ELSEVIER

Pharmacology, Biochemistry and Behavior 67 (2000) 271–279

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

Anabolic androgenic steroids affects alcohol intake, defensive behaviors and brain opioid peptides in the rat

Pia Johansson[a], Ann-Sophie Lindqvist[b], Fred Nyberg[a], Claudia Fahlke[b,*]

[a]*Department of Pharmaceutical Biosciences, Division of Biological Research on Drug Dependence, Uppsala University, Uppsala, Sweden*
[b]*Department of Psychology, Göteborg University, P.O. Box 500, SE-405 30 Göteborg, Sweden*

Received 25 February 2000; received in revised form 15 May 2000; accepted 24 May 2000

Abstract

The present study investigated whether a relationship exists between nandrolone decanoate and voluntary ethanol intake in laboratory rats. Animals were subjected to daily subcutaneous injections with nandrolone decanoate (15 mg/kg) during 2 weeks. One group of animals was tested for voluntary alcohol intake 1 week after the end of the 2-week treatment period and another group received alcohol 3 weeks after the treatment. In addition, assessment of defensive behaviors and immunoreactivity (ir) levels of the brain opioid peptides dynorphin B and Met-enkephalin-Arg-Phe (MEAP) were performed. The nandrolone decanoate-treated animals were significantly more aggressive and showed lower fleeing and freeezing reaction than the oil-treated controls. Treatment with nandrolone decanoate enhanced voluntary alcohol intake, regardless if it was presented 1 or 3 weeks after end of the treatment period. These animals had a decreased activity of dynorphin B-ir in the nucleus accumbens, decreased levels of MEAP-ir in the periaqueductal gray (PAG) and higher levels of MEAP-ir in the hypothalamus compared to controls. In line with previous studies, this suggests that the altered dynorphin B-ir activity may promote the rewarding effects of ethanol and thereby increasing alcohol intake, whereas MEAP-ir may be associated with the ability to control the aggressive reaction. Abuse of nandrolone decanoate may thus constitute a risk factor for increased alcohol consumption and defensive aggression. In human, this constellation of behavioral symptoms is closely related to acts of crimes and violence and is often observed among those abusing anabolic androgenic steroids.

Keywords: Anabolic androgenic steroids; Nandrolone decanoate; Alcohol intake; Defensive aggression; Freezing; Fleeing; Aggression; Brain peptides

Anabolic androgenic steroids (AAS) have become widely used and abused not only by professional athletes, but by recreational athletes as well. AAS have both anabolic (protein-synthesizing) and androgenic (masculinizing) action in the body [55]. It has been reported that some athletes consume AAS in massive quantities (10–100 times the therapeutic dose) in order to enhance physical performance, improve strength and increase muscle mass [30,32,55]. It is also common that athletes combine different AAS substance with each other (stacking) and with other doping agents [21].

Chronic steroid use causes several physical side effects such as heart disease, high blood pressure, liver dysfunction and severe acne [39]. In addition, prolonged abuse of AAS also leads to adverse effects on mental health. For example, personality changes, extreme cases of aggressive behavior and unprovoked rage attacks, known as roid rage, have been reported after abuse of AAS [10,16,28,32,56]. Furthermore, AAS abuse has been connected with crimes and acts of violence [6,10]. Animal experimental studies have also shown that AAS treatment results in a significant increase in aggression [8,31]. A common method for activating defensive aggression is the use of a predator that attacks or threats the experimental animal. In the present study, defensive aggression was operationalized as defensiveness elicited by innocuous stimuli [1]. Recent studies have shown that different types of brain damage produces uncontrollable rage attacks in response to innocuous stimuli such as the experimenter [3,13,24]. Additionally, we examined whether nandrolone decanoate exposure was associated with alterations in fleeing and freezing, two other expressions of the defensive motivational system [5].

* Corresponding author. Tel.: +46-31-773-4289; fax: +46-31-773-4628.

E-mail address: claudia.fahlke@psy.gu.se (C. Fahlke).

0091-3057/00/$ – see front matter
PII: S0091-3057(00)00365-8

During recent years, concurrent abuse of AAS with other drugs has been observed among teenagers and adults not connected to sport [12,27,33]. Whether abuse of other drugs may constitute a risk factor for misuse of AAS, or vice versa, is still unknown. In the present study, we tested the hypothesis that exposure of nandrolone decanoate induces high intake of alcohol when ethanol was presented in a free choice situation. More specifically, one group of animals was tested for voluntary alcohol intake 1 week after a 2-week treatment period with daily injection of nandrolone decanoate and another group received alcohol 3 weeks after the end of AAS treatment period. These two time intervals were selected because recent evidence have suggested that AAS steroids may give rise to long-term effects on various central nervous system function [33].

Recent studies suggest that AAS interact with the brain reward system [9] by affecting the endogenous opioid peptide system [37]. For example, when using radioimmunoassay (RIA) procedures, it has been shown that nandrolone decanoate treatment increases immunoreactivity (ir) levels of β-endorphin, dynorphin B and Met-enkephalin-Arg-Phe (MEAP) in various brain area [25,26]. Also alcohol has been shown to interact with the endogenous opioid peptide system (e.g. Refs. [17,22,54]). Thus, ir-levels of the opioid peptides dynorphin B and MEAP were examined in various brain tissues known to be involved in the reward system. Dynorphin B-ir may serve as a marker of the activity of the endogenous kappa-opioid system whereas the MEAP-ir is a marker of activity mediated through the mu- and delta-opioid receptors [17,52,53].

1. Methods

1.1. Animals

Male Wistar rats purchased from Möllegard Breeding Laboratories (Denmark) served as subjects. They were 80–90 days of age and weighing 250–300 g at the beginning of the experiments. Animals were housed in an air-conditioned colony room (lights-off 10:00 A.M.–10:00 P.M.) at a temperature of 23°C and a humidity of 50–60%. The rats had free access to water and R34 food pellets (Labfor, Lactamin, Vadstena, Sweden). The animals were initially housed in groups of four per cage (clear plastic cages; $53 \times 31 \times 25$ cm) for 2 weeks to adapt to the novel laboratory conditions before the experiments started. The experiments were approved by the local ethical committee of the Swedish National Board for Laboratory Animals.

1.2. AAS treatment

Animals were randomly divided into two groups, each consisting of 20 rats. One group received daily injections (sc) of nandrolone decanoate (Deca-Durabol®, Organon, Oss, Netherlands) of 15 mg/kg for 14 days. This dose affects the neurochemistry in the rat brain [25,26,37]. The other group of animals was given daily injections of oil (sterile arachidis oleum) for 14 days and served as controls.

1.3. Behavioral tests

Tests for defensive behaviors (fleeing, freezing and aggression) were conducted immediately after the end of the 2-week treatment period with nandrolone decanoate. Thereafter, the AAS and control groups were each randomly divided in two subgroups. One subgroup from the AAS ($n = 10$) and one from the control group ($n = 10$) were tested for voluntary ethanol intake 1 week after the last nandrolone decanoate injection. The remaining AAS ($n = 10$) and control ($n = 10$) subgroups were tested 3 weeks after the last injection. A second test for defensive aggression was performed immediately after the end of alcohol consumption period (i.e. 6 or 8 weeks after the last nandrolone decanoatetreatment injection). All behavioral tests were conducted during the dark phase of the light–dark cycle.

1.3.1. Fleeing and freezing

The rat was lifted by its tail from the home cage, placed in an opaque holding cage and carried to the test room. The rat was placed in a circular Plexiglas cage (diameter 39 cm) with the floor covered by filter paper which was divided by two lines to form four 90° sectors (e.g. Refs. [13,20]). The cage was enclosed in a soundproof test chamber. The test chamber was illuminated by a 15-W white light bulb, suspended together with a door bell under the chamber roof. The behavior of the animal was observed through a Plexiglas window on the front wall. The rat was allowed 5 min adaptation period in the test chamber. During that time, the following open-field behavioral items were observed: first crossing (latency to leave the sector where the animal was first placed), locomotor activity (number of lines crossed by the hind legs), rearing (number of raisings on hind legs), grooming (cumulative time recorded) and defecation (number of boli deposited). After the adaptation period, the door bell (95 dB) was subsequently sounded for 6 s and the rat would attempt to flee. The number of lines crossed by the animal during the signal was recorded and used as a measure of flight distance. Concurrently with, or slightly before the termination of the bell signal, the rat freezed. In both cases, the duration of freezing reaction was defined as beginning at the cessation of the bell sound and ending with the first distinct movement of some part of the body (usually the head), excluding eye blinks and respiratory or vibrissae movements.

1.3.2. Defensive aggression

For the defensive aggression tests (i.e. reactivity to experimenter), the rat was lifted by its tail and placed in a Plexiglas cage ($60 \times 31 \times 41$ cm high) and allowed to habituate for 30 s. The rats' reaction to four different stimuli was then assessed (modified from Refs. [3,13,29]): (a) a

wooden rod was slowly moved to approach and touch the rat's snout (0: no response or sniffs at the rod; 1: intermittently bites or attacks the rod and/or adopts a defensive upright posture; 2: continuously bites/attacks the rod); (b) startle to an air puff (air blown from a 50-ml syringe) at the back (0: no response or some movement; 1: jumping response; 2: exaggerated jumping response); (c) poking with wooden rod at the flanks (0: no response or sniffing at the rod; 1: defensive upright posture; 2: defensive upright posture together with biting/attack); and (d) capturing with a gloved hand (0: very easy to capture; 1: easy to capture but some resistance and/or prolonged vocalization; 2: difficult to capture because of escape; 3: difficult to capture because of attacking or biting; 4: very difficult to capture because of continuous violent attacks/bites). The averages of the total scores (maximum total score 10) from each individual stimuli test was used in the statistical calculations.

1.3.3. Voluntary alcohol intake

One subgroup from the AAS ($n=10$) and one from the control group ($n=10$) were given continuous access to a second bottle containing an ethanol solution, in addition to the water bottle, 1 week after the last nandrolone decanoate injections. The ethanol concentration was gradually increased (2–4–6% v/v) over a 2-week period. The animals were subsequently housed individually in clear plastic cages ($42 \times 25 \times 14$ cm) during 3 consecutive weeks. During this time, animals had continued access to two bottles (plastic 300-ml bottles with ballvalve spouts; ALAB, Sweden) containing tap water or 6% ethanol solution. This particular ethanol concentration stimulates peak levels of consumption in the present strain of rats [19]. Fluid consumption was recorded daily at 9:00 A.M. and the bottles were cleaned and refilled with fresh beverages twice a week. Ethanol intake is expressed as gram per kilogram per day (g/kg/day) of absolute ethanol, and ethanol preference as, i.e. proportion of ethanol solution intake relative to total fluid consumption in percent. The remaining AAS ($n=10$) and control ($n=10$) subgroups were tested for voluntary ethanol intake 3 weeks after end of the period of nandrolone decanoate injections.

1.4. Biological measurements

1.4.1. Tissue dissection

Before decapitation, ethanol solution was removed to give the animals a 3-day alcohol washout period. All animals were decapitated in a separate room. The systemic effect of the nandrolone decanoate was investigated by weighing the wet thymus gland. It is well known that steroids bind to glucocorticoid receptors in thymus and by a negative feedback mechanism induce a thymus atrophy [2,15]. Brains were also rapidly dissected out using a brain matrix (Activational System, Mortella Drive Warren, MI, USA) and immediately placed on dry ice. The tissue parts (hypothalamus, nucleus accumbens, striatum and periaqueductal gray [PAG]) were put into eppendorf tubes and kept at -80°C until further analysis of the opioid peptide concentrations of dynorphin B and MEAP were assessed.

1.4.2. Preparation of brain tissue sample

Tissue samples from each independent rat were homogenized in 1 M heated acetic acid (90°C) as described elsewhere [7]. The hypothalamus, nucleus accumbens and PAG were homogenized by ultrasonification in a volume of 500 μl, whereas the striatum was homogenized in 1 ml acetic acid. The homogenates from all tissues were centrifuged at $12000 \times g$ for 20 min (4°C). Following centrifugation, the supernatants were diluted (1:1) with 0.1 M formic acid and 0.018 M pyridine, pH 3.0 (buffer I). Small plastic columns were packed with SP-Sephadex C-25 (packed gel volume = 1 ml) and washed with 20 ml buffer I before the sample were added. After additional washing with 10 ml buffer I, the fraction containing MEAP was eluted in 4 ml of 0.35 M formic acid and 0.35 M pyridine, pH 4.4 (buffer III). Elution of dynorphin B containing material was done with 4 ml of 1.6 M formic acid and 1.6 M pyridine, pH 4.4 (buffer V) after additional washing with 6 ml buffer III. All buffers (I, III and V) contained 0.01% mercaptoethanol. The eluate samples were evaporated in a Speed Vac centrifuge (Savant, Hicksville, NY, USA) and subsequently analyzed by RIA.

1.4.3. Radioimmunoassay

The dynorphin B was measured in fraction V from the ion exchange procedure. The dynorphin B RIA was based on the double-antibody precipitation [40]. Briefly, the sample or standard peptide (25 μl), dissolved in methanol/HCI 0.1 M (1:1) was incubated with 100 μl of antiserum and 100 μl of labeled peptide (5000–5500 cpm/100 μl) for 24 h. After the 24-h incubation with antiserum and labeled peptide, the samples were incubated 1 h (4°C) with 100 μl of sheep anti-rabbit antiserum (Pharmacia decanting suspension 3, Pharmacia Biotech, Uppsala, Sweden), to separate free and antibody-bound peptide. Following centrifugation the radioactivity in the pellets was counted in a gamma counter.

Before analysis by the MEAP RIA, the evaporated tissue extracts from fraction III were oxidized by dissolving in 100 μl, 1 M acetic acid and incubated for 30 min at 37°C with 10 μl 30% H_2O_2. The samples were evaporated a second time and redissolved in methanol/HCI 0.1 M (1:1). The MEAP RIA was based on the charcoal absorption technique [40,50]. After the 24-h incubation with the sample or standard, antiserum and the labeled peptide, a charcoal suspension (250 and 25 mg dextran T-70 in 100 ml of 0.05 M sodium phosphate buffer) was added to the samples (200 μl/sample). This was incubated for 10 min and thereafter centrifuged for 1 min. The radioactivity in the supernate (300 μl) was counted in a gamma counter.

All antisera were raised in rabbits against the peptide–thyroglobulin conjugate as described in Glämsta et al. [18].

The MEAP antiserum (90-3DII) was raised against the oxidized analogue of MEAP, the final dilution in the RIA was 1:70,000. The cross-reactivity of the MEAP antisera was less than 0.1% with Met-enkephalin, Met-enkephalin-Lys^6, Leu-enkephalin-Arg^6 and Leu-enkephalin [40,50]. The final dilution of the dynorphin B antiserum (113B) was 1:330,000. The cross-reactivity with dynorphin 32 was 100% and with dynorphin B 29% [40].

1.5. Statistics

Between-group comparisons of defensive behaviors and biological measurements were employed by the Student's *t*-test (StatView, Abacus). For within-group comparisons, paired *t*-test was used. The data are presented as mean ± standard error of the mean (SEM). Because the distribution of ethanol intake is too skewed [19] to permit statistical testing with parametric tests, nonparametric methods (Mann–Withney *U*-test and Wilcoxon matched-pairs signed-ranks test; [51]) were used in the statistical treatment of the drinking data. Results of drinking data are presented as median ± median absolute deviation (MAD; i.e. the median of the set of differences between each data point and the median of the data). Two-tailed levels of significance were used in all statistical calculations.

2. Results

2.1. Fleeing and freezing

The results of the open-field behavior tests are shown in Table 1. AAS animals displayed prolonged latency to leave the sector (first crossing) compared to controls ($t=3.23$, $p<0.01$). AAS rats also showed significantly less locomotor activity and rearing behavior than the control group ($t=7.77$, $p<0.0001$; $t=3.35$, $p<0.01$, respectively). No group differences were observed in numbers of boli deposited or amount of grooming behavior.

The flight reaction (number of lines crossed) during the door bell signal was less in the AAS group than in the control group ($t=3.33$, $p<0.01$; Fig. 1, upper panel) and the freezing reaction was shortened in the AAS group compared to controls ($t=2.79$; $p<0.01$; Fig. 1, lower panel).

Table 1
Mean ± SEM of performance of various open-field behaviors (first crossing, locomotion, rearing, defecation and grooming) in rats treated with the AAS ($n=20$) nandrolone decanoate (daily injections of 15 mg/kg for 14 days) or with oil (controls; $n=20$)

	AAS	Controls
First crossing (latency, s)	35.4 ± 8.9 **	6.6 ± 0.9
Locomotion (number of crossings)	12.9 ± 1.84***	31.5 ± 1.52
Rearing (frequency)	11.8 ± 1.1 **	16.8 ± 1.0
Defecation (number of boli)	4.4 ± 0.6	3.5 ± 0.6
Grooming (cumulative time, s)	5.2 ± 2.0	5.0 ± 0.9

** $p<0.01$ vs. controls (Student's *t*-test).
*** $p<0.001$ vs. controls (Student's *t*-test).

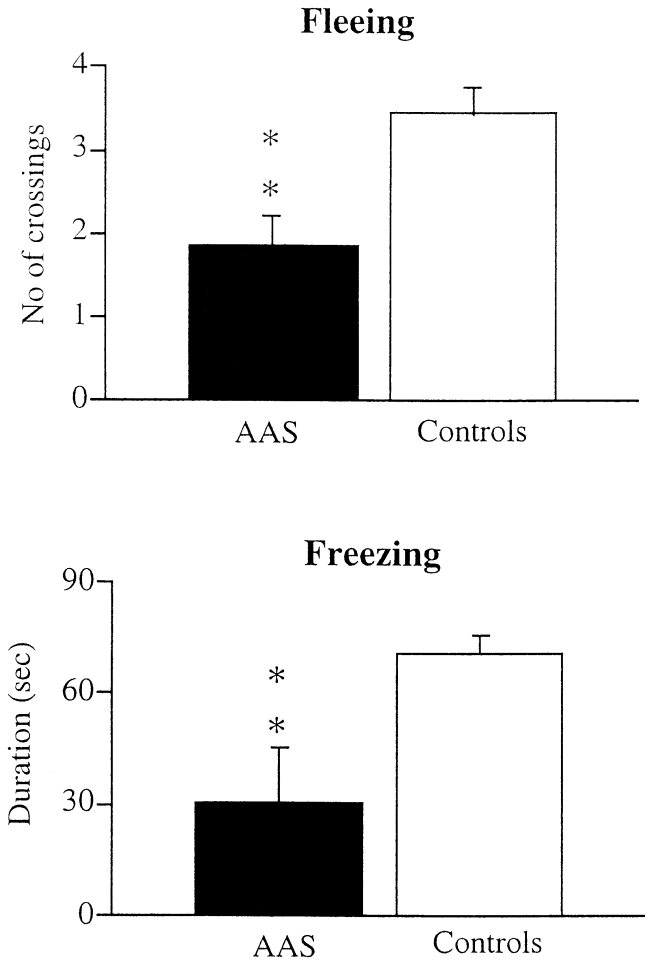

Fig. 1. Mean ± SEM of flight reaction (number of lines crossed; upper panel) and duration (s) of freezing reaction (lower panel) in rats treated with the AAS ($n=20$) nandrolone decanoate (daily injections of 15 mg/kg for 14 days) or with oil (controls; $n=20$). *** $p<0.001$ (Student's *t*-test).

2.2. Defensive aggression

As seen in Fig. 2, AAS animals showed more defensive aggression immediately after the end of the 2-week injection period and after the 3-week alcohol consumption period than controls ($t=5.92$, $p<0.001$; $t=6.04$, $p<0.001$, respectively). Table 2 shows the reactivity scores on individual tests. AAS animals differed significantly from the control group on all individual tests at both test occasions, except in their reactivity toward the approaching rod. There were no significant differences in the second test of defensive aggression between the two subgroups of AAS treated animals (conducted immediately after end of the alcohol consumption period, i.e. 6 or 8 weeks after last nandrolone decanoate injection), or between the two subgroups of controls.

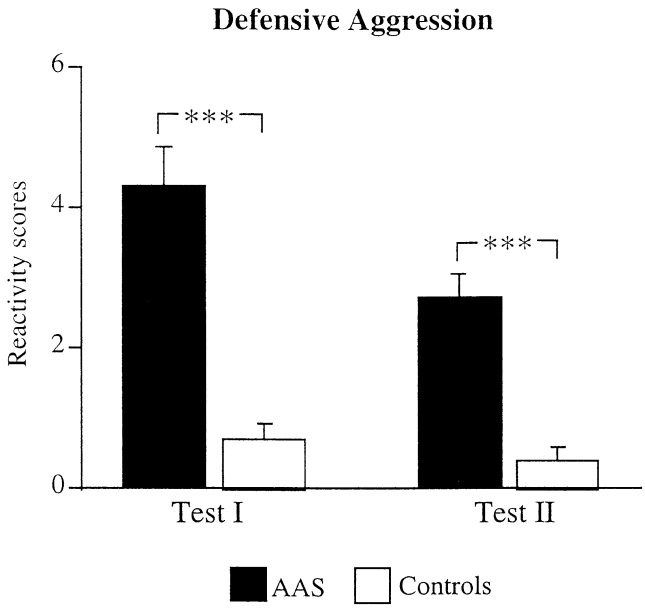

Fig. 2. Mean ± SEM of defensive behavior in rats treated with the AAS ($n = 20$) nandrolone decanoate (daily injections of 15 mg/kg for 14 days) or with oil (controls; $n = 20$). Test I: immediately after the end of the 2-week AAS injection period; Test II: immediately after the end of the 3-week alcohol consumption period. *** $p < 0.001$ (Student's t-test).

Within-group comparisons showed that the AAS animals were most aggressive on the first test occasion ($t = 2.79$, $p < 0.02$), with significantly higher scores on the individual test 'flank prods' ($t = 3.19$, $p < 0.01$) and 'capturing' ($t = 2.66$, $p < 0.02$). There were no within-group differences for the control rats.

Table 2
Mean ± SEM defensive aggression in response to an approaching rod, an air puff, flank prods and capturing with gloved hand in rats treated with the AAS ($n = 20$) nandrolone decanoate (daily injections of 15 mg/kg for 14 days) or with oil (controls; $n = 20$)

	Test I	Test II
Approaching rod		
AAS	0.00 ± 0.00	0.00 ± 0.00
Controls	0.00 ± 0.00	0.00 ± 0.00
Air puff		
AAS	0.30 ± 0.13 *	0.20 ± 0.09 *
Controls	0.00 ± 0.00	0.00 ± 0.00
Flank prods		
AAS	0.65 ± 0.11***	0.30 ± 0.10 **
Controls	0.00 ± 0.00	0.00 ± 0.00
Capturing		
AAS	3.20 ± 0.14***	2.85 ± 0.13***
Controls	0.35 ± 0.11	0.20 ± 0.09

Test I: immediately after the end of the 2-week AAS injection period; Test II: immediately after the end of the 3-week alcohol consumption period.
* $p < 0.05$ vs. controls (Student's t-test).
** $p < 0.01$ vs. controls (Student's t-test).
*** $p < 0.001$ vs. controls (Student's t-test).

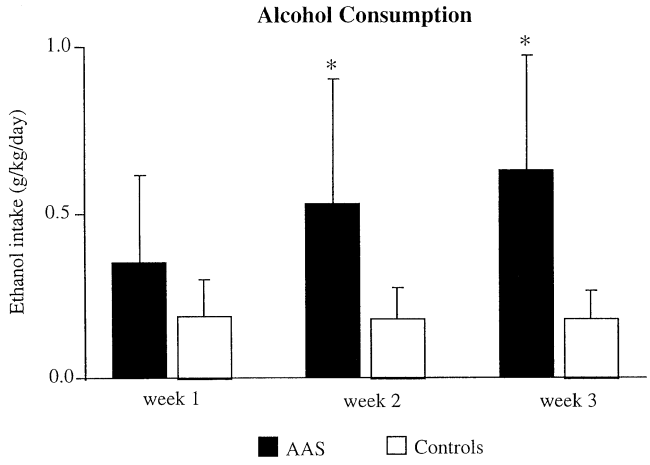

Fig. 3. Median ± MAD ethanol intake (expressed as g/kg/day of absolute ethanol) during 3 weeks in rats treated with the AAS ($n = 20$) nandrolone decanoate (daily injections of 15 mg/kg for 14 days) or with oil (controls; $n = 20$). * $p < 0.05$ (Mann–Whitney U-test).

2.3. Voluntary alcohol intake

There were no significant differences in alcohol intake between the two subgroups of AAS-treated animals (i.e. tested for voluntary ethanol consumption 1 or 3 weeks after the end of the injection period), or between the two matching control subgroups. Thus, subgroups were therefore combined to their original cohorts (AAS: $n = 20$; controls: $n = 20$).

As seen in Fig. 3, there was no significant differences in ethanol intake during the first week between AAS and control animals. However, AAS animals drank more ethanol during the second week ($U = 126$, $p < 0.05$) and the last week ($U = 105$, $p < 0.02$) compared to the controls. There were no significant alteration in total fluid, water intake and ethanol preference, except for week 3; AAS animals significantly increased their preference for ethanol compared to control animals ($U = 125$, $p < 0.05$; Table 3).

Table 3
Median ± MAD ethanol preference (% of total fluid intake), total fluid intake (ml/kg/day) and water intake (ml/kg/day) during 3 weeks in rats treated with the AAS ($n = 20$) nandrolone decanoate (daily injections of 15 mg/kg for 14 days) or with oil (controls; $n = 20$)

	Week 1	Week 2	Week 3
Ethanol preference			
AAS	39.8 ± 24.6	56.9 ± 26.5	65.4 ± 21.4 *
Controls	29.5 ± 11.9	34.7 ± 9.9	35.72 ± 8.8
Total fluid intake			
AAS	27.9 ± 5.4	27.8 ± 9.6	37.1 ± 11.0
Controls	25.5 ± 3.9	24.7 ± 6.8	26.8 ± 6.6
Water intake			
AAS	16.0 ± 6.3	13.9 ± 9.0	19.9 ± 13.3
Controls	21.7 ± 4.6	27.1 ± 4.9	29.8 ± 6.1

* $p < 0.05$ vs. controls (Mann–Whitney U-test).

In comparison to the first week, there was a gradual increase in alcohol consumption within the AAS group over the subsequent weeks, which was significantly enhanced during the third week (ethanol intake: $t=2.37$, $p<0.02$; ethanol preference: $t=2.01$; $p<0.05$; total fluid intake: $t=2.73$, $p<0.01$). Water intake did not change significantly during the corresponding period. There were no within-group differences in fluid intakes over the subsequent weeks for the control rats.

2.4. Biological measurements

There were no differences in body weight between the AAS and control animals at the start of the experiment (268 ± 9 and 271 ± 6 g, respectively). Throughout the experiment period, the AAS-treated group did not gain as much weight as the controls. Thus, at decapitation, the body weights of the formerly AAS-treated rats were 336 ± 7 g and controls were 417 ± 8 g ($t=3.67$, $p<0.0001$). The systemic effect of nandrolone decanoate treatment was measured by weighing the thymus gland. A significant reduction in thymus weight was observed in the AAS animals (27.2 ± 1.9 mg/100 g body weight) compared to controls (105.5 ± 4.7 mg/100 g body weight; $t=15.45$, $p<0.0001$).

The results showed that the nandrolone decanoate treatment decreased levels of dynorphin B-ir in nucleus accumbens compared to the controls ($t=3.36$, $p<0.01$; Fig. 4). A decreased level of the peptide MEAP-ir was observed in PAG in the AAS animals compared to controls ($t=2.04$, $p<0.05$), whereas AAS animals had a higher level of MEAP-ir in the hypothalamus compared to controls ($t=2.44$, $p<0.02$; Fig. 4). No other significant differences were observed.

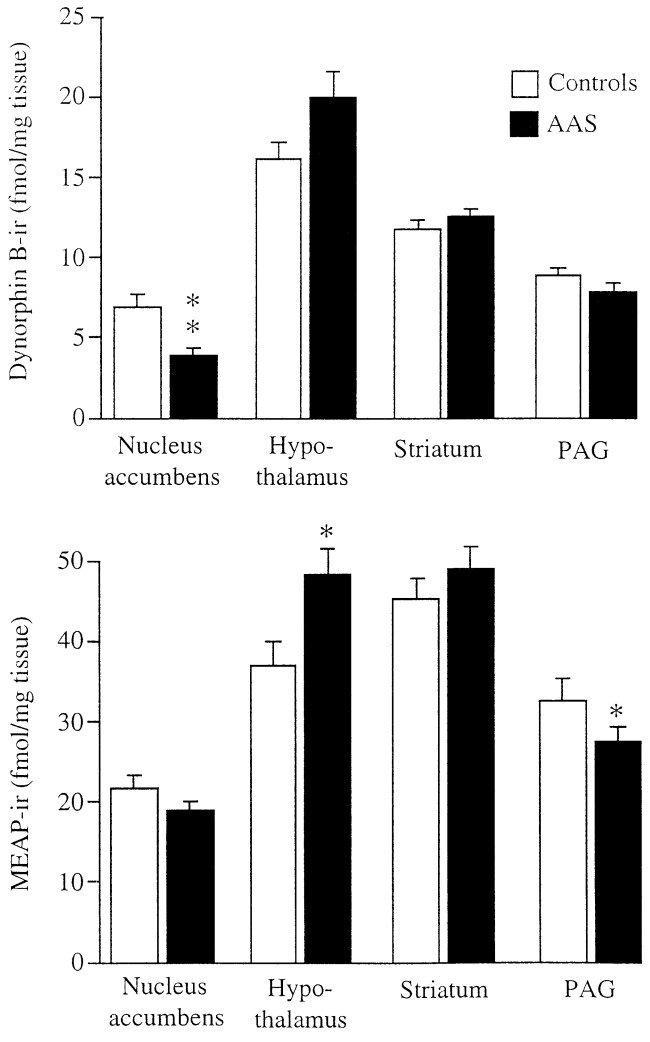

Fig. 4. Mean ± SEM of ir-levels of dynorphin B (upper panel) and MEAP (lower panel) in rats treated with the AAS ($n=20$) nandrolone decanoate (daily injections of 15 mg/kg for 14 days) or with oil (controls; $n=20$). * $p<0.05$; ** $p<0.01$ (Student's t-test).

3. Discussion

The present study examined the effects of nandrolone decanoate on defensive behaviors, voluntary alcohol consumption, brain opioid peptides and thymus weights. The results show that exposure to nandrolone decanoate affects defensive behavior in rats. Specifically, AAS-treated animals were significantly more defensive aggressive when exposed to innocuous stimuli (i.e. reactivity to experimenter) as compared to controls. This effect sustained several weeks after end of the nandrolone decanoate treatment. It may be argued that the enhanced aggression, observed after the ethanol intake period, is due to alcohol consumption. Bergvall et al. [3], however, recently found that there is no relationship between high alcohol intake and defensive aggression in untreated rats. On the other hand, it is possible that ethanol reinforces the effect of AAS on behavior. In fact, an increased anger has been observed in AAS abusers with concurrent intake of alcohol (e.g. Ref. [33]). Furthermore, the present results of enhanced aggression are in line with earlier reports showing that aggressive behavior is affected by AAS in rodents [8,31,34–36], monkeys [23,46,47] and in humans [10,16,28,32,42–45,56]. Interestingly, it is suggested that defensive aggression, rather than social aggression (i.e. natural states of aggressiveness towards conspecifics; [4]), may constitute the closest approximation to human aggression in nonprimate species [1].

The AAS-treated animals exhibited less locomotor activity in a novel situation than controls. This is in contrast to earlier studies reporting no main effect of steroid treatment on activity [8,38]. A likely reason for this discrepancy is that the present study assessed locomotor activity during the dark phase of the light–dark cycle when animals are naturally awake and peak levels of corticosterone is increased [14]. Thus, it is possible that tests performed during the light phase of the light–dark cycle eliminates possible differences between experimental and control animals. In fact, observations from our laboratory have shown no

difference in locomotor activity between animals with high or low preference for ethanol after an amphetamine injection when tested during the light phase (unpublished data), whereas an amphetamine-induced hyperactivity was observed in the high preferring group when tested during the dark phase [15]. When testing the two other expressions of the defensive motivational system, fleeing and freezing [5], the AAS group in contrast to the controls showed less escape responses and lower freezing reaction. The latter behavior is a naturally occurring defense reaction, presumably reflecting fear in a threatening situation (e.g. the likelihood of detection by predators guided by visual cues). Due to its sensitivity to the anxiolytic drug diazepam [20], the freezing reaction may be classified as fear/anxious reaction. The decreased duration of the freezing reaction in the AAS rats therefore suggests a lower potential for fear reactions in a threatening situation.

The enhanced alcohol intake in the AAS group as observed in this study is of particular interest since it has been observed among teenagers and adults, not connected to any sport, a concurrent abuse of AAS and other drugs such as alcohol (e.g. Refs. [12,27,33]). Whether abuse of other drugs may constitute as a risk factor for misuse of AAS, or vice versa, is unknown. Our results from the present study, however, is to our knowledge the first to report that abuse of nandrolone decanoate may constitute as a risk factor for onset of enhanced alcohol consumption later on in life. It is therefore important to further investigate if this association could be replicated, not only with alcohol but also with other drugs of abuse.

The increased ethanol intake can be related to the effect of AAS on the endogenous opioid system, especially those related to the reward system. Results show that the activity of dynorphin B-ir was lowered in the nucleus accumbens. A decrease in kappa-agonist like dynorphin B-ir in this area of the brain would be compatible with an increase in the dopaminergic activity [11] leading to reward and euphoria. Therefore, it is possible that the altered dynorphin B-ir activity may, in interaction with the dopaminergic system, promote the rewarding effects of ethanol and reinforce the act of drinking, and thereby increasing alcohol intake. In addition, it has been found that administration of kappa opioid agonist decreases ethanol preference and this effect was reversed by administration of a kappa antagonist [48]. Repeated administration of ethanol has been shown not to effect the expression of neither preprodynorphin nor preproenkephalin mRNA in the rat [35]. Therefore, the found effect on the dynorphin B-ir (but also MEAP-ir) is not likely to be mediated by ethanol itself, but possibly by the nandrolone decanoate pretreatment or the combination of nandrolone decanoate and ethanol.

The effect of nandrolone decanoate on the opioid peptide MEAP-ir may affect other dimensions of behaviors. For instance, the decreased MEAP-ir in PAG may be associated with reduced ability of this system to control the aggressive reaction. In fact, it has been shown that enhanced enkephalin activity within the PAG suppresses defensive aggression in cats [49]. The enhanced activity of MEAP-ir in the hypothalamus may reflect an increased enkephalin response to stress [41]. The fact that the opioid peptide system of the AAS animals remained affected up to about 8 weeks after last nandrolone decanoate injection is in line with a recent study by Johansson et al. [25] showing that treatment with nandrolone decanoate induces increased levels of endogenous kappa- as well as mu/delta-opioid receptor agonists in brain areas such as the hypothalamus and PAG.

It should be noted that one of the limitations with the RIA technique is the difficulty to determine whether the found increase in immunoreactivity correspond to an increased secretion or biosynthesis of the neuropeptides. However, based on the chronic treatment, we believe that the altered levels seen in this study correspond to an increased biosynthesis. The fact that the altered levels remained in some tissues after the recovery period further supports this hypothesis, since an increased secretion is more likely to be an acute effect.

Taken together, the present study indicates that chronical treatment of nandrolone decanoate affects the opioid peptide system, which is related to various behaviors such as voluntary intake of alcohol and defensive behaviors. Furthermore, these findings suggest that abuse of nandrolone decanoate may constitute a risk factor for enhanced alcohol consumption and defensive aggression. In human, this constellation of behavioral symptoms is closely related to acts of crimes and violence and is often observed among those abusing AAS. Further studies are needed in order to clarify the relationship between AAS, other drugs of abuse and behavior, and the long-term effects on various brain functions.

Acknowledgments

This study was supported by the Swedish Medical Research Council (K-99-04X-09495-09C and K-98-21P-11842-03A), Trygg Hansa Research Foundation, Fredrik and Ingrid Thurings Foundation, Radman and Mrs Ernst Collianders Foundation, Wilhem and Martina Lundgrens Research Foundation, Stiftelsen Längmanska Kulturfonden and Stiftelsen Lars Hiertas Minne.

References

[1] Albert DJ, Walsh ML, Jonik RH. Aggression in humans: what is its biological foundations? Neurosci Biobehav Rev 1993;17:405–25.

[2] Bell PA, Munck A. Steroid-binding properties and stabilization of cytoplasmic glucocoticoid receptors from rat thymus cells. Biochem J 1973;136:97–107.

[3] Bergvall ÅH, Fahlke C, Hansen S. An animal model for type 2 alcoholism? Alcohol consumption and aggressive behavior following lesions in the raphe nuclei, medial hypothalamus, or ventral striatum-septal area. Physiol Behav 1996;60:1125–35.

[4] Bergvall ÅH, Fahlke C, Jönsson L, Hansen S. In quest for a possible association between heightened social aggression and excessive alcohol drinking in the rat. Physiol Behav 1996;59:807–12.
[5] Bolles RC. Species-specific defence reactions and avoiding learning. Psychol Rev 1970;77:32–48.
[6] Choi PYL, Pope HG. Violence toward women and illicit androgenic anabolic steroid use. Ann Clin Psychiatry 1994;6:21–5.
[7] Christensson-Nylander I, Nyberg F, Ragnarsson U, Terenius L. A general procedure for analysis of proenkephalin B derived peptides. Regul Pept 1985;11:65–76.
[8] Clark AS, Barber DM. Anabolic–androgenic steroids and aggression in castrated male rats. Physiol Behav 1994;56:1107–13.
[9] Clark AS, Lindenfeld RC, Gibbons CH. Anabolic–androgenic steroids and brain reward. Pharmacol Biochem Behav 1996;53:741–5.
[10] Conacher GH, Workman DG. Violent crime possibly associated with anabolic steroid use. Am J Psychiatry 1989;146:679.
[11] Das D, Rogers J, Michael-Titus AT. Comparative study of the effects of mu, delta and kappa opioid agonists on 3H-dopamine uptake in rat striatum and nucleus accumbens. Neuropharmacology 1994;33:221–6.
[12] DeRant RH, Vaughn RI, Ashworth CS, Newman C, Slavens G. Use of multiple drugs among adolescents who use anabolic steroids. N Eng J Med 1993;32:922–6.
[13] Fahlke C, Hansen S. Alcohol responsiveness, hyperactivity, and motor restlessness in an animal model for attention-deficit hyperactivity disorder. Psychopharmacology 1999;146:1–9.
[14] Fahlke C, Hard E, Eriksson CJP, Engel JA, Hansen S. Consequence of long-term exposure to corticosterone or dexamethasone on ethanol consumption in the adrenalectomized rat, and the effect of Type I and Type II corticosteroid receptor antagonists. Psychopharmacology 1995;117:216–24.
[15] Fahlke C, Hard E, Eriksson CJP, Engel JA, Hansen S. Amphetamine-induced hyperactivity: differences between rats with high or low preference for alcohol. Alcohol 1995;12:363–7.
[16] Galligani N, Renck A, Hansen S. Personality profile of men using anabolic androgenic steroids. Horm Behav 1996;30:170–5.
[17] Gianoulakis C. Implications of endogenous opioids and dopamine in alcoholism: human and basic science studies. Alcohol Alcohol 1996;1:33–42.
[18] Glämsta E-L, Mörkrid L, Lantz I, Nyberg F. Concomitant increase in blood plasma levels of immunoreactive hemorphin-7 and β-endorphin following long distance running. Regul Pept 1993;49:9–18.
[19] Hansen S, Fahlke C, Hård E, Engel JA. Adrenal corticosteroids modulate the consumption of ethanol in the rat. In: Palomo T, Archer T, editors. Strategies for studying brain disorders: depressive, anxiety and abuse disorders. London: Farand Press, 1994. pp. 465–79.
[20] Hard E, Engel JA, Larsson K, Musi B. Effect of diazepam, apomorphine and haloperidol on the audogenic immobility reaction and on open field behavior. Psychopharmacology 1985;85:106–10.
[21] Haupt HA, Rovere GD. Anabolic steroids: a review of the literature. Am J Sports Med 1984;12:469–84.
[22] Herz A. Opioid reward mechanisms: a key role in drug abuse? Can J Physiol Pharmacol 1998;76:252–8.
[23] Higley JD, Mehlman PT, Poland RE, Taub DM, Vickers J, Suomi SJ, Linnoila M. CSF testosterone and 5-HIAA correlate with different types of aggressive behaviors. Biol Psychiatry 1996;40:1067–82.
[24] Johansson ÅK, Bergvall AH, Hansen S. Behavioral disinhibition following basal forebrain excitotoxin lesions: alcohol consumption, defensive aggression, impulsivity and serotonin levels. Behav Brain Res 1999;102:17–29.
[25] Johansson P, Hallberg M, Kindlundh A, Nyberg F. The effect on opioid peptides in the rat brain following chronic treatment with the anabolic androgenic steroid, nandrolone decanoate. Brain Res Bull 2000;51:413–8.
[26] Johansson P, Ray A, Zhou Q, Huang W, Karlsson K, Nyberg F. Anabolic androgenic steroids increase β-endorphin levels in the ventral tegmental area in the male rat brain. Neurosci Res 1997;27:185–9.
[27] Kindlundh AMS, Isacson DGL, Berglund L, Nyberg F. Factors associated with adolescent use of doping agents: anabolic–androgenic steroids. Addiction 1999;94:543–53.
[28] LaBree M. A review of anabolic steroids: use and effects. J Sports Med Phys Fitness 1991;31:618–26.
[29] Lee SC, Yamamoto T, Ueki S. Characteristics of aggressive behavior induced by nucleus accumbens septi lesions in rats. Behav Neural Biol 1983;37:237–45.
[30] Lindström M, Nilsson AL, Katzman PL, Janzon L, Dymling J-F. Use of anabolic–androgenic steroids among body builders — frequency and attitudes. J Int Med 1990;227:407–11.
[31] Long SF, Davis WM. Cocaine and nandrolone increase aggression in male rats. J Neurosci Abstr 1994;227:594.
[32] Lukas SE. Current perspectives on anabolic–androgenic steroid abuse. Trends Pharmacol Sci 1993;14:61–8.
[33] Lukas SE. CNS effects and abuse liability of anabolic–androgenic steroids. Annu Rev Pharmacol Toxicol 1996;36:333–57.
[34] Lumia AR, Thorner KM, McGinnis MY. Effects of chronically high doses of the anabolic steroid, testosterone, on intermale aggression and sexual behavior in male rats. Physiol Behav 1994;55:331–5.
[35] Mathieu-Kia A-M, Besson M-J. Repeated administration of cocaine, nicotine and ethanol: effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. Mol Brain Res 1998;54:141–51.
[36] Melloni RH, Connor DF, Hang PTX, Harrison RJ, Ferris CF. Anabolic–androgenic steroid exposure during adolescence and aggressive behavior in golden hamster. Physiol Behav 1997;61:359–64.
[37] Menard CS, Hebert TJ, Dohanich GP, Harlan RE. Androgenic–anabolic steroids modify β-endorphin immuno-reactivity in the rat brain. Brain Res 1995;669:255–6.
[38] Minkin D, Meyer M, van Haaren F. Behavioral effects of long-term administration of an anabolic steroid in intact and castrated male Wistar rats. Pharmacol Biochem Behav 1993;44:959–63.
[39] Narducci WA, Wagner JC, Hendrickson TP, Jeffrey TP. Anabolic steroids — a review of the clinical toxicology and diagnostic screening. Clin Toxicol 1990;28:287–310.
[40] Nylander I, Stenfors C, Tan-No K, Mathe AA, Terenius L. A comparison between microwave irradiation and decapitation: basal levels of dynorphin and enkephalin and the effect of chronic morphine treatment on dynorphin peptides. Neuropeptides 1997;31:357–65.
[41] Pohorecky LA, Skiandos A, Zhang X, Rice KC, Benjamin D. Effect of chronic social stress on delta-opioid receptor function in the rat. J Pharmacol Exp Ther 1999;290:196–206.
[42] Pope HG, Katz DL. Bodybuilders psychosis. Lancet 1987;11:863.
[43] Pope HG, Katz DL. Affective and psychotic symptoms associated with anabolic steroid use. Am J Psychiatry 1988;145:487–90.
[44] Pope HG, Katz DL. Psychiatric and medical effects of anabolic–androgenic steroid use. Arch Gen Psychiatry 1994;51:375–82.
[45] Pope HG, Katz DL, Champoux R. Anabolic–androgenic steroid use among 1010 college men. Physician Sports Med 1988;16:75–81.
[46] Rejeski WJ, Brubaker PH, Herb RA, Kaplan JR, Koritnik D. Anabolic steroids and aggressive behavior in cynomolgus monkeys. J Behav Med 1988;11:95–105.
[47] Rose RM, Bernstein IS, Holiday JW. Plasma testosterone dominance rank, and aggressive behavior in a group of male rhesus monkeys. Nature 1971;231:366–8.
[48] Sandi C, Borrell J, Guaza C. Involvement of kappa type opioids on ethanol drinking. Life Sci 1988;42:1067–75.
[49] Shaikh MB, Shaikh AB, Siegel A. Opioid peptides within the midbrain periaqueductal gray suppress affective defensive behavior in the cat. Peptides 1988;9:999–1004.
[50] Sharma HS, Nyberg F, Thörnwall M, Olsson Y. Met-enkephalin-Arg-Phe in spinal cord and brain following traumatic injury to the

spinal cord: influence of *p*-chlorophenylalanine. Neuropharmacology 1993;32:711–7.
[51] Siegel S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill, 1956.
[52] Spanagel R, Herz A, Shippenberg TS. Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc Natl Acad Sci USA 1992;89:2046–50.
[53] Steiner H, Gerfen CR. Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. Exp Brain Res 1998;123:60–76.
[54] Terenius L. Alcohol addiction (alcoholism) and the opioid system. Alcohol 1996;13:31–41.
[55] Wilson JD. Androgen abuse by athletes. Endocr Rev 1988;9:181–99.
[56] Yesalis CE, Bahrke MS. Anabolic–androgenic steroids, current issues. Sports Med 1995;19:326–40.