceeding not only their post-infusion locomotor activity ( $100.0\pm 6.1$  grid-crossings/10 min), but also their baseline locomotion ( $188.6\pm 4.7$  grid-crossings/10 min). This increase in locomotor activity after naloxone remained consistent throughout the 15-day test period. It is important to note that one male (no. 18) failed to respond to controlled infusions of testosterone. In this individual, locomotion, respiration and body temperature remained at baseline values throughout 15 days of i.c.v. testosterone. However, like the other testosterone-infused males, his locomotor activity increased acutely following naloxone challenge.

Fig. 4 illustrates organ weights in testosterone- and vehicle-treated animals. Males receiving testosterone infusions weighed significantly less ( $132.4\pm 2.8\text{ g}$ ) than vehicle-treated males ( $156.1\pm 1.2\text{ g}$ ,  $P<0.05$ ). As well, epididymal fat pad weight in testosterone-treated males ( $0.8\pm 0.1\text{ g}/100\text{ g BW}$ ) was significantly less than in vehicle-treated animals ( $1.5\pm 0.1\text{ g}/100\text{ g BW}$ ,  $P<0.05$ ). Despite the difference in body weight, males receiving testosterone had significantly larger seminal vesicles ( $1.5\pm 0.1\text{ g}/100\text{ g BW}$ ) than vehicle-infused males ( $0.6\pm 0.1\text{ g}/100\text{ g BW}$ ,  $P<0.05$ ). Although paired testes weights/100 g BW were significantly larger in testosterone-treated males ( $2.6\pm 0.1\text{ g}$ ) compared with vehicle-infused controls ( $2.4\pm 0.1\text{ g}$ ), this effect was largely due to the smaller body weight of testosterone-infused males (85% of controls). Paired testes weights uncorrected for BW in testosterone- and vehicle-treated males were similar ( $3.4\pm 0.1\text{ g}$  and  $3.7\pm 0.1\text{ g}$ , respectively).

### Experiment 3: opioid blockade during controlled infusion

Fig. 5 illustrates the effects of pre-treatment with naltrexone on the response to controlled infusion of testosterone. Before exposure to naltrexone or testosterone, baseline measurements for locomotion, body temperature and respiration averaged  $179.3\pm 3.9$  grid-crossings/10 min,  $36.0\pm 0.1\ ^\circ\text{C}$ , and  $116.1\pm 1.3$  breaths/min, respectively. When males were pre-treated with 10 mg/kg naltrexone, controlled infusion of testosterone had minimal behavioral or physiologic effects. Locomotion ( $183.7\pm 1.8$  crossings/10 min), body temperature ( $36.1\pm 0.04\ ^\circ\text{C}$ ), and respiration ( $116.9\pm 0.3$  breaths/min) were comparable to baseline values throughout the 10 days of testing. However, males did not develop tolerance to testosterone during 10 mg/kg naltrexone pre-treatment. When naltrexone was discontinued, controlled infusion of testosterone significantly depressed locomotion ( $16.8\pm 3.1$  counts/10



**Fig. 3.** (A) Experimental design: testosterone- and vehicle-treated males were tested for locomotor behavior following a 4 h controlled infusion session. Males receiving 40  $\mu$ g testosterone or 40  $\mu$ l vehicle were tested for locomotor behavior a second time following a naloxone challenge. All experiments occurred during the dark phase of the light cycle. For further discussion, please see Experimental Procedures. (B) Behavioral and physiological effects of daily i.c.v. infusions of 40  $\mu$ g testosterone ( $n=9$ , filled circles) or 40  $\mu$ l vehicle ( $n=7$ , open circles) for 15 days in male hamsters: locomotor behavior (Loc., top left), body temperature ( $^{\circ}$ C, bottom left), and respiration (Resp., bottom right). Shaded line indicates baseline measures obtained from all animals before infusion. Top right: effects of the opioid antagonist naloxone (2.5 mg/kg) on locomotor activity following each infusion session. (C) Behavioral and physiological effects of i.c.v. infusions of 40  $\mu$ g testosterone (T40,  $n=9$ ), 40  $\mu$ l vehicle (V40,  $n=7$ ), and 80  $\mu$ l vehicle (V80,  $n=6$ ) for 5 days in male hamsters: locomotor behavior (left), body temperature (middle), and respiration (right).

min), body temperature ( $33.5 \pm 0.3$   $^{\circ}$ C), and respiration ( $69.2 \pm 3.4$  breaths/min,  $P < 0.05$ ) on the first day of testing. By

the 10th day of testosterone infusion without naltrexone, locomotion ( $129.2 \pm 13.3$  crossings/10 min), body temperature

**Table 1.** Mean  $\pm$  S.E.M. locomotor behavior (grid-crossing/10 min) in male hamsters ( $n=7$ ) following 4 h controlled infusion of testosterone (40  $\mu$ g of 1  $\mu$ g/ $\mu$ l) or vehicle (40  $\mu$ l) on alternate days for 8 days<sup>a</sup>

Time	Testosterone	Vehicle
30 Min	10.3 $\pm$ 0.6*	183.8 $\pm$ 2.7
90 Min	9.4 $\pm$ 0.9*	185.5 $\pm$ 3.3
120 Min	9.3 $\pm$ 1.1*	189.2 $\pm$ 2.3
150 Min	9.2 $\pm$ 0.8*	186.4 $\pm$ 3.0

<sup>a</sup> Asterisks indicate significant difference from vehicle controls.

(35.5 $\pm$ 0.1  $^{\circ}$ C), and respiration (110.5 $\pm$ 3.8) had significantly increased, compared with the first day of testosterone infusion ( $P<0.05$ ).

In contrast to the effects of naltrexone at 10 mg/kg, naltrexone pretreatment at 1 mg/kg failed to block the sedative effects of testosterone. During 5 days of testosterone infusion following 1 mg/kg naltrexone pretreatment, locomotion (20.4 $\pm$ 3.8 grid-crossings/10 min), respiration (60.2 $\pm$ 2.8 breaths/min) and body temperature (33.6 $\pm$ 0.1  $^{\circ}$ C) were all significantly below baseline values (193.0 $\pm$ 6.5 grid-crossings/10 min, 111.2 $\pm$ 1.0 breaths/min, 37.2 $\pm$ 0.1  $^{\circ}$ C).

#### Experiment 4: opioid blockade during testosterone self-administration

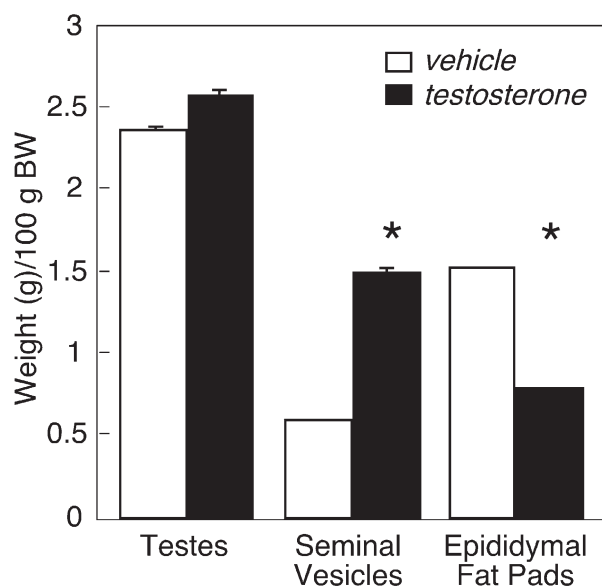
Fig. 6 illustrates i.c.v. self-administration of testosterone in the presence and absence of an opioid antagonist. When pretreated with naltrexone, males failed to develop a significant preference for the active nose-poke hole during 10 days of i.c.v. testosterone self-administration. On the last day of naltrexone pre-treatment, responses on the active and inactive nose-poke holes averaged 12.0 $\pm$ 2.1 and 14.5 $\pm$ 3.1 per 4 h, respectively. Operant responses were comparable to re-

sponses for self-administration of the cyclodextrin vehicle alone (Wood et al., 2004). However, when naltrexone pretreatment was discontinued, males developed a significant preference for the active nose-poke within 5.0 $\pm$ 1.0 days of testosterone self-administration. On the 10th day of vehicle pretreatment, males made significantly more responses on the active nose-poke (27.0 $\pm$ 5.7 per 4 h), compared with the inactive nose-poke (9.4 $\pm$ 1.6 per 4 h). Operant responding on the active nose-poke averaged 23.5 $\pm$ 2.1 nose-pokes/4 h during 20 days of self-administration ( $P<0.05$ ). Responding on the inactive nose-poke remained constant (9.1 $\pm$ 0.4 nose-pokes/4 h). By contrast, pretreatment with naltrexone at 1 mg/kg failed to block testosterone self-administration. Operant responses on the active nose-poke (30.4 $\pm$ 3.3 per 4 h) were significantly higher than responses on the inactive nose-poke (15.5 $\pm$ 3.3 per 4 h) during days 6–10 of testosterone self-administration.

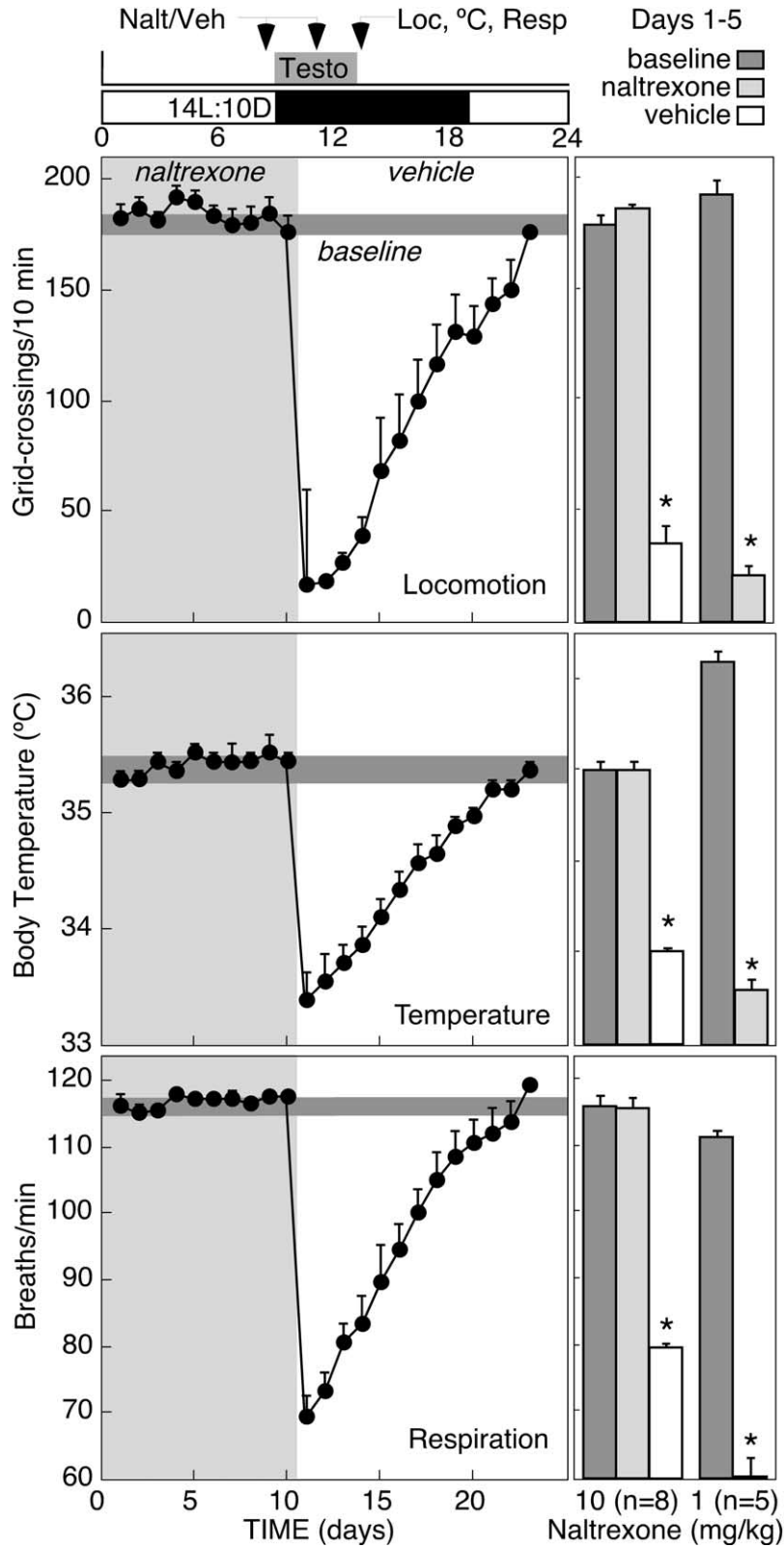
## DISCUSSION

The present study demonstrates the potential for fatal testosterone overdose during i.c.v. self-administration in hamsters. Deaths correlated with maximal daily testosterone intake (>60  $\mu$ g/day), rather than average androgen consumption. Moreover, fatalities were not due to i.c.v. fluid intake, as determined by controlled infusion of testosterone or vehicle. Symptoms of androgen overdose resembled those of opiate intoxication, including reduced locomotion, respiration and body temperature. However, with repeated exposure, males developed tolerance to the depressive effects of testosterone infusion. Administration of the opioid antagonist naloxone acutely reversed testosterone-induced locomotor depression. Likewise, pretreatment with the long-acting opioid blocker naltrexone blocked the physiologic and behavioral symptoms of testosterone infusion and prevented the reinforcing effects of testosterone self-administration. These data suggest the potential for testosterone addiction, which may be mediated through opioidergic mechanisms.

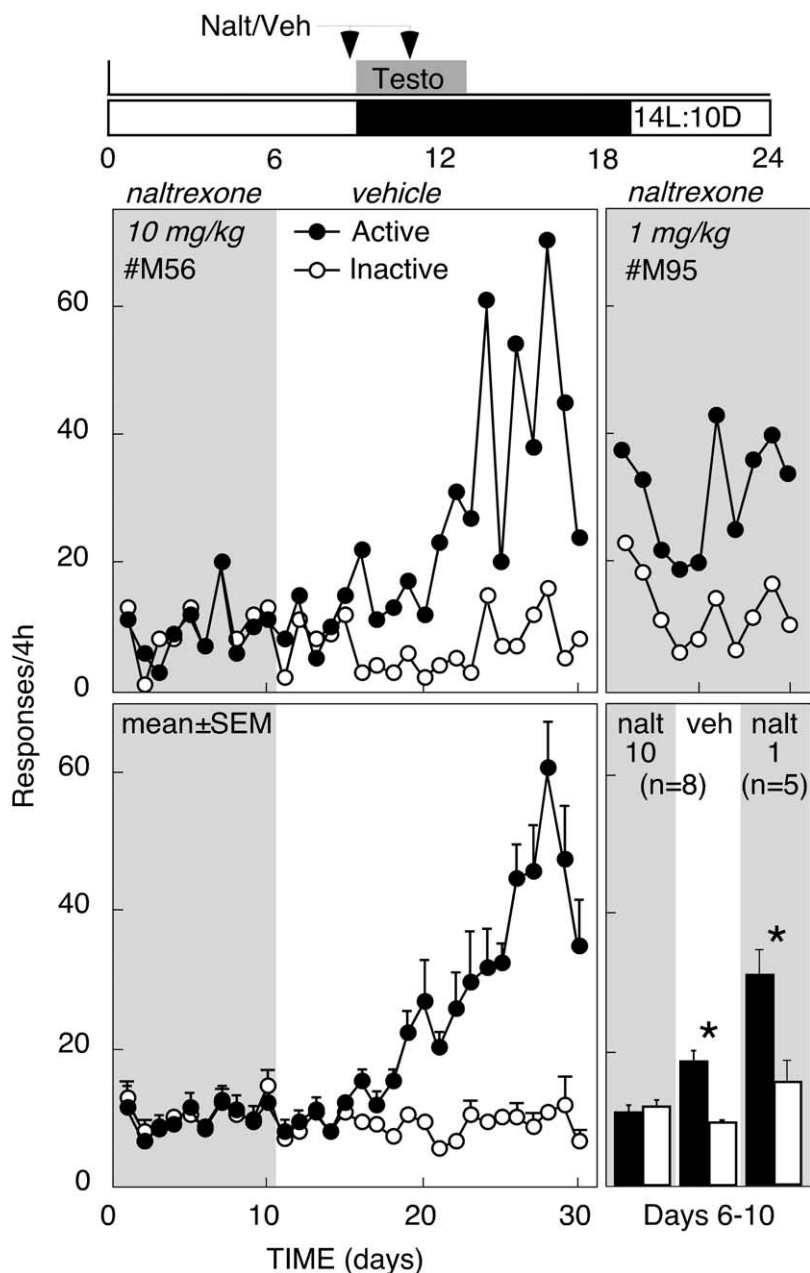
The present study is part of an ongoing series of investigations to determine the potential for addiction to anabolic steroids, and to determine brain mechanisms underlying androgen reinforcement. In humans, anabolic steroids are drugs of abuse. Recently, Brower (2002) proposed a two-stage model of AAS dependence. According to the model, anabolic effects of AAS on muscle growth account for the initial stage of steroid use. However, with chronic exposure, users develop dependence on the psychoactive effects of AAS. In this regard, there are reports that AAS may possess euphorogenic effects (Cicero and O'Connor 1990; Brower et al., 1991; see also Kashkin and Kleber, 1989; Galloway, 1997; Leshner, 2000; Doweiko, 2002). Moreover, AAS users frequently experience withdrawal when steroids are discontinued. Nonetheless, it is difficult in humans to separate direct psychoactive effects of AAS from the user's psychological dependence on the anabolic effects of AAS. In laboratory rodents, testosterone reward and reinforcement have been established previously by conditioned place preference (rats: Alexander et



**Fig. 4.** Effects of 15 daily i.c.v. infusions of 40  $\mu$ g testosterone ( $n=9$ , filled bars) or 40  $\mu$ l vehicle ( $n=7$ , open bars) on paired weights of testes, seminal vesicles, and epididymal fat pads (mean  $\pm$  S.E.M.) in male hamsters. Asterisks indicate significant differences between groups.



**Fig. 5.** Left: behavioral and physiological effects of daily i.c.v. infusions of 40 µg testosterone (Testo;  $n=8$ ) for 15 days in male hamsters: locomotor activity (Loc., top), body temperature (°C, middle), and respiration (Resp., bottom). For the first 10 days (shaded box), animals were pre-treated with the long-acting opioid antagonist, naltrexone (10 mg/kg). Open box represents testosterone infusion without naltrexone pre-treatment. Shaded line indicates baseline measures obtained before infusion. Right: group means during days 1–5 in males pretreated with vehicle or naltrexone (10 or 1 mg/kg).



**Fig. 6.** Operant responses (mean±S.E.M.) for i.c.v. testosterone ( $1 \mu\text{g}/1 \mu\text{l}$  injection) in male hamsters. Closed circles represent operation of the active nose-poke; responses on the inactive nose-poke are indicated by open circles. For the first 10 days (shaded box), animals were pre-treated with the long-acting opioid antagonist, naltrexone (Nalt). Open box represents testosterone infusion without Nalt pre-treatment (Veh). Top: daily responses in representative hamsters pretreated with Nalt at 10 (left) and 1 mg/kg (right). Bottom: group means ( $n=8$ ). Asterisks indicate significant differences in operant responses on the active (closed bars) and inactive (open bars) nose-pokes during days 6–10 of testing.

al., 1994; Packard et al., 1997; King et al., 1999; Rosellini et al., 2001; mice: Arnedo et al., 2000), and by oral (Johnson and Wood, 2001), i.v. and i.c.v. self-administration in rats and hamsters (Wood et al., 2004). Conditioned place preference with testosterone appears to involve dopamine release in the nucleus accumbens (Packard et al., 1997, 1998), which acts on D1 and D2 types of dopamine receptors (Schroeder and Packard, 2000).

Although the foregoing studies suggest that testosterone is reinforcing, many substances (e.g. sucrose) that are

reinforcing are not necessarily addictive. Results of the present study offer the first evidence for testosterone addiction in experimental animals. Addiction is characterized by loss of control over use, such that subjects continue to seek out the drug despite adverse consequences (Institute of Medicine, 1996). In the present study, self-administration of testosterone to the point of death suggests the potential for androgen addiction. Other criteria to establish addiction in animal studies include tolerance, withdrawal and sensitization (Koob and Nestler, 1997). It is

significant that hamsters in experiment 2 developed physical and behavioral tolerance to repeated infusions of testosterone. After the first day of infusion, testosterone-treated males were flaccid, while vehicle infusions had no effect. However, testosterone and vehicle-treated males were indistinguishable after 15 days of exposure.

Nonetheless, it is evident that androgen reinforcement is not comparable to that of cocaine or heroin. In part, this may be due to the slow time-course of testosterone effects. While cocaine and heroin produce a “rush” in human users, former opiate addicts did not report euphoria following an injection of testosterone (Fingerhood et al., 1997). Likewise, death can occur rapidly with cocaine due to sudden cardiac death (Eickelberg and Mayo-Smith, 1998) or by heroin due to severe respiratory depression (O'Connor and Kosten, 1998). By contrast, deaths due to testosterone overdose in the present study typically occurred several days after peak androgen intake. Moreover, whether by oral (Johnson and Wood 2001), i.v., or i.c.v. self-administration, rats and hamsters show only a modest preference for testosterone (Wood et al., 2004). It may be that the magnitude of steroid reinforcement is similar to that of other mild reinforcers, such as caffeine, nicotine, or benzodiazepines.

At high doses, the behavioral and physiologic effects of testosterone were reminiscent of narcotics or sedatives. Opiates cause CNS depression, particularly in respiratory control, and testosterone overdose appears to have similar effects. Previous investigators have postulated an interaction of AAS and opioids. In humans, it has been suggested that AAS abuse may lead to abuse of opioids (Arvary and Pope, 2000), and naloxone treatment produced withdrawal symptoms in a single case study of an AAS user (Tennant et al., 1988). In rat brain, AAS increase levels of endogenous opioids in the hypothalamus, striatum and periaqueductal gray (Johansson et al., 1997, 2000). In the present study, the ability of naloxone and naltrexone to block testosterone intoxication and self-administration suggests that testosterone modulation of the opioid system may be behaviorally relevant. Nonetheless, testosterone does not precisely mimic opioid effects. It is significant that we did not observe classic symptoms of opiate withdrawal [wet-dog shakes, paw shakes, teeth chattering, abdominal writhing, yawning, and defecation (Schnur, 1991)] after naloxone treatment of testosterone-infused hamsters. Similar findings have been reported for rhesus monkeys treated with naloxone following testosterone propionate (Negus et al., 2001). As a further caveat, we have not tested other neurotransmitter antagonists. In this regard, previous studies have also suggested parallels between testosterone and sedative-hypnotics such as benzodiazepines. Like benzodiazepines, testosterone and its derivatives have anxiolytic and analgesic effects, as demonstrated with an elevated plus-maze in mice (Aikey et al., 2002) and with open field, tail flick, paw lick, defensive burying, and social interaction tests in rats (Frye and Seliga, 2001). Androgens also modulate activity of the GABA/benzodiazepine receptor (Jorge-Rivera et al., 2000).

To date, deaths with central autonomic depression similar to that observed in hamsters with i.c.v. testosterone infu-

sions have not been described in humans. AAS users may take androgenic compounds at up to 100× the dose used for medical purposes (Brower et al., 1990). However, most AAS users begin with small doses of less potent androgens, and gradually increase their intake. Based on results of the present study, androgen overdose would be expected only with initial exposure to high doses of steroids. Most clinical studies have either administered more modest doses (300 mg) to normal volunteers, or have recruited current steroid users, who have presumably already developed tolerance to AAS (Parssinen and Seppala, 2002).

Nonetheless, there is a precedent for androgen-related deaths in human users (reviewed in Parssinen and Seppala, 2002) and laboratory animals (Bronson and Matherne, 1997). While early studies focused on steroid-induced hepatic dysfunction (Hickson et al., 1989), recent studies and case reports describe fatalities due to myocardial infarction and cerebrovascular accident. Among athletes and body builders, AAS abuse can precipitate sudden cardiac death during exercise (Kennedy and Lawrence, 1993; Dickerman et al., 1995; Fineschi et al., 2001), with cardiac hypertrophy noted at autopsy. The anabolic effects of androgens include hypertrophy of cardiac myocytes (Maron et al., 1986) and androgen-stimulated thrombosis (Dickerman et al., 1995). In the present study testosterone infused i.c.v. was not restricted to the brain. While we cannot exclude the possibility of disturbances in cardiovascular function due to i.c.v. self-administration, we have not observed fatalities in rats and hamsters self-administering testosterone i.v. (Wood et al., 2004). With i.v. self-administration, androgen intake averaged >300 µg/day, far beyond the lethal dose for most hamsters self-administering testosterone i.c.v.. Thus, it appears that fatal androgen overdose in the present study is largely due to effects on the brain.

*Acknowledgments*—We thank Jennifer L. Triemstra and Lucy Chu for assistance with infusions and self-administration. This work supported by a grant from the NIH (DA12843) to R.I.W.

## REFERENCES

- Aikey JL, Nyby JG, Anmuth DM, James PJ (2002) Testosterone rapidly reduces anxiety in male house mice (*Mus musculus*). *Horm Behav* 42:448–460.
- Alexander GM, Packard MG, Hines M (1994) Testosterone has rewarding affective properties in male rats: implications for the biological basis of sexual motivation. *Behav Neurosci* 108:424–428.
- Arnedo MT, Salvador A, Martinez-Sanchez S, Gonzalez-Bono E (2000) Rewarding properties of testosterone in intact male mice: a pilot study. *Pharmacol Biochem Behav* 65:327–332.
- Arvary D, Pope HG Jr (2000) Anabolic steroid: a possible gateway to opioid dependence. *N Engl J Med* 342:1532–1533.
- Avis HH, Peeke HV (1975) Morphine withdrawal induced behavior in the Syrian hamster (*Mesocricetus auratus*). *Pharm Biochem Behav* 11:11–15.
- Bronson FH, Matherne CM (1997) Exposure to anabolic-androgenic steroids shortens life span of male mice. *Med Sci Sports Exerc* 29:615–619.
- Brower KJ (2002) Anabolic steroid abuse and dependence. *Curr Psychiatry Rep* 4:377–387.
- Brower KJ, Blow FC, Young JP, Hill EM (1991a) Symptoms and correlates of anabolic-androgenic steroid dependence. *Br J Addict* 86:759–768.

- Brower KJ, Catlin DH, Blow FC, Eliopoulos GA, Beresford TP (1991b) Clinical assessment and urine testing for anabolic-androgenic steroid abuse and dependence. *Am J Drug Alcohol Abuse* 17: 161–171.
- Brower KJ, Eliopoulos GA, Blow FC, Catlin DH, Beresford TP (1990) Evidence for physical and psychological dependence on anabolic androgenic steroids in eight weight lifters. *Am J Psychiatry* 147:510–512.
- Brower KJ, Catlin DH, Blow FC, Eliopoulos GA, Beresford TP (1991) Clinical assessment and urine testing for anabolic-androgenic steroid abuse and dependence. *Am J Drug Alcohol Abuse* 17: 161–171.
- Bruning PF, Jonker KM, Boerema-Baan AW (1981) Adsorption of steroid hormones by plastic tubing. *J Steroid Biochem* 14:553–555.
- Cicero TJ, O'Connor LH (1990) Abuse liability of anabolic steroids and their possible role in the abuse of alcohol, morphine, and other substances. In: *Anabolic steroid abuse: NIDA research monograph 102* (Lin GC, Erinoff L, eds), pp 1–28. Rockville, MD: NIH.
- Creagh TM, Rubin A, Evans DJ (1988) Hepatic tumours induced by anabolic steroids in an athlete. *J Clin Pathol* 41:441–443.
- Dickerman RD, Schaller F, Prather I, McConathy WJ (1995) Sudden cardiac death in a 20-year-old bodybuilder using anabolic steroids. *Cardiology* 86:172–173.
- Doweiko HE (2002) *Concepts of chemical dependency*. Florence, KY: Brooks/Cole.
- Eickelberg SJ, Mayo-Smith MF (1998) Management of stimulant intoxication and withdrawal. In: *Principles of addiction medicine* (Graham AW, Schultz TK, eds), pp 441–455. Chevy Chase, MD: American Society for Addiction Medicine.
- Fineschi V, Baroldi G, Monciotti F, Reattelli LP, Turillazzi E (2001) Anabolic steroid abuse and cardiac sudden death. *Arc Pathol Lab Med* 125:253–255.
- Fingerhood MI, Sullivan JT, Testa M, Jasinski DR (1997) Abuse liability of testosterone. *J Psychopharmacol* 11:59–63.
- Frye CA, Seliga AM (2001) Testosterone increases analgesia, anxiety, and cognitive performance of male rats. *Cog Affect Behav Neurosci* 1:371–381.
- Galloway GP (1997) Anabolic-androgenic steroids. In: *Substance abuse: a comprehensive textbook* (Lowinson JH, Ruiz P, Millman RB, Langrod JG, eds), pp 308–318. MD: Williams & Wilkins.
- Hickson RC, Ball KL, Falduto MT (1989) Adverse effects of anabolic steroids. *Med Toxicol Adverse Drug Exp* 4:254–271.
- Institute of Medicine (1996) *Pathways of Addiction: opportunities in drug addiction research*. Washington, DC: National Academy Press.
- Ishak KG, Zimmerman HJ (1987) Hepatotoxic effects of the anabolic/androgenic steroids. *Semin Liver Dis* 7:230–236.
- Johansson P, Hallberg M, Kindlundh A, Nyberg F (2000) The effect on opioid peptides in the rat brain, after chronic treatment with the anabolic androgenic steroid, nandrolone decanoate. *Brain Res Bull* 51:413–418.
- Johansson P, Ray A, Zhou Q, Huang W, Karlsson K, Nyberg F (1997) Anabolic androgenic steroids increase B-endorphin levels in the ventral tegmental area in the male rat brain. *Neurosci Res* 27:185–189.
- Johnson LR, Wood RI (2001) Oral testosterone self-administration in male hamsters. *Neuroendocrinology* 73:285–292.
- Johnston LD, O'Malley PM, Bachman JG (2003) *Monitoring the future national survey results on drug use, 1975–2002: volume I: secondary school students*. Bethesda, MD: National Institute on Drug Abuse.
- Jorge-Rivera JC, McIntyre KL, Henderson LP (2000) Anabolic steroids induce region- and subunit-specific rapid modulation of GABA(A) receptor-mediated currents in the rat forebrain. *J Neurophysiol* 83:3299–3309.
- Kashkin KB, Kleber HD (1989) Hooked on hormones? Anabolic steroid addiction hypothesis. *JAMA* 262:3166–3170.
- Kennedy MC, Lawrence C (1993) Anabolic steroid abuse and cardiac death. *Med J Aust* 158:346–348.
- King BE, Packard MG, Alexander GM (1999) Affective properties of intra-medial preoptic area injections of testosterone in male rats. *Neurosci Lett* 269:149–152.
- Koch JJ (2002) Performance-enhancing substances and their use among adolescent athletes. *Pediatr Rev* 23:310–317.
- Koob GF, Nestler EJ (1997) The neurobiology of drug addiction. *J Neuropsych Clin Neurosci* 9:482–497.
- Leshner AI (2000) *Anabolic steroid abuse: NIDA research report series*. NIH Publication 00–3721.
- Lowy MT, Yim GKW (1982) Drinking, but not feeding, is opiate-sensitive in hamsters. *Life Sci* 30:1639–1644.
- Lowy MT, Sangiah S, Yim GKW (1985) Naltrexone fails to suppress spontaneous locomotor activity in hamsters. *Pharmacol Biochem Behav* 22:399–401.
- Maron BJ, Epstein SE, Roberts WC (1986) Causes of sudden death in competitive athletes. *J Am Coll Cardiol* 7:204–214.
- Morin LP, Wood RI (2001) *A stereotaxic atlas of the golden hamster brain*. San Diego, CA: Academic Press.
- Negus SS, Pope HG Jr, Kanayama G, Wines JD Jr, Fischer BD (2001) Lack of evidence for opioid tolerance or dependence in rhesus monkeys following high-dose anabolic-androgenic steroid administration. *Psychoneuroendocrinology* 26:789–796.
- NIDA Research Report Series (2002) <http://165.112.78.61/ResearchReports/Steroids/Anabolicsteroids.html>.
- O'Connor PG, Kosten TR (1998) Management of opioid intoxication and withdrawal. In: *Principles of addiction medicine* (Graham AW, Schultz TK, eds), pp 457–464. Chevy Chase, MD: American Society for Addiction Medicine.
- Packard MG, Cornell A, Alexander GM (1997) Rewarding affective properties of intra-accumbens injections of testosterone. *Behav Neurosci* 111:219–224.
- Packard MG, Schroeder JP, Alexander GM (1998) Expression of testosterone conditioned place preference is blocked by peripheral or intra-accumbens injection of a-flupenthixol. *Horm Behav* 34:39–47.
- Parssinen M, Seppala T (2002) Steroid use and long-term health risks in former athletes. *Sports Med* 32:83–94.
- Rosellini RA, Svare BB, Rhodes ME, Frye CA (2001) The testosterone metabolite and neurosteroid 3 $\alpha$ -androstenediol may mediate the effects of testosterone on conditioned place preference. *Brain Res Rev* 37:162–171.
- Schnur P (1991) Acute morphine dependence in the hamster. *Pharmacol Biochem Behav* 38:711–713.
- Schnur P, Barela P (1984) Locomotor activity and opiate effects in male and female hamsters. *Pharmacol Biochem Behav* 21:369–374.
- Schnur P, Bravo F, Trujillo M (1983) Tolerance and sensitization to the biphasic effects of low doses of morphine in the hamster. *Pharmacol Biochem Behav* 19:435–439.
- Schroeder JP, Packard MG (2000) Role of dopamine receptor subtypes in the acquisition of a testosterone conditioned place preference in rats. *Neurosci Lett* 282:17–20.
- Sullivan ML, Martinez CM, Gennis P, Gallagher EJ (1998) The cardiac toxicity of anabolic steroids. *Prog Cardiovasc Dis* 41:1–15.
- Tennant F, Black DL, Voy RO (1988) Anabolic steroid dependence with opioid-type features. *N Engl J Med* 319:578.
- Triemstra JL, Wood RI (2004) Testosterone self-administration in female hamsters. *Behav Brain Res* 154(1):221–229.
- Wood RI, Johnson LR, Chu L, Schac CA, Self DW (2004) Testosterone reward: intravenous and intracerebroventricular self-administration. *Psychopharmacology* 171:298–305.