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ORIGINAL INVESTIGATION

Subchronic nandrolone administration reduces cocaine-induced dopamine and 5-hydroxytryptamine outflow in the rat nucleus accumbens

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Abstract
Rationale The abuse of anabolic androgenic steroids (AASs) is not only a problem in the world of sports but is associated with the polydrug use of nonathletes. Investigations of the neurochemical effects of AAS have focused in part on the monoaminergic systems, involving, among other things, the development of dependence. We have previously shown that pretreatment with nandrolone decanoate attenuates dose-dependently the increase in extracellular dopamine (DA) concentration evoked by amphetamine and 3,4-methylenedioxymethamphetamine in the nucleus accumbens (NAc).
Objectives The aim of this study was to investigate whether the nandrolone pre-exposure modulates the acute neurochemical and behavioral effects of cocaine in rats and whether the effects are long lasting.
Methods DA, 5-hydroxytryptamine (5-HT), and their metabolites were measured from samples collected from the NAc by microdialysis. The behavior of the animals was recorded.
Results The present study demonstrates that five injections of nandrolone (5 and 20 mg/kg) inhibited cocaine-evoked DA and 5-HT outflow in the NAc, locomotor activity (LMA), and stereotyped behavior in experimental animals, and that these effects are seen even after elimination of nandrolone from bloodstream.
Conclusions Given that accumbal outflow of DA and 5-HT, as well as LMA and stereotyped behavior, is related to gratification of stimulant drugs, this study suggests that nandrolone, at the doses tested, has a significant effect on the pleasurable properties of cocaine. Furthermore, because neurochemical and behavioral responses were still attenuated after a fairly long recovery period, it seems that nandrolone may induce long-lasting changes in the brains of rat.

Keywords Anabolic androgenic steroids · Cocaine · Dopamine · Locomotor · Activity · Nucleus accumbens · Microdialysis · 5-HT

Introduction

Anabolic androgenic steroids (AASs) are defined as synthetic derivatives of the endogenous sex hormone testosterone. These compounds have been used clinically to treat, e.g., hypogonadism, anemia, and protein deficiency, as well as severe weight loss associated with chronic diseases (Basaria et al. 2001; Shahidi 2001). The anabolic effects of AASs have made these substances attractive also outside clinical use. However, beside these desired physical effects, AASs can affect multiple brain functions (e.g., neuroendocrine and behavioral functions) via intracellular receptors. The steroid receptors, when occupied by a ligand, are classically translocated to the cell nucleus where they function as a transcription factor and modulate gene expression, which in turn results in many neurochemical changes. There is evidence that dopaminergic as well as serotonergic activities in the brain are influenced by sex steroids, for instance, there are studies where androgens are shown to have direct stimulating effects on central dopamine (DA) and 5-hydroxytryptamine (5-HT) release (de Souza Silva et al. 2008, 2009). However, the central nervous system (CNS) actions of AASs are complex, and the response depends not only on their interaction with ARs in the brain. For example, testosterone has shown to induce conditioned place preference

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(CPP), and this could be blocked by the mixed dopamine D_1/D_2 receptor antagonist (Packard et al. 1998; Schroeder and Packard 2000). Pre-exposure to AASs has been demonstrated to affect also the response to other substances of abuse in experimental animals. Nandrolone decanoate is shown to block CPP induced by food, tetrahydrocannabinol, and morphine (Celerier et al. 2003, 2006), and male adult rats have been shown to increase voluntary alcohol intake after cessation of AAS administration (Johansson et al. 2000).

According to recent surveys, people who abuse AASs also tend to abuse psychotropic drugs such as cocaine, heroin, amphetamine, and 3,4-methylenedioxymethamphetamine (MDMA) or "ecstasy" (DuRant et al. 1995; Kindlundh et al. 2001a; Thevis et al. 2008). It has been hypothesized that steroid hormones are important determinants of cocaine's effects on behavior through their influence on neuronal activity and plasticity (Hruska and Pitman 1982; Perrotti et al. 2000; Quinones-Jenab et al. 2001). Female rats have been shown to develop CPP at cocaine doses of 5 and 10 mg/kg, while male rats require higher cocaine doses (20 mg/kg; Russo et al. 2003). In this context, it is not surprising that recent animal studies have indicated that AASs, which resemble male gonadal hormones, are able to modulate the central dopaminergic and serotonergic neuronal function involved in reward, a key phenomenon in drug dependence. For example, in our microdialysis study, subchronic nandrolone pretreatment attenuated the dopaminergic effects of amphetamine or MDMA in the nucleus accumbens (NAc; Kurling et al. 2008), which is believed to be a critical locus in the functional anatomy of drug reward.

One aim of this study was to determine whether the effects of supraphysiological nandrolone administration observed with amphetamine and MDMA can be extended to cocaine, a stimulant drug that has a different mechanism of action from the amphetamines. Another aim was to evaluate whether the AAS pretreatment-induced changes in the brain's reward circuitry, measured as an altered response to the drug of abuse, are reversible. This study focused on the hypothesis that DA and 5-HT projections to the NAc play an essential role in the mechanisms underlying the effects of drugs of abuse. We used an in vivo microdialysis technique on fully conscious rats to monitor whether subchronic nandrolone treatment modulates the dopaminergic and serotonergic effects of acute injections of cocaine and to evaluate whether these changes are persistent.

Methods

Animals

Seventy-two adult male Wistar rats, weighing 300–380 g, were supplied by Harlan Netherlands B.V. (The Netherlands) at least 1 week before the experiments. Animals were housed three per transparent cage (Techniplast Eurostandard type IV cage: 595×380×200 mm, floor area 1,820 cm^2) in a temperature- and humidity-controlled room (21±2°C) with a 12-h light cycle. The lights were on from 06:00 a.m. to 06:00 p.m., during which time, all the experiments were conducted. Standard laboratory chow (Altromin Nr. 1314; Chr. Petersen A/S, Ringsted, Denmark) and tap water were freely available. After surgery, the rats were housed individually. The State Provincial Office of Southern Finland Animal Experiment Board approved the animal experiments, and they were conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Drugs and treatments

Nandrolone was administered at doses of 0 (vehicle), 5, and 20 mg/kg (calculated as free base) intramuscularly (i.m.) every other day over a 10-day period (total of five injections per animal). The injections were given in the left and right hind leg alternately. Nandrolone decanoate (Deca-Durabolin®) was a commercial preparation supplied by NV Organon (Oss, the Netherlands). The matching vehicle for nandrolone decanoate, a mixture of arachnoid oil and benzylalcohol, was prepared by the University Pharmacy (Helsinki, Finland) for control purposes. Following the 10-day administration period, the animals were randomly assigned to two groups: In group 1, the microdialysis experiments were conducted 6 days after the last nandrolone injection, and in group 2, the microdialysis experiments were conducted 28 days after the last nandrolone injection. A recovery period of 28 days was used to evaluate whether the effects of subchronic nandrolone treatment were reversible. The doses of nandrolone were chosen to correspond to dosages used in preliminary studies without any observable adverse effects on animal welfare.

Cocaine HCl (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) was dissolved in saline (0.9% NaCl) to be injected at the dose of 20 mg/kg dose (calculated as free base). The drug was racemic mixture. The drug was injected intraperitoneally (i.p.) in a volume of 1 ml/kg of body weight during the microdialysis experiment, at the sixth or 28th day from the last nandrolone injection. The animals were weighed before each injection with the volume administered adjusted accordingly. The cocaine dose chosen represents the minimum dose that produces stereotyped behavior in rats and sufficient increase in the extracellular DA in the NAc without any observable adverse effects on animal welfare (as determined by preliminary studies).

Microdialysis surgery and experiments

The rats were anesthetized using 5% halothane gas (Halothane Liquid BP; Rhodia Organique Fine Ltd., Bristol, UK) and placed in a stereotactic instrument. A guide cannula (CMA/12; CMA Microdialysis, Solna, Sweden) was implanted 2 mm above the NAc [A, +1.9; L, −1.0; D, −6.0] as calculated relative to the bregma and skull surface according to Paxinos and Watson (1986) and secured with two small screws and dental cement (Aqualox; VOCO, Cuxhaven, Germany). During surgery, halothane gas was administered at a concentration of 2.5%. The animals received subcutaneously (s.c.) 0.05 ml of buprenorphium preparation (Temgesic®, 0,3 mg/ml; Schering-Plough Europe, Brussels, Belgium) to alleviate the pain and were allowed to recover from the surgery for at least 5 days.

One day before the experiment, the rats were allowed to habituate to the test cage, and a microdialysis probe (CMA/12, membrane length 2 mm; CMA Microdialysis, Solna, Sweden) was inserted through the guide cannula into the NAc shell. The next day, the rats were placed in the test cage, and the probes were connected to a CMA/100 microinjection pump and perfused with modified Ringer's solution (147 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, and 1.0 mM $MgCl_2$, pH 6) at a flow rate of 2 µl/min. In order to prevent degradation of monoamine transmitters, a 6.5-µl aliquot of an antioxidant solution (1.0 mM oxalic acid, 3.0 mM L-cysteine, and 0.1 mM acetic acid) was added to each vial before collecting the dialysate samples.

The perfusate was discarded during the first 60 min, after which, the samples were collected at 20-min intervals. An i.p. injections of cocaine or saline was given after the collection of four basal samples (2 h 20 min from the beginning of the perfusion). Animal behavior was video recorded from 20 min before the injection until 160 min after the injection. At the end of the experiment, the animals were anesthetized with 5% halothane gas, and blood samples for drug measurements were drawn using cardiac puncture. After decapitation, their brains were dissected out and immersed in buffered 10% formalin solution to verify the correct placement of the probes. Only data from animals with accurate probe placements were included in statistical analyses.

Analytical procedures

Determination of DA, 5-HT, and their metabolites in dialysate samples

In order to quantify DA, 5-HT, and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA), 20-µl aliquots of dialysate samples were injected into an ESA (ESA Inc. Chelmsford, MA, USA) high-performance liquid chromatography apparatus equipped with an Inertsil ODS-3V 5 µm (4.6×250 mm ID) reverse-phase column (GL-Sciences Inc., Tokyo, Japan) and a coulometric ESA Coulochem III detector. The mobile phase was a mixture of a buffer containing 50 mM NaH_2PO_4, 0.1 mM Na_2 EDTA, 2.3 mM octanesulfonic acid, and acetonitrile (14% *v*/*v* in the final solution), with the pH adjusted to 3.0 with orthophosphoric acid (H_3PO_4). The mobile phase was filtered through a 47-mm hydrophilic polypropylene membrane filter with pore size of 0.22 µm (Gelman Sciences, Ann Arbor, MI, USA) and degassed under vacuum. The flow rate was 1.2 ml/min, and the detector potentials of the two electrodes were −175 and +250 mV.

Determination of cocaine, benzoylecgonine, and ecgonine methyl ester concentrations in blood samples

Trunk blood concentrations of cocaine and its metabolites benzoylecgonine and ecgonine methyl ester were measured using gas chromatography–mass spectrometry (GC–MS). Briefly, cocaine and the metabolites were extracted from 1 ml of whole blood by solid phase extraction as follows: first, blood samples were acidified with sodium acetate buffer (pH 4) containing the internal standard (IS) benzoylecgonine-d3. After shaking and centrifuging, the liquid phase was extracted with an IST Isolute HCX solid phase extraction column (International Sorbent Technology Ltd, Mid Glamorgan, UK) with a cation exchanger and C8 as an adsorbent. The eluates were evaporated to dryness and derivatized with pentofluoro-1-propanol and pentafluoropropionic anhydride and incubated. The excess derivatization reagents were evaporated, and residues were resuspended in ethyl acetate. Then, a 2-µl aliquot of the sample was injected into a GC–MS.

The analysis was performed with an Agilent (Agilent Technologies, Palo Alto, CA, USA) 6890N GC, an Agilent 5973 mass selective detector (EI, positive ions, 70 eV), and an Agilent ChemStation data system. The system was operated in the splitless injector mode. Helium was used as the carrier gas. The GC column was a DB-35MS, with a length of 30 m, internal diameter of 0.32 mm, and film thickness of 0.25 µm (J&W Scientific Inc., Folsom, CA, USA). The column temperature was initially 120°C with a hold time of 2 min and was increased 15°C/min, with a final hold time of 2 min at 325°C. The inlet and MSD transfer line heater temperatures were maintained at 250°C and 300°C, respectively. MS detection was performed in the selected ion monitoring mode. The limit of quantitation was 10 ng/ml for all the analytes.

Cocaine hydrochloride, benzoylecgonine, and ecgonine methyl ester hydrochloride standard substances were donated by the UN Narcotics Laboratory (Vienna, Austria). The IS was purchased from Cerilliant (Round Rock, TX, USA) All the reagents used were of the highest quality.

Determination of nandrolone concentrations in blood samples

Blood nandrolone concentrations were analyzed with GC–MS by using a method described earlier (Kurling et al. 2008). Briefly, nandrolone was extracted from blood samples as follows: 5 ml of toluene containing IS (tetrahydrocannabinol-d3) was vortexed with 0.5 ml of the sample. After centrifugation, the toluene layer was transferred into a clean test tube and evaporated to dryness. The dry residue was dissolved in 60 μl of butyl acetate, and nandrolone (and IS) was derivatized with 15 μl of *N*-methyl-*n*-(trimethylsilyl) trifluoroacetamide (Sigma Aldrich Co Ltd, Dorset, UK). A 2-μl aliquot of the mixture was injected into an Agilent GC–MS (EI) apparatus equipped with a DB-35MS capillary column of length 30 m, internal diameter of 320 μm, and film thickness of 0.25 μm (J&W Scientific Inc., Folsom, CA, USA). The oven temperature was initially 150°C with a hold time of 1.0 min and was increased 15°C/min to 320°C, with a final hold time of 3.0 min. The lower limit of detection was set at 0.5 μg/l. At a concentration level of 1.0 μg/l, bias (percent) and precision (RSD%) were −1.0% and 3.5%, respectively.

Nandrolone (19-nortestosterone) and tetrahydrocannabinol (THC-d3) were purchased from Fluka Chemie GmbH (Buchs, Switzerland) and Cerilliant (Round Rock, TX, USA), respectively.

Characterization of behavioral changes

Motor activity and the behavior of the animal were characterized from video tapes by an observer blind to drug conditions. Recording was begun 20 min before injection and continued for 160 min after the injection. Monitoring was discontinued for 20 min following the injection to exclude the effect of the injection. The locomotor activity (LMA) of the animals was estimated from the number of complete passes across midline in the test cage at intervals of 20 min (corresponding to the sampling interval in the microdialysis experiments). In addition, a more detailed behavioral analysis was performed at the same time intervals. The beginning, frequency, and/or duration of different behavior were monitored visually for 1 min every 5 min. The behavioral patterns were scored according to a rating scale modified from the one used in our previous study (Kurling et al. 2008) and the one described by Chin et al. (2002). The rats were given a single behavioral score for each 1-min observation point, and mean values were calculated for each 20-min sampling interval. Behavioral scores were 0, passive motionlessness; 1, active motionlessness; 2, active motionlessness with occasional movements; 3, sniffing, grooming, occasional LMA; 4, LMA with burst of rearing, slight agitation; 5, stereotyped behavior; 6, intense stereotyped behavior; and 7, ataxia. When rated as passive motionless, the animal was stationary, lying, or sleeping, whereas active motionlessness included alertness. Active motionlessness with occasional movements was defined as movements of the head or occasional movement of the animal. LMA was defined as movement of the animal over the surface of the observation cage floor. Stereotyped behavior was defined as compulsive-like, rapid, and repetitive purposeless behavior, such as intensive sniffing (e.g., during a prolonged rearing), head or body weaving, or head bobbing. Intense stereotyped behavior was defined as compulsive-like, rapid, and repetitive purposeless behavior as mentioned above, but higher density. Ataxia was defined according to Sturgeon et al. (1979) as impairment in the ability of the animal to execute coordinated motor responses leading, in the extreme, to incapacitation.

Statistics

In the microdialysis experiments, the mean of the four samples before the drug treatments was considered as basal release (100%), according to which, relative changes after the injections were calculated. The absolute basal releases were calculated based on the absolute values. In the LMA test, the absolute number of passes across the midline was counted, and for the more detailed behavioral analysis, scoring was conducted as described above. For statistical evaluations, both neurochemical and behavioral data were calculated as areas under the curves (AUCs) with the trapezoidal method. The microdialysis data were then subjected to a two-tailed *t* test (the effect of cocaine versus vehicle treatment, and 6-day versus 28-day recovery period), or one-way analysis of variance (ANOVA) followed by Tukey's test (the effects of different doses of nandrolone alone and the effects of the nandrolone doses on drug-induced changes). The behavioral scores were analyzed with the Mann–Whitney *U* test, or Kruskall–Wallis nonparametric ANOVA followed by the Mann–Whitney *U* test with Tukey's adjustment for multiple comparisons. The results from measurements of nandrolone and cocaine blood concentrations were analyzed with one-way ANOVA followed by Tukey's test. The correlation between neurochemical and behavioral data were analyzed with linear correlation method, using two-tail tests. The results are presented as means ± SEM (standard error of the mean), and the level of statistical significance was set at $p \leq 0.05$.

Results

Blood drug concentrations

The measured blood cocaine concentrations were below the limit of detection, as was expected because cocaine

is quickly metabolized to benzoylecgonine and ecgonine methyl ester. The concentrations of benzoylecgonine (mean, 99.25±24.0 ng/ml) or ecgonine methyl ester (mean, 31.91±9.0 ng/ml) did not statistically differ between treatment groups. Concentrations of nandrolone are show in Table 1. All nandrolone groups differed drastically from the vehicle group ($p<0.001$ ANOVA) with the exception of the 5-mg/kg 28-day recovery group. Every 28th day group differed statistically from every sixth day group ($p<0.001$ ANOVA). There were no traces of metabolites, noretiocholanolone, and norandrosterone in the collected samples.

Table 1 The effects of subchronic nandrolone (5 and 20 mg/kg) i.m. injections on blood concentration (micrograms per liter) of nandrolone and its metabolites 19-noretiocholanolone and 19-norandrosterone 4 h after cocaine administration (n=11–12)

Nandrolone	
Vehicle	0
Nandrolone	
5 mg/kg 28th day	0.31±0.2***
5 mg/kg sixth day	4.16±0.3*,**
Vehicle 28th day	0
Nandrolone	
20 mg/kg 28th day	4.15±0.4*,**
20 mg/kg sixth day	12.73±0.7*,**

The values are expressed as mean ± SEM

nd under measurable value

*$p\leq0.001$ vs. vehicle, Tukey's test

**$p\leq0.001$, six recovery days vs. 28 recovery days, Tukey's test

Microdialysis experiments

The absolute basal accumbal extracellular concentrations of DA, 5-HT, and their metabolites did not differ significantly between the treatment groups (Table 2). The levels remained unaltered in groups injected with saline after basal samples. Even if there is some fluctuation in the basal concentrations of the metabolites, these changes were not statistically significant. Administration of cocaine, at the dose of 20 mg/kg, affected spontaneous increase in the extracellular concentration of DA and 5-HT, as well as the DA metabolites DOPAC and HVA in the NAc. The cocaine in a concentration of 20 mg/kg elevated DA ($p<0.001$) and 5-HT levels ($p<0.001$) when compared with saline in the NAc (AUC; one-way ANOVA). Cocaine decreased the concentrations of DOPAC ($p<0.001$) and HVA ($p<0.010$), while the 5-HT metabolite 5-HIAA remained unchanged. Nandrolone pretreatment had no effect on extracellular levels of DA, 5-HT, or metabolites, as compared to vehicle pretreatment.

The temporal profiles of the effects of nandrolone pretreatments and cocaine injections on extracellular DA, DOPAC, and HVA levels after 6- and 28-day recovery periods are shown in Fig. 1. Nandrolone pretreatment decreased dose-dependently the cocaine-induced elevation of extracellular DA levels as compared to the vehicle (Fig. 1, $p<0.001$ AUC ANOVA). The temporal profiles of the effects of nandrolone pretreatments and cocaine injections on extracellular 5-HT and 5-HIAA levels are shown in Fig. 2. Nandrolone pretreatment also decreased cocaine-induced elevation of extracellular 5-HT levels dose-dependently ($p<0.001$ AUC ANOVA), but it seems that nandrolone 20 mg/kg influenced 5-HT efflux to a greater extent than DA.

Effects of nandrolone pretreatment on cocaine-induced behavioral changes

Nandrolone pretreatment per se or acute saline injections did not alter the behavior of the rats, while administration of cocaine induced profound effects on both LMA and stereotyped behavior. As shown in Fig. 3, administration of cocaine increased significantly the behavioral scores ($p=0.004$; Mann–Whitney U test) and LMA ($p=0.017$, t test), as compared with saline. Cocaine-induced behavior is clearly distinguishable from the behavior of the saline-treated rats. While saline-treated animals were mostly sleeping or awake but motionless, the cocaine-treated animals exhibited behavioral patterns such as increased LMA with burst of rearing, slight agitation, and stereotyped behavior such as intensive sniffing, head or body weaving, or head bobbing.

Nandrolone pre-exposure modified the ability of cocaine to increase LMA and stereotyped behavior as seen in Fig. 4 ($p<0.001$; LMA $p=0.004$ behavior scores; ANOVA). Less stereotyped behavior was observed; the frequency and duration of rapid and repetitive purposeless behavior and intensive sniffing were reduced, head and body weaving were not so broad, and head bobbing was no longer observed. These effects were nandrolone dose related. Because, for example, high-dose nandrolone caused complete and sustained suppression of cocaine-induced 5-HT efflux (Fig. 2) and LMA (Fig. 4) while seeming to have more modest effects on DA and behavioral scores, correlation analysis between neurochemical and behavioral data was carried out (Table 3). In all treatment groups, the DA and 5-HT concentrations correlate strongly with LMA and stereotyped behavior. The correlation is positive and strong, since the Pearson correlation score is positive number and near zero. Metabolites concentration correlates negatively with the measured parameter.

Table 2 The means (±SEM; n=11–12) of the basal concentrations in the NAc dialysate in different treatment groups

Treatment	DA	DOPAC	HVA	5-HT	5-HIAA
Vehicle sixth	9.0±1.2	18.3±3.4	7.3±1.2	6.4±2.1	8.4±2.0
Vehicle 28th	9.2±1.4	17.7±3.1	8.0±1.5	5.6±1.8	7.2±1.8
Nandrolone 5 mg/kg sixth	9.4±2.0	16.6±2.9	7.4±1.4	6.8±2.0	7.1±1.5
Nandrolone 5 mg/kg 28th	10.1±1.9	17.1±3.2	7.7±1.7	5.5±1.9	7.7±1.0
Nandrolone 20 mg/kg sixth	9.3±1.7	15.4±4.1	7.8±1.8	7.2±1,8	7.9±1.3
Nandrolone 20 mg/kg 28th	9.2±1.5	16.3±3.1	6.9±1.2	7.3±1.9	8.2±1.6

Concentration fmol/40 µl

Effects of nandrolone pretreatment on cocaine-induced behavioral and neurochemical changes after a recovery period of 28 days

The length of the recovery period (6 fc. 28 days) had no effect on cocaine's action per se. The attenuating effect of nandrolone pretreatment on cocaine-induced elevation of extracellular DA remained persistent over the 28-day recovery period (Fig. 1). The DA concentration collected from the NAc after the 20-mg/kg cocaine injection did not differ statistically between group of the 6-day recovery period group and the 28-day recovery period group. According to DA results, the extracellular levels of DA metabolites HVA and DOPAC after the 20-mg/kg cocaine injection did not differ statistically between the 6-day recovery group and the 28-day recovery group. The attenuating effect of nandrolone on cocaine-induced elevation of extracellular 5-HT remained persistent also over the 28-day recovery period as seen in Fig. 2.

The effect of nandrolone on cocaine-induced behavioral changes also remained persistent over the 28-day recovery

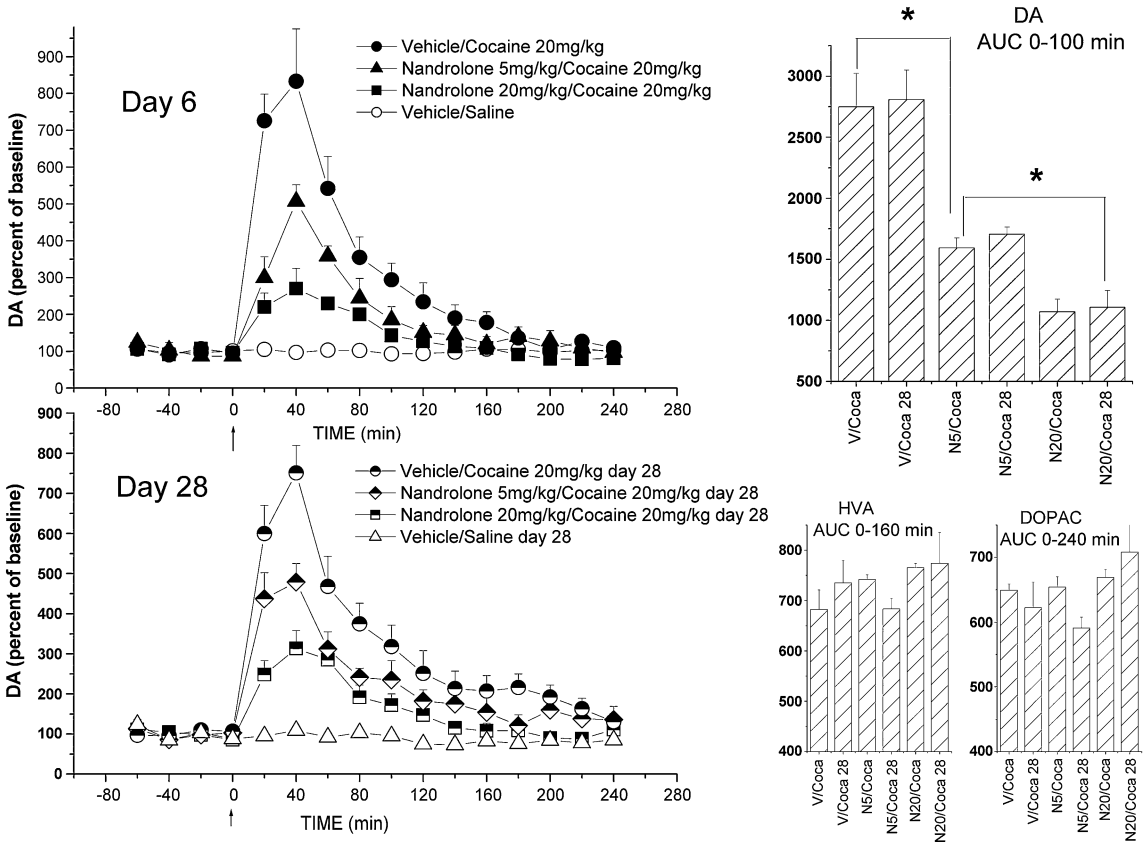

Fig. 1 The effects of subchronic nandrolone and acute cocaine (20 mg/kg) injections on extracellular dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid levels in the nucleus accumbens. The times of the cocaine injections are indicated by *arrows*. Data expressed as percentages of basal release are given as means ± SEM (n=6). Histograms represent the area under the curve (AUC) after injection of the drug, and the minutes where AUC are shown in the figure. *Single asterisk* $p \leq 0.05$, Tukey's test. Vehicle-saline groups are added to the figure as baseline references. V/Coca=vehicle+cocaine 20 mg/kg, V/Coca 28=vehicle+cocaine 20 mg/kg after 28th day, N5/Coca=nandrolone 5 mg/kg+cocaine 20 mg/kg, N5/Coca 28=nandrolone 5 mg/kg+cocaine 20 mg/kg after 28th day, N20/Coca=nandrolone 20 mg/kg+cocaine 20 mg/kg, N20/Coca 28=nandrolone 20 mg/kg+cocaine 20 mg/kg after 28th day

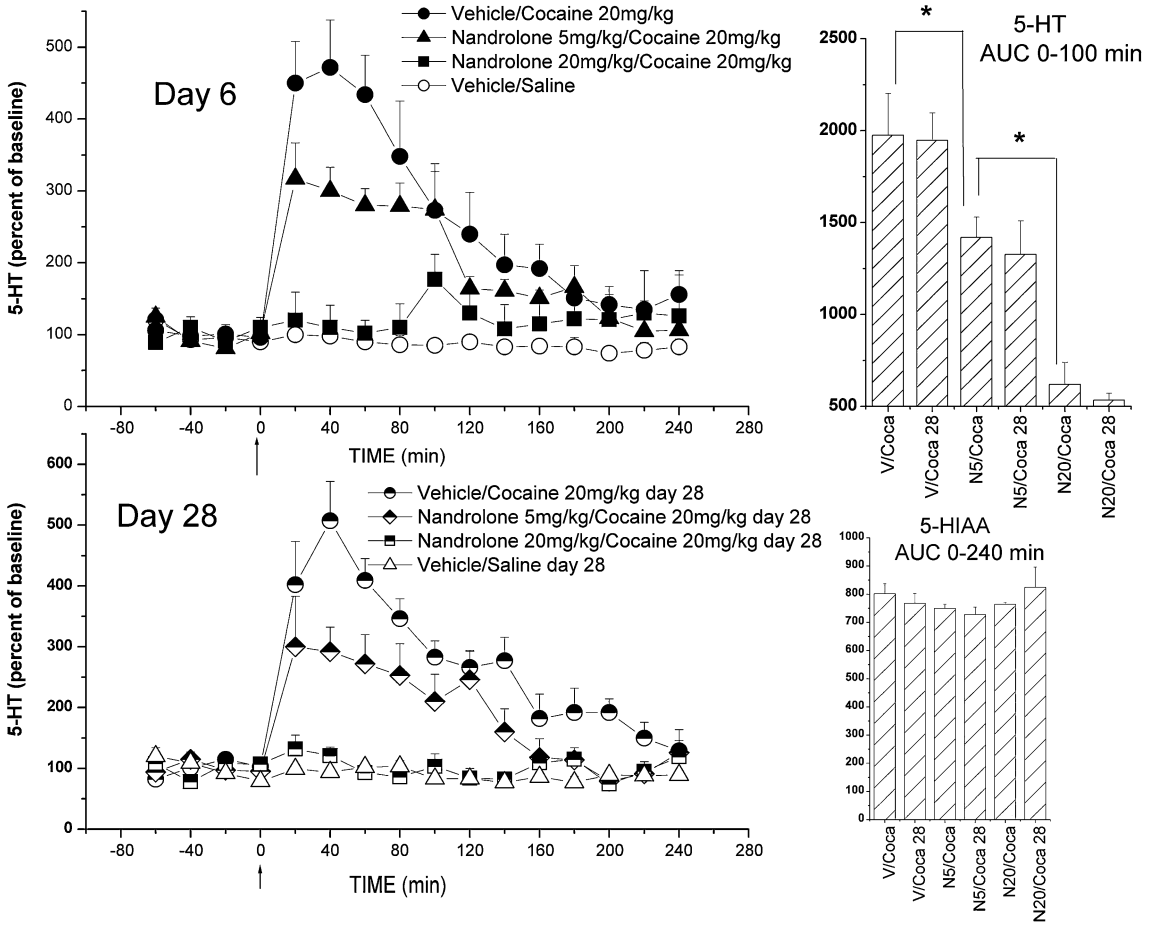

Fig 2 The effects of subchronic nandrolone and acute cocaine (20 mg/kg) injections on extracellular 5-HT and 5-HIAA levels in the nucleus accumbens. The times of the cocaine injections are indicated by *arrows*. Data expressed as percentages of basal release are given as means ± SEM (n=6). Histograms represent the area under the curve (AUC) after injection of the drug, and the minutes where AUC are shown in the figure. *Single asterisk* $p \leq 0.05$, Tukey's test. Vehicle-saline groups are added to the figure as baseline references. V/Coca=vehicle+cocaine 20 mg/kg, V/Coca 28=vehicle+cocaine 20 mg/kg after 28th day, N5/Coca=nandrolone 5 mg/kg+cocaine 20 mg/kg, N5/Coca 28=nandrolone 5 mg/kg+cocaine 20 mg/kg after 28th day, N20/Coca=nandrolone 20 mg/kg+cocaine 20 mg/kg, N20/Coca 28=nandrolone 20 mg/kg+cocaine 20 mg/kg after 28th day

period as seen in Fig. 4. The only exception was the nandrolone 5-mg/kg 28-day recovery group, which failed to attenuate statistically the LMA induced by cocaine.

Discussion

The main findings of the present series of experiments are that subchronic pretreatment with nandrolone decanoate attenuates dose-dependently the reward-related neurochemical and behavioral effects of acute cocaine administration, and both the neurochemical and behavioral effects of nandrolone were seen for at least 28 days after the last nandrolone injection. Despite the significant decreases in blood nandrolone concentration, the attenuation of cocaine's effects remained unchanged, suggesting that nandrolone effects are long lasting. These findings are in line with the reported ability of nandrolone to decrease morphine reward (Celerier et al. 2003). Taking into account that the mesocorticolimbic dopaminergic system is considered to be involved in reward-related associative learning (Di Chiara 1999), reinforcement (Koob 1992), and incentive salience (Berridge and Robinson 1998), our present study suggests that AASs may induce some changes in brain reward systems contributing to the maintenance of drug dependence.

The whole cascade of monoamine action from synthesis to receptor activation provides several possible mechanisms by which nandrolone might affect the dopaminergic response to cocaine. Neither the mRNA of tyrosine hydroxylase nor the accumulation of L-dopa has been shown to change in the rat brain after repeated nandrolone administration (Kindlundh et al. 2003b; Thiblin et al. 1999). The possible activation of monoamine oxidase

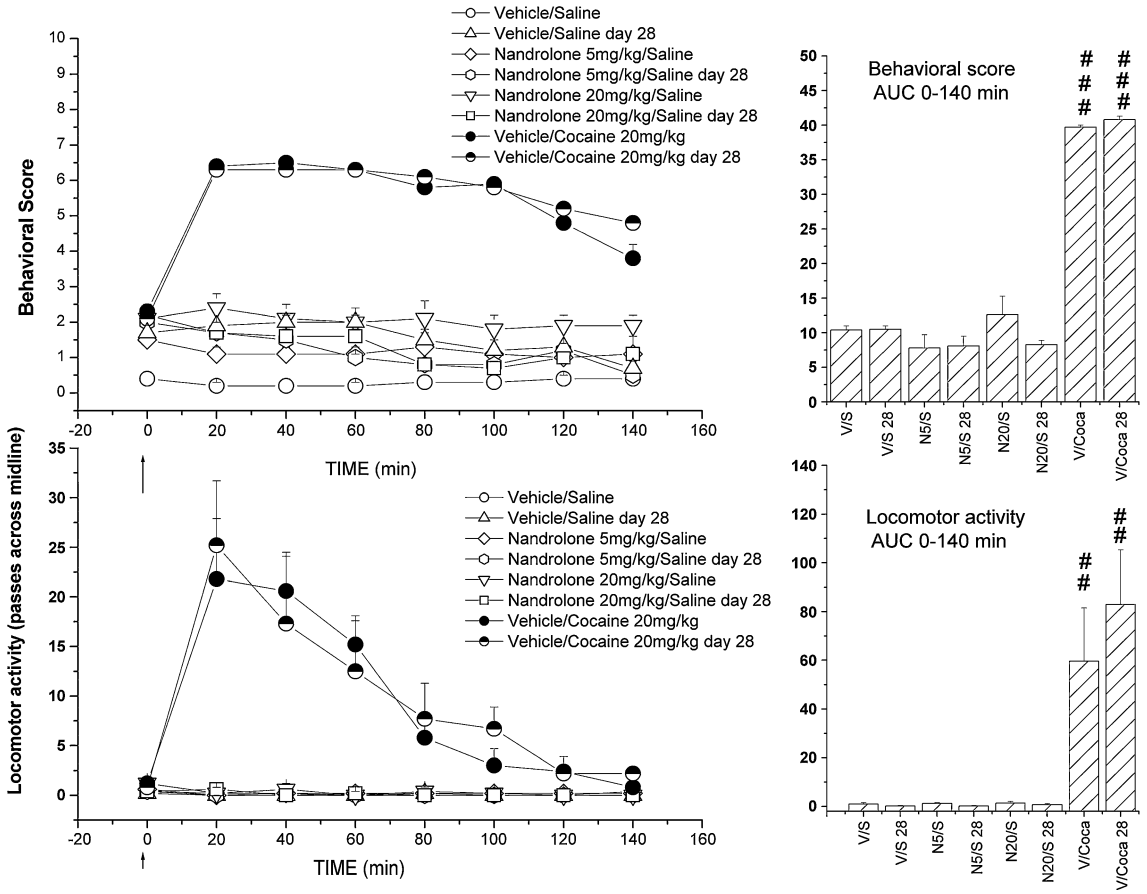

Fig. 3 The effects of subchronic nandrolone and acute cocaine (20 mg/kg) injections on locomotor activity and stereotyped behavior. The rats were given a single behavioral score per each 1-min observation point, and mean values were calculated per each 20-min sampling interval. Cocaine or saline was administered at 0 min and is indicated by *arrows*. Data expressed as absolute behavioral scores and passes across midline (n=5–6). Histograms represent AUC 0 to 140 min effect after injection of the drug. *Single number sign* $p \leq 0.05$, *double number sign* $p \leq 0.01$, *triple number sign* $p \leq 0.001$ compared with vehicle-saline group, t test. V/S=vehicle+saline, V/S 28=vehicle+saline after 28th day, N5/S=nandrolone 5 mg/kg+saline, N5/S 28=nandrolone 5 mg/kg+saline after 28th day, N20/S=nandrolone 20 mg/kg+saline, N20/S 28=nandrolone 20 mg/kg+saline after 28th day, V/C=vehicle+cocaine 20 mg/kg, V/C 28=vehicle+cocaine 20 mg/kg after 28th day

(MAO) would increase the metabolism of both 5-HT and DA, but this is not supported by the results of Thiblin et al. (1999), where nandrolone did not change MAO activity. However, there is evidence that dopaminergic as well as serotonergic activities in the brain are influenced by sex steroids. There is a study where intranasal administration of testosterone is shown to have direct stimulating effects on central DA and 5-HT release (de Souza Silva et al. 2009). Even then, these steps of DA and 5-HT transmission, in all likelihood, are not the primary target of nandrolone action, since the basal levels of DA, 5-HT, and their metabolites were unaltered in this study, and our earlier study showed that the accumbal tissue content of DA remained unaltered in AAS treated animals (Kurling et al. 2005).

Cocaine is a stimulant of the CNS that increases synaptic concentration of DA by blocking its reuptake into nerve endings (Glowinski and Axelrod 1965; Heikkila et al. 1975) by binding to the dopamine transporter (DAT). Nandrolone decanoate has been shown to increase DAT density in the male rat brain in the striatum and NAc (Kindlundh et al. 2004), which might lead to increased reuptake capacity leading to decreased DA concentration in the extracellular space after cocaine administration. The role of DAT is also supported by our study where nandrolone was shown to change the DA response for amphetamine-like compounds (Kurling et al. 2008). However, some studies done with genetically modified mice would likely lead to the opposite expectation. For instance, Salahpour et al. (2008) has shown that overexpression of DAT leads to a marked increase in the amount of DA release after amphetamine. However, this increased release was measured only in striatum, and as seen in study of Carboni et al. (2001), there can be remarkable differences between brain areas. Carboni et al. showed that results seen in DAT knockout mice can be opposite in Caudate Putamen, where DA release was decreased in DAT

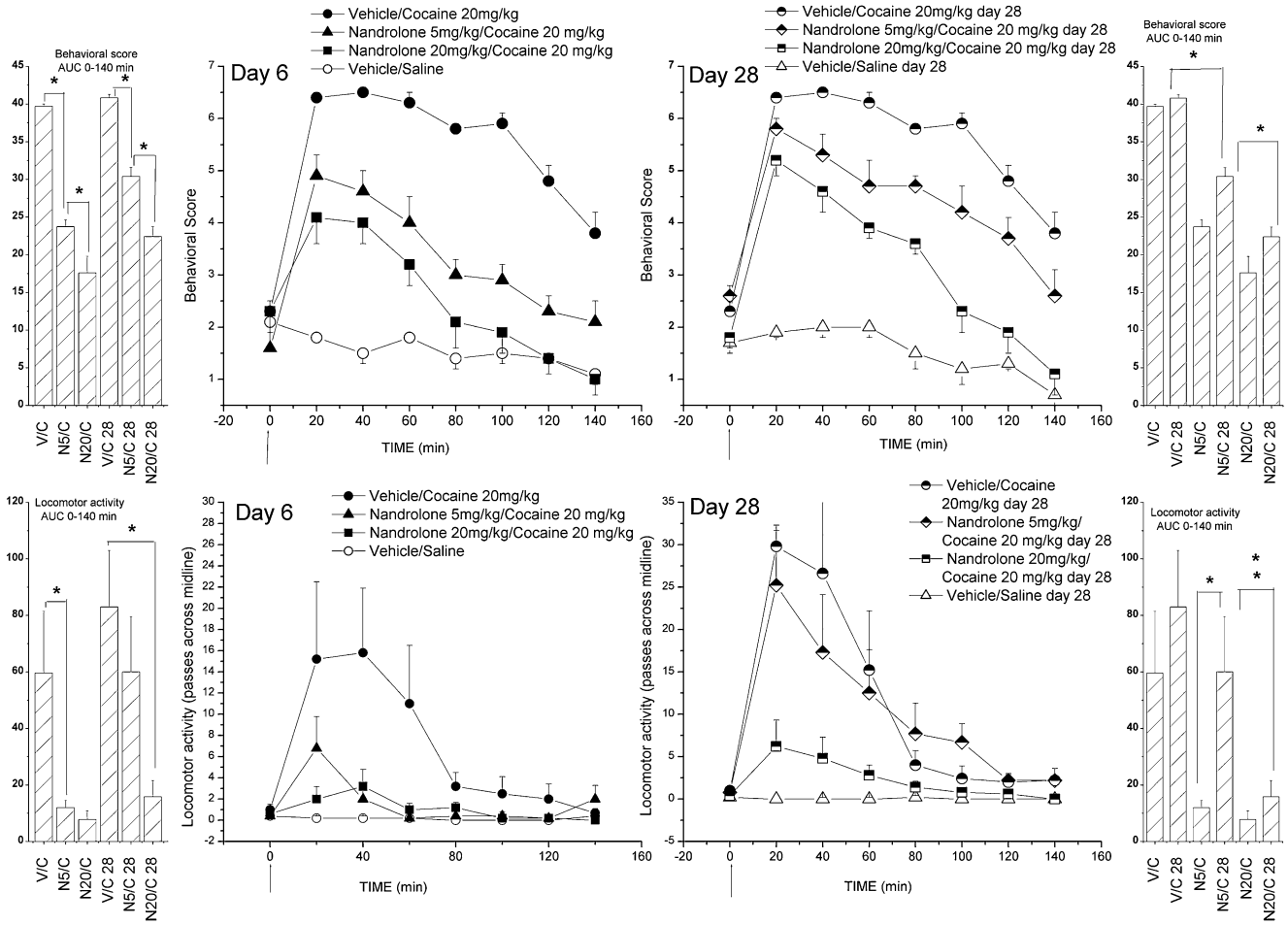

Fig. 4 The effects of subchronic nandrolone and acute cocaine (20 mg/kg) injections on locomotor activity and stereotyped behavior. The rats were given a single behavioral score per each 1-min observation point, and mean values were calculated per each 20-min sampling interval. Cocaine or saline was administered at 0 min and is indicated by *arrows*. Data expressed as absolute behavioral scores and passes across midline (n=5–6). Histograms represent AUC 0 to 140 min effect after injection of the drug. *Single number sign* $p \leq 0.05$, *double number sign* $p \leq 0.01$, *triple number sign* $p \leq 0.001$ compared with vehicle-saline group, t test. *Single asterisk* $p \leq 0.05$, *double asterisk* $p \leq 0.01$, Tukey's test. V/S=vehicle+saline, V/S 28=vehicle+saline after 28th day, V/C=vehicle+cocaine 20 mg/kg, V/C 28=vehicle+cocaine 20 mg/kg after 28th day, N5/C=nandrolone 5 mg/kg+cocaine 20 mg/kg, N5/C 28=nandrolone 5 mg/kg+cocaine 20 mg/kg after 28th day, N20/C=nandrolone 20 mg/kg+cocaine 20 mg/kg, N20/C 28=nandrolone 20 mg/kg+cocaine 20 mg/kg after 28th day

knockout mice, compared to the NAc, where DA output was increased in knockouts compared to wild type after cocaine. This gives support to our conclusion that more DAT would lead to reduction of the effects of cocaine on DA concentration. Also, the study of Ji and Dluzen (2008) can be seen as support to our conclusion. They showed that heterozygote knockout mice, which have less DAT, released more DA after methamphetamine injections than

Table 3 Correlation between neurochemical and behavioral data (n=69)

Correlation between	DA	DOPAC	HVA	5-HT	5-HIAA
All animals; behavior	0.890**	−0.636*	−0.468*	0.871*	−0.261
All animals; LMA	0.721**	−0.521*	−0.414*	0.701*	−0.273

The correlations in this table represent tests of hypotheses which assume causation flowing from increasing of DA and 5-HT and decreasing of DOPAC, HVA, and 5-HIAA concentrations on extracellular space to cause more locomotor activity and stereotyped behavior

*$p \leq 0.05$

**$p \leq 0.001$

wild type mice. When expected that knockout mice should response less to stimulant drugs than wild type mice, usually the studies are done with homozygote mice, which lack the DAT in its entirety (see as an example Drago et al. 1998; Morice et al. 2007; Thomsen et al. 2009).

Pretreatment with nandrolone also attenuated the cocaine-induced elevation of extracellular 5-HT in the NAc dose-dependently. Because cocaine increases synaptic concentration of 5-HT almost exclusively by blocking its reuptake into nerve endings (Glowinski and Axelrod 1965; Heikkila et al. 1975), the present data offers a better basis for discussing the possible role of the serotonin transporter (SERT) in these phenomena than our earlier results with amphetamine (Kurling et al. 2008). To our knowledge, no studies have been carried out with nandrolone, but testosterone has been shown to increase the expression of SERT mRNA and the density of SERT sites in the forebrain (McQueen et al. 1999). Therefore, it seems plausible that—analogously with dopaminergic effects—the possible increase in SERT density might lead to increased reuptake capacity and hence to decreased 5-HT concentration in the extracellular space after cocaine administration.

In addition to monoamine transporters, others possible sites for nandrolone modulation are the DA and 5-HT receptors. Indeed, there are studies indicating that nandrolone increases the levels of the D_2 receptor (autoreceptor) transcript and protein, in addition to decreasing levels of the D_1 receptor transcript and protein in the mesocorticolimbic and nigrostriatal systems (Kindlundh et al. 2001b, 2003b). It has also been shown to induce alterations in the density of 5-HT receptors: 5-HT_{1B} receptors were downregulated, and $5HT_2$ receptors were upregulated in the rat brain after nandrolone dosing (Kindlundh et al. 2003a). Concerning 5-HT autoreceptors (5-HT_{1A}) or 5-HT_3 receptors, to our knowledge, no studies have been done with nandrolone, but chronic administration of testosterone propionate was found to decrease the concentration of [3H]quipazine binding at 5-HT_3 receptors (Mendelson and McEwen 1990). It would also be of the utmost interest to elucidate the role of androgen receptors or other neuronal systems such as the GABAergic or glutamatergic systems that have been reported to be modulated by AASs (Le Greves et al. 2002; Masonis and McCarthy 1995) in the reward-related effects of nandrolone.

In this study, cocaine produced a rapid and long-lasting increase in LMA and stereotypic behavior, as was to be expected since cocaine has been shown to produce LMA in rats (Bedford et al. 1980), and with high doses, stereotyped behavior (Wellman et al. 2002). Correlation analysis between neurochemical and behavioral data was carried out, and as seen from results, DA, its metabolites, and 5-HT concentrations correlates strongly with LMA and stereotyped behavior seen after stimulant dosing. These correlation results empower us to make suggestion that nandrolone is able to strongly uncover a serotonergic component of cocaine-induced LMA. However, it should be remembered that LMA after cocaine is not behavior indicative of abuse potential.

In conclusion, the present study demonstrates that nandrolone dose-dependently inhibited cocaine-evoked DA and 5-HT outflow in the NAc in experimental animals. Nandrolone also decreased the behavioral effects induced by cocaine. Given that accumbal outflow of DA and 5-HT, as well as stereotyped behavior, are all considered to be related gratification of stimulant drugs, this study suggests that nandrolone may modulate the pleasurable properties of cocaine. Further, because cocaine-induced neurochemical and behavioral responses were still attenuated after a fairly long period without nandrolone (28 cf. 6 days), it seems that nandrolone may induce long-lasting changes in the brains of rats. Conclusions on the endurance of these effects can be determined using a longer abstinence time ensuring total clearance of the drug from the system. The reduced activation of monoaminergic neurons may contribute to the increased prevalence of cocaine usage among people who already self-administer AASs and stimulant drugs. However, because the pretreatment with nandrolone substantially decreased the efficacy of cocaine on extracellular DA or 5-HT and related behaviors, it could be speculated that compounds acting like steroids at central receptors or transporter proteins may provide a useful approach in the research of stimulant abuse treatment. These findings provide insight into the physiological role of steroids and the biology of stimulant dependence and may have some clinical implications.

References

Basaria S, Wahlstrom JT, Dobs AS (2001) Clinical review 138: anabolic-androgenic steroid therapy in the treatment of chronic diseases. J Clin Endocrinol Metab 86:5108–5117

Bedford JA, Borne RF, Wilson MC (1980) Comparative behavioral profile of cocaine and norcocaine in rats and monkeys. Pharmacol Biochem Behav 13:69–75

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev 28:309–369

Carboni E, Spielewoy C, Vacca C, Nosten-Bertrand M, Giros B, Di Chiara G (2001) Cocaine and amphetamine increase extracellular dopamine in the nucleus accumbens of mice lacking the dopamine transporter gene. J Neurosci 21:RC141: 1–4

Celerier E, Yazdi MT, Castane A, Ghozland S, Nyberg F, Maldonado R (2003) Effects of nandrolone on acute morphine responses, tolerance and dependence in mice. Eur J Pharmacol 465:69–81

Celerier E, Ahdepil T, Wikander H, Berrendero F, Nyberg F, Maldonado R (2006) Influence of the anabolic-androgenic steroid nandrolone on cannabinoid dependence. Neuropharmacology 50:788–806

Chin J, Sternin O, Wu HB, Burrell S, Lu D, Jenab S, Perrotti LI, Quinones-Jenab V (2002) Endogenous gonadal hormones mod-

ulate behavioral and neurochemical responses to acute and chronic cocaine administration. Brain Res 945:123–130
de Souza Silva MA, Topic B, Huston JP, Mattern C (2008) Intranasal administration of progesterone increases dopaminergic activity in amygdala and neostriatum of male rats. Neuroscience 157:196–203
de Souza Silva MA, Mattern C, Topic B, Buddenberg TE, Huston JP (2009) Dopaminergic and serotonergic activity in neostriatum and nucleus accumbens enhanced by intranasal administration of testosterone. Eur Neuropsychopharmacol 19:53–63
Di Chiara G (1999) Drug addiction as dopamine-dependent associative learning disorder. Eur J Pharmacol 375:13–30
Drago J, Padungchaichot P, Accili D, Fuchs S (1998) Dopamine receptors and dopamine transporter in brain function and addictive behaviors: insights from targeted mouse mutants. Dev Neurosci 20:188–203
DuRant RH, Escobedo LG, Heath GW (1995) Anabolic-steroid use, strength training, and multiple drug use among adolescents in the United States. Pediatrics 96:23–28
Glowinski J, Axelrod J (1965) Effect of drugs on the uptake, release, and metabolism of h3-norepinephrine in the rat brain. J Pharmacol Exp Ther 149:43–49
Heikkila RE, Orlansky H, Cohen G (1975) Studies on the distinction between uptake inhibition and release of (3H)dopamine in rat brain tissue slices. Biochem Pharmacol 24:847–852
Hruska RE, Pitman KT (1982) Distribution and localization of estrogen-sensitive dopamine receptors in the rat brain. J Neurochem 39:1418–1423
Ji J, Dluzen DE (2008) Sex differences in striatal dopaminergic function within heterozygous mutant dopamine transporter knock-out mice. Journal Neural Transm 115:809–817
Johansson P, Lindqvist A, Nyberg F, Fahlke C (2000) Anabolic androgenic steroids affects alcohol intake, defensive behaviors and brain opioid peptides in the rat. Pharmacol Biochem Behav 67:271–279
Kindlundh AM, Hagekull B, Isacson DG, Nyberg F (2001a) Adolescent use of anabolic-androgenic steroids and relations to self-reports of social, personality and health aspects. Eur J Public Health 11:322–328
Kindlundh AM, Lindblom J, Bergstrom L, Wikberg JE, Nyberg F (2001b) The anabolic-androgenic steroid nandrolone decanoate affects the density of dopamine receptors in the male rat brain. Eur J NeuroSci 13:291–296
Kindlundh AM, Lindblom J, Bergstrom L, Nyberg F (2003a) The anabolic-androgenic steroid nandrolone induces alterations in the density of serotonergic 5HT1B and 5HT2 receptors in the male rat brain. Neuroscience 119:113–120
Kindlundh AM, Lindblom J, Nyberg F (2003b) Chronic administration with nandrolone decanoate induces alterations in the gene-transcript content of dopamine D(1)- and D(2)-receptors in the rat brain. Brain Res 979:37–42
Kindlundh AM, Rahman S, Lindblom J, Nyberg F (2004) Increased dopamine transporter density in the male rat brain following chronic nandrolone decanoate administration. Neurosci Lett 356:131–134
Koob GF (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 13:177–184
Kurling S, Kankaanpaa A, Ellermaa S, Karila T, Seppala T (2005) The effect of sub-chronic nandrolone decanoate treatment on dopaminergic and serotonergic neuronal systems in the brains of rats. Brain Res 1044:67–75
Kurling S, Kankaanpaa A, Seppala T (2008) Sub-chronic nandrolone treatment modifies neurochemical and behavioral effects of amphetamine and 3, 4-methylenedioxymethamphetamine (MDMA) in rats. Behav Brain Res 189:191–201
Le Greves P, Zhou Q, Huang W, Nyberg F (2002) Effect of combined treatment with nandrolone and cocaine on the NMDA receptor gene expression in the rat nucleus accumbens and periaqueductal gray. Acta Psychiatr Scand Suppl 412:129–32
Masonis AE, McCarthy MP (1995) Direct effects of the anabolic/androgenic steroids, stanozolol and 17 alpha-methyltestosterone, on benzodiazepine binding to the. gamma-aminobutyric acid(a) receptor. Neurosci Lett 189:35–38
McQueen JK, Wilson H, Sumner BE, Fink G (1999) Serotonin transporter (SERT) mRNA and binding site densities in male rat brain affected by sex steroids. Brain Res Mol Brain Res 63:241–247
Mendelson SD, McEwen BS (1990) Chronic testosterone propionate treatment decreases the concentration of [3H]quipazine binding at 5-HT3 receptors in the amygdala of the castrated male rat. Brain Res 528:339–343
Morice E, Denis C, Giros B, Nosten-Bertrand M (2007) Evidence of long-term expression of behavioral sensitization to both cocaine and ethanol in dopamine transporter knockout mice. Psychopharmacology 208:57–66
Packard MG, Schroeder JP, Alexander GM (1998) Expression of testosterone conditioned place preference is blocked by peripheral or intra-accumbens injection of alpha-flupenthixol. Horm Behav 34:39–47
Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic, San Diego
Perrotti LI, Beck KD, Luine VN, Quinones V (2000) Progesterone and cocaine administration affect serotonin in the medial prefrontal cortex of ovariectomized rats. Neurosci Lett 291:155–158
Quinones-Jenab V, Perrotti LI, Fabian SJ, Chin J, Russo SJ, Jenab S (2001) Endocrinological basis of sex differences in cocaine-induced behavioral responses. Ann N Y Acad Sci 937:140–171
Russo SJ, Jenab S, Fabian SJ, Festa ED, Kemen LM, Quinones-Jenab V (2003) Sex differences in the conditioned rewarding effects of cocaine. Brain Res 970:214–220
Salahpour A, Ramsey AJ, Medvedev IO, Kile B, Sotnikova TD, Holmstrand E, Ghisi V, Nicholls PJ, Wong L, Murphy K, Sesack SR, Wightman RM, Gainetdinov RR, Caron MG (2008) Increased amphetamine-induced hyperactivity and reward in mice overexpressing the dopamine transporter. Proc Natl Acad Sci USA 105:4405–4410
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
Shahidi NT (2001) A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. Clin Ther 23:1355–1390
Sturgeon RD, Fessler RG, Meltzer HY (1979) Behavioral rating scales for assessing phencyclidine-induced locomotor activity, stereotyped behavior and ataxia in rats. Eur J Pharmacol 59:169–179
Thevis M, Sauer M, Geyer H, Sigmund G, Mareck U, Schanzer W (2008) Determination of the prevalence of anabolic steroids, stimulants, and selected drugs subject to doping controls among elite sport students using analytical chemistry. J Sports Sci 26:1059–1065
Thiblin I, Finn A, Ross SB, Stenfors C (1999) Increased dopaminergic and 5-hydroxytryptaminergic activities in male rat brain following long-term treatment with anabolic androgenic steroids. Br J Pharmacol 126:1301–1306
Thomsen M, Hall FS, Uhl GR, Caine SB (2009) Dramatically decreased cocaine self-administration in dopamine but not serotonin transporter knock-out mice. J Neurosci 29:1087–1092
Wellman P, Ho D, Cepeda-Benito A, Bellinger L, Nation J (2002) Cocaine-induced hypophagia and hyperlocomotion in rats are attenuated by prazosin. Eur J Pharmacol 455:117–126