

Psychomotor and rewarding properties of the neurosteroids dehydroepiandrosterone sulphate and androsterone: Effects on monoamine and steroid metabolism

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The neurosteroids, dehydroepiandrosterone sulfate (DHEAS) and androsterone, are implicated in drug addictions. We examined their influence on locomotor activity and reward in male Wistar rats, and on steroid and monoamine metabolism in the hippocampus and striatum. In the open field test, DHEAS injections (10, 40, 80 mg/kg, i.p.) 30 min prior the test had no significant effect on ambulation, but androsterone (10 mg/kg) increased general locomotion and at doses 1–10 mg/kg, increased central field activity, suggestive of an anxiolytic action. In the conditioned place preference test, both steroids had a biphasic effect: DHEAS was rewarding at doses of 10 and 40 mg/kg, but not at 80 mg/kg, while androsterone was rewarding at doses of 1 and 10 mg/kg, but aversive at 40 mg/kg. Monoamine and steroid concentrations were analyzed in homogenates from the hippocampus and striatum of DHEAS and androsterone injected rats. DHEAS reduced the hippocampal dopamine level, increased striatal homovanilic acid (HVA) and decreased the striatal serotonin concentrations. Androsterone did not affect dopamine levels or turnover, but increased noradrenaline concentration and serotonin turnover in the hippocampus. DHEAS administration augmented concentrations of DHEA, pregnenolone, androstendiol and androstentriol in both brain structures, while androsterone injections increased brain levels of androsterone, epiandrosterone, 5 α -dihydrotestosterone, and androstendiol. Present data document that although psychobehavioral and neurochemical effects of DHEAS and androsterone differ in several aspects; both neurosteroids have rewarding properties at certain dose ranges, suggesting their likely involvement in addictions, which entail different mechanisms.

Key words: neurosteroids, dehydroepiandrosterone sulphate, androsterone, reward, locomotion, monoamines

ABBREVIATIONS

CPP – conditioned place preference test
DA – dopamine
DHEAS – dehydroepiandrosterone sulphate
DHEA – dehydroepiandrosterone
5 α DHT – 5-alpha-dihydrotestosterone
DOPAC – 3,4-dihydroxyphenylacetic acid
GABA – γ -Aminobutyric acid
5HIAA – 5-hydroxyindolacetic acid

5HT – serotonin
HP- β -CD – hydroxypropyl- β -cyclodextrine
HVA – homovanilic acid
3MT – 3-methoxy-4-hydroxyphenethylamine
NA – noradrenaline
NMDA – N-methyl-D-aspartic acid

INTRODUCTION

Steroids influence neuronal functions by binding to intracellular receptors that can act as transcription factors and regulate gene expression. In addition, some so-called ‘neuroactive steroids’ or neurosteroids (Robel and Baulieu 1994) are potent modulators of several

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ligand-gated ion channel-receptors, such as the GABA_A, glycine, NMDA, nicotinic acetylcholine (nACh) and 5HT₃ (serotonin) receptors, as well as voltage gated Ca²⁺ channels (Majewska et al. 1986, Majewska 1992, French-Mullen et al. 1994, Monnet et al. 1995) and distinct G-protein coupled receptors, acting *via* nongenomic mechanisms (Ueda et al. 2001). By these diverse mechanisms of action neurosteroids can influence many functions of the central nervous system (CNS).

Clinical and preclinical studies revealed that neurosteroids can alter susceptibility to seizures, modulate anxiety, affect sleep and activity phases, and influence learning and memory (for example: Bäckström et al. 1984, Mendelson et al. 1987, Herzog 1995, Kokate et al. 1996, Edgar et al. 1997, Lancel et al. 1997, Czlonkowska et al. 2001, Damianisch et al. 2001, Huppert and van Niekerk 2001, Kaminski et al. 2003, Biagini et al. 2006). Particular attention in research was focused on the weak androgen, dehydroepiandrosterone and its sulfate derivative (DHEAS), which are interconvertible in the body and will be further named collectively DHEA(S). Several studies documented that higher circulating levels of DHEA(S) are associated with better physical and mental health and greater longevity (Haren et al. 2007, Enomoto et al. 2008) while treatment of aging patients with exogenous DHEA produced perception of physical and psychological well-being (Morales et al. 1994). Although DHEAS is the most abundant steroid hormone in the blood of adult humans, it still remains enigmatic. It is a multifunctional steroid, which serves as substrate for synthesis of other androgens and estrogens. It crosses the blood-brain-barrier, but it is also synthesized *de novo* in the CNS (Baulieu and Robel 1998), where it acts as an allosteric antagonist of GABA_A receptors (Majewska et al. 1990) and agonist of the NMDA receptors (Monnet et al. 1995, Chen et al. 2006), exerting a synergistic neuroexcitatory action (Majewska 1995) among others. It is believed that in the CNS and in the periphery, DHEA(S) can be metabolized to androsterone, which acts as a potent allosteric agonist of GABA_A receptors and is neuroinhibitory (Harrison et al. 1987, Majewska 1992). Many effects of androsterone on the central nervous system resemble those of benzodiazepines or barbiturates (Harrison et al. 1987, Majewska 1992).

DHEA(S) is not only important as a hormone and neuromodulator. It is also often used and abused as a popular food supplement to enhance mood and physical performance (Bahrke and Yesalis 2004).

Several animal studies demonstrated involvement of neurosteroids in drug addictions (Budziszewska et al. 1996, Reddy and Kulkarni 1997, Yadid et al. 2010). Previous clinical studies documented an ambiguous involvement of DHEA(S) in psychostimulant addiction (Shoptaw et al. 2004, Wilkins et al. 2005), where high endogenous levels of this steroid in blood correlated with protection of cocaine addicts from relapse (Wilkins et al. 2005), but treatment of addicts with high dose of DHEA seemed to augment the rewarding effect of cocaine (Shoptaw et al. 2004). Because DHEA(S) can be metabolized to androsterone, which has opposite neuronal activity, we were interested in comparing the direct effects of both these steroids on psycho-behaviors intrinsically linked with addiction processes such as locomotor activity, reward and anxiety. In the present study we examined the acute effects of peripherally administered DHEAS and androsterone on locomotion in the open field and assessed their rewarding potential in the conditioned place preference test in male rats. To better understand the neurochemical bases of these steroids' actions on behaviors, we also assessed their influence on turnover of monoamines and steroid metabolism in the hippocampus and striatum.

The behavioral tests are chosen as they are standard methods used to assess anxiogenic and anxiolytic properties as well as rewarding action of different substances. The key neurotransmitter included in both actions is dopamine, therefore to assess the influence of neurosteroids on both monoamine turnover and steroid metabolism the striatum (the structure of the central nervous system with the highest dopamine level) was chosen. The hippocampus was included for its role in memory as it also plays crucial role in the development of reward. It is also important to note that DHEA(S) acts on the dopamine level differently in both structures (Perez-Neri et al. 2008b).

METHODS

Animals

Experimentally naïve male Wistar rats (Institute of Experimental and Clinical Medicine, Polish Academy of Sciences, Warsaw, Poland) weighing: 250–350 g were used. Animals were housed 4 per cage in a room maintained at about 22°C with an alternating 12 h light-dark cycle with lights on at 07:00 AM, food and

water were available *ad libitum*. All experiments were conducted according to the ethical standards laid down in respective Polish and European (directive No. 86/609/EEC) regulations. All procedures were reviewed and approved by the ethics committee on animal studies. The behavioral tests were performed between 02:00 PM and 07:00 PM.

Drug doses and drug administration

DHEAS and androsterone were purchased from the Steraloids Inc (UK). DHEAS was dissolved in water for injections, while androsterone in 20% hydroxypropyl- β -cyclodextrin (Sigma-Aldrich). Both substances were injected intraperitoneally (i.p.) in volume 1 ml/kg of body weight. DHEAS was injected at doses 10.0, 40.0 and 80.0 mg/kg and androsterone at doses 1.0, 10.0 and 40.0 mg/kg.

Open field test

All experimental animals were habituated for a week by the experimenter. The experiment was conducted in black open-field square boxes 100 cm \times 100 cm. Positioned above the open field was a camera, which tracked the rat's movement using the Videomot System (VideoMot2 from TSE video tracking), attached to the computer. Animals were injected with either neurosteroids or their respective vehicles 30 min prior to experiment and left in their cages in the open field experimenting room for habituation. The locomotor activity was automatically measured and recorded by the system for another 30 minutes (Członkowska et al. 1999). There were 8 to 10 animals per group used in this experiment.

Conditioned Place Preference Test

The experiment consisted of 3 phases: pre-conditioning, conditioning and post-conditioning, according to the methods previously described (Steinpreis et al. 1996, Russo et al. 2008). The schematic procedure is shown in Figure 1. Injections of vehicle or drug were done only during the conditioning phase. A three-compartment box (composed of 2 visually distinct conditioning compartments: 50 \times 20 \times 30 cm, separated by a neutral wooden compartment: 10 \times 20 \times 30 cm) was used in experiments. One compartment had black walls with black wooden floor

and the other – black and white striped walls with a black plexiglass floor. During the pre-conditioning phase (3 days) rats were allowed to freely explore all 3 compartments for 15 minutes and time spent in each conditioning compartment was measured. For further experiments only the animals that did not show significant preference between the compartments were used (between 50 and 70% time in the more preferred compartment). The conditioning sessions were carried out on experimental days 4 to 11. The box was divided into compartments by putting down 2 sliding separating walls. The sessions were conducted once daily. Rats were injected with either DHEAS or androsterone, or their respective vehicles 30 minutes before the conditioning session, and then they were placed in one of the two conditioning compartments. The conditioning session lasted for 30 minutes. Animals receiving only vehicles served as controls. The post-conditioning session was carried out on experimental day 12 and was exactly the same as pre-conditioning – the animals were not administered any drug, but were punctured with a needle only. Scores for the drug-paired place were then calculated by subtraction of the pre-conditioning score from post-conditioning score. Scores are presented as the median time, in seconds, spent in the drug-paired, initially slightly less preferred (30–50% time spent) compartment. A positive score represented conditioned place preference, while a negative score represented conditioned place aversion. There were 8 experimental groups, counting each 10–13 animals (8 animals had to be excluded from further experimental procedures due to very significant preference of one compartment).

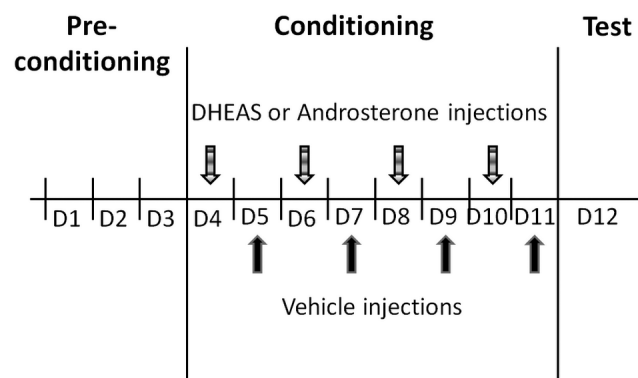


Fig. 1. Conditioned place preference experimental scheme. (D) day.

Determination of monoamines in brain homogenates

For determination of monoamines and their metabolites, each tissue sample (from the hippocampus and striatum – the tissues were obtained using the method described by Kostowski et al. 2004; for further details see also Kolomanska et al. 2011, Rok-Bujko et al. 2012) was weighed, placed in a dry-cooled polypropylene vial, and homogenized in 20 volumes of an ice cold 2% perchloric acid with internal standard added (DHBA-dihydroksybenzylamine) (30 s, 4°C). The tissues were then centrifuged for 8 min at 4°C. After centrifugation, the supernatants were collected from homogenates and filtered through 0.45 µm filter (Millipore). Samples were immediately frozen and kept at –70°C until assay.

Concentrations of monoamines and their metabolites were measured using a modified high pressure liquid chromatography (HPLC) method reported by Kaneda and coauthors (1986). The HPLC system consisted of Shimadzu LC-10AD VP pump, electrochemical detector with flow-through cell (Decade - Antec

Leyden). A high-density glassy carbon-working electrode was operated at +760 mV. The sample was injected manually, using Rheodyne 7725i injection valve, with a 10 µl sample loop. Separation of monoamines and their metabolites was obtained on Phenomenex Luna C18, 150 × 3 mm with a Phenomenex KJO-4286 precolumn. The column temperature was 32°C. The mobile phase consisted of 55.3 mM disodium phosphate (Na_2HPO_4), 107 mM citric acid ($\text{C}_6\text{H}_8\text{O}_7$), 0.027 mM EDTA, 0.517 mM octane sulphonic acid ($\text{C}_8\text{H}_{17}\text{NaSO}_4$) and 12% methanol. It was filtrated through 0.45 µm filters (Millipore). The flow rate was 0.4 ml/min. The mobile phase was degassed with helium. Chromatogram registration and analysis was done using ChromaX2004 software. The concentration of monoamines and their metabolites was calculated as ng/g of brain tissue.

Determination of steroid concentrations in brain homogenates

The homogenates from the striatum and hippocampus samples (10% w/v in ethanol) were prepared from com-

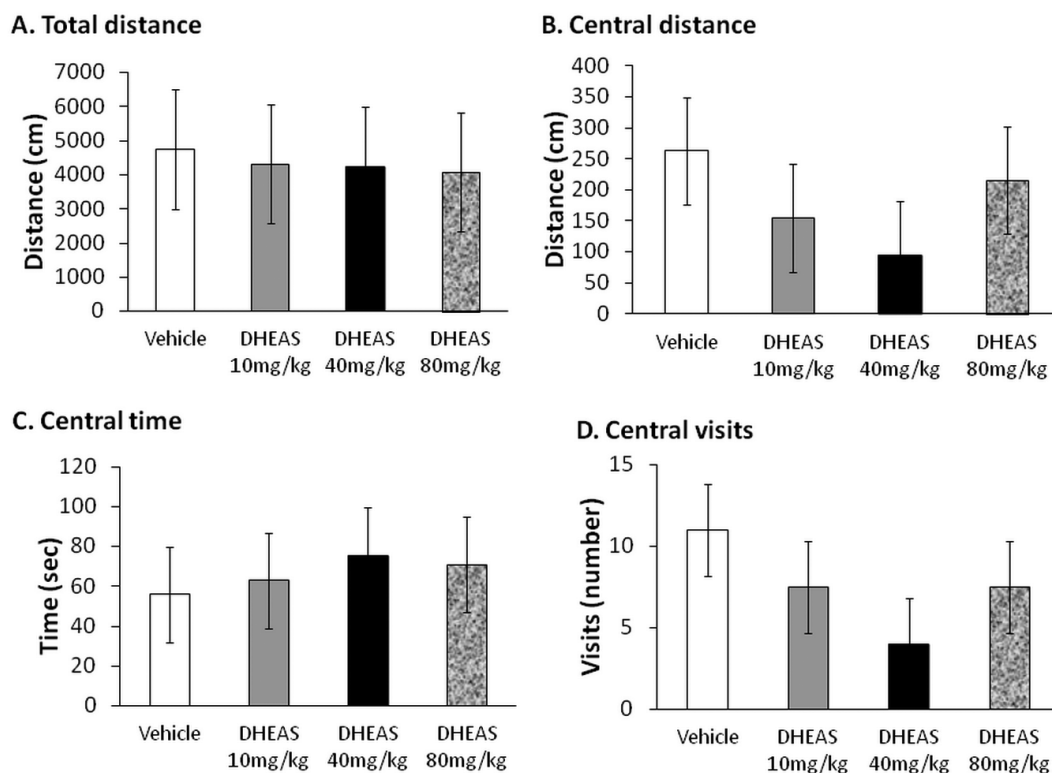


Fig. 2. Dose-response effects of DHEAS on locomotor activity in the open field test. (A) Total distance traveled; (B) Distance traveled in the central field; (C) Time spent in the central compartment; (D) Visits in the central compartment. Data are means ± SEM; $n=9-13$. No significant effect of DHEAS on locomotor activity was observed ($P>0.05$).

Table I
Effect of peripheral DHEAS and androsterone administration on monoamine levels in brain tissue homogenates

| ng/g tissue | Hippocampus | | | | | Striatum | | | |
|-------------|-----------------|-----------------|-----------------|-----------------|-------------------|-------------------|--------------------|--------------------|--|
| | Saline | DHEAS | HP-β-CD | Androsterone | Saline | DHEAS | HP-β-CD | Androsterone | |
| NA | 195.17 ± 65.97 | 220.76 ± 29.27 | 214.66 ± 39.81 | 318.62* ± 59.82 | not detected | not detected | not detected | not detected | |
| DA | 29.08 ± 18.16 | 7.77* ± 4.04 | 14.23 ± 10.41 | 13.84 ± 8.55 | 8076.60 ± 1699.64 | 8355.40 ± 3791.80 | 10495.80 ± 2478.83 | 10523.50 ± 4882.16 | |
| DOPAC | 15.38 ± 5.97 | 14.12 ± 2.43 | 13.91 ± 3.90 | 17.14 ± 4.80 | 3712.67 ± 1248.22 | 4570.60 ± 804.03 | 4005.00 ± 523.04 | 4285.67 ± 1811.18 | |
| DOPAC/DA | 0.74 ± 0.69 | 2.32* ± 1.38 | 1.31 ± 0.89 | 1.81 ± 1.22 | 0.46 ± 0.27 | 0.63 ± 0.25 | 0.40 ± 0.09 | 0.49 ± 0.31 | |
| 3MT | not detected | not detected | not detected | not detected | 339.17 ± 82.34 | 305.60 ± 104.04 | 353.60 ± 105.6825 | 380.67 ± 219.62 | |
| 3MT/DA | not detected | not detected | not detected | not detected | 0.04 ± 0.010 | 0.38 ± 0.01 | 0.034 ± 0.01 | 0.036 ± 0.01 | |
| HVA | not detected | not detected | not detected | not detected | 954.50 ± 257.15 | 1336.17* ± 287.43 | 1247.60 ± 473.06 | 1220.33 ± 689.39 | |
| HVA/DA | not detected | not detected | not detected | not detected | 0.11 ± 0.02 | 0.16* ± 0.09 | 0.12 ± 0.05 | 0.13 ± 0.08 | |
| 5HT | 95.18 ± 50.58 | 54.09 ± 26.15 | 79.75 ± 27.48 | 77.09 ± 18.22 | 152.00 ± 70.83 | 55.60* ± 28.38 | 124.33 ± 33.08 | 109.33 ± 18.62 | |
| 5HIAA | 383.84 ± 106.93 | 219.98* ± 73.52 | 312.35 ± 114.97 | 428.38 ± 109.90 | 166.60 ± 30.33 | 160.40 ± 87.25 | 150.33 ± 86.92 | 155.67 ± 70.33 | |
| 5HIAA/5HT | 4.48 ± 1.21 | 4.69 ± 1.81 | 3.94 ± 0.75 | 5.67* ± 1.18 | 1.24 ± 0.40 | 3.05* ± 1.30 | 1.14 ± 0.42 | 1.41 ± 0.58 | |

DHEAS was injected ip at dose 80 mg/kg. Androsterone was injected ip at dose 40 mg/kg. As vehicle hydroxypropyl-β-cyclodextrin (20%) was used. Asterisks denote a statistically significant difference ($P < 0.05$) compared to control. Data are means ± SEM; $n = 4-5$. (5HT) serotonin; (5HIAA) 5-hydroxyindolacetic acid; (DA) dopamine; (3MT) 3-methoxy-4-hydroxyphenethylamine; (DOPAC) 3,4-dihydroxyphenylacetic acid; (HVA) homovanillic acid; (NA) noradrenaline; (HP-β-CD) hydroxypropyl-β-cyclodextrin; (DHEAS) dehydroepiandrosterone sulphate

bined tissues, extracted from both sides of the brain, separately from each experimental animal. The homogenates were kept frozen at temperature -70°C until analyzed.

Steroid concentrations were measured in the brain homogenates by the combined GC-MS method, using the method and equipment described in detail elsewhere (Hill et al. 2010). The following steroids were analyzed: pregnenolone, dehydroepiandrosterone (DHEA), androstendiol, androstentriol, allopregnanolone, isopregnanolone, 5α -dihydrotestosterone, androsterone, epiandrosterone, and androstandiol. The steroid concentrations in tissues were expressed as nmoles/g.

Statistical analysis

Conditioning scores were expressed as means \pm SEM. One way analysis of variance (ANOVA) followed by NIR and Rir-Tukey *post hoc* test have been used. $P < 0.05$ was considered a significant difference.

RESULTS

Open field activity

The open field test measures animals' spontaneous locomotor and exploratory activity, as well as provides information about their state of anxiety in novel environment (Crawley 1985). Administration of DHEAS at doses of 10, 40 or 80 mg/kg did not significantly alter general locomotor activity (measured as total distance traveled) in this test. Although at doses 10 and 40 mg/kg DHEAS slightly reduced activity in the central field, this effect did not reach statistical significance (for 40 mg/kg $P = 0.07$; $F = 2.547$ for the central distance traveled). These data suggest that, in this experimental system DHEAS manifests neither clear anxiolytic nor anxiogenic prosperities (Fig. 2).

In contrast, androsterone had a statistically significant biphasic effect on locomotion. At a dose of 10 mg/kg, it increased general ambulatory activity and at

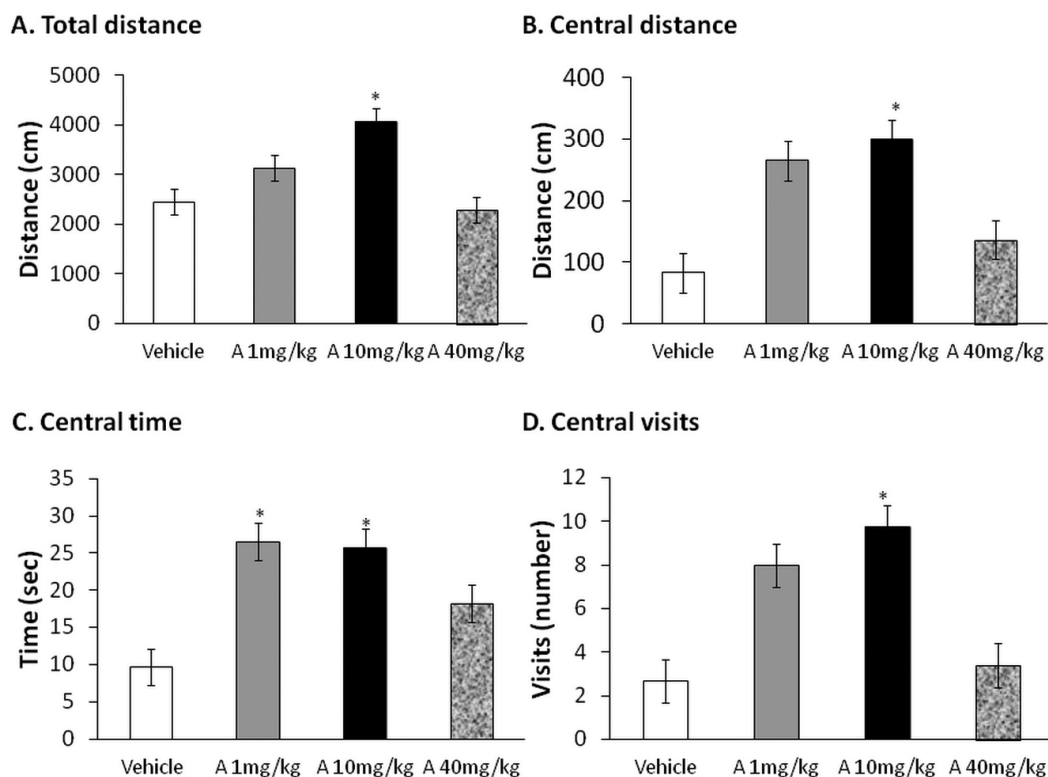


Fig. 3. Dose- response effects of androsterone on locomotor activity in the open field test (A) Total distance traveled; (B) Distance traveled in the central field; (C) Time spent in the central compartment; (D) Visits in the central compartment. Data are means \pm SEM; $n = 7-8$. Statistically significant effects on total locomotor activity and the pronounced anxiolytic effects were observed for androsterone dose of 10 mg/kg. Asterisks denote a statistically significant difference ($P < 0.05$) compared to control.

doses 1 and 10 mg/kg, it also augmented activity in the central area ($P=0.02$; $F=4.810$; Fig. 3). However, this effect was lost at 40 mg/kg dose of androsterone. Dose-dependent increased activity in the central field suggests an anxiolytic property of this steroid. Cyclodextrin, used as a vehicle in experiments testing the effects of androsterone (but not DHEAS), by itself also affected locomotor activity, as values of total distance traveled and of central field activities were all lower in rats, which received cyclodextrin alone, when compared to animals which received water as a vehicle ($P=0.005$; $F=10.407$). These data indicate that cyclodextrin alone has an intrinsic weak sedative effect, consistent with its previously reported positive modulatory effect on the GABA_A receptors (Pytel et al. 2006) (Figs 2 and 3).

Conditioned Place Preference

The potential reinforcing/hedonic or aversive properties of DHEAS and androsterone were examined in the conditioned place preference test (CPP). DHEAS had a biphasic dose-dependent effect on reward. It increased preference for the site linked to its injection at doses of 10 and 40 mg/kg, yet only in the higher dose was the effect statistically significant ($P<0.05$; $F=3.956$). At a dose of 80 mg/kg the reinforcing effect of DHEAS disappeared (Fig. 4 A).

Androsterone had a similar biphasic effect in the CPP test. It increased preference for the place associated with its administration at doses of 1 and 10 mg/kg, although the effect was statistically significant only for the higher dose ($P<0.05$; $F=7.215$). However, at an androsterone dose of 40 mg/kg, significant place aversion was observed ($P<0.05$; $F=7.215$); (Fig. 4B). Thus, for both steroids, the dose response curves for reward have an inverted U shape, which suggests either that these steroids actions may be controlled by two opposing mechanisms or that they may have mixed agonistic-antagonistic properties.

Monoamine levels and turnover

In order to evaluate the potential role of biogenic amines in the behavioral effects of DHEAS and androsterone, monoamine levels were measured by HPLC in homogenates from the hippocampus and striatum of animals sacrificed 30 min after steroid injection. The monoamine turnover rates (DOPAC/DA; 3MT/DA;

HVA/DA and 5HIAA/5HT) were calculated for each animal separately and the mean rates were calculated from these individual values. Administration of DHEAS and androsterone caused rapid, but distinct changes in brain monoamine levels. DHEAS altered metabolism of dopamine (DA) in both brain structures, although in different patterns (Table I). In the hippocampi of DHEAS-treated rats, DA concentrations markedly decreased ($P=0.03$; $F=6.500$), resulting in an increased DOPAC/DA ratio ($P=0.035$; $F=6.151$). In the striatum, the DOPAC/DA ratio also increased, but this effect was not statistically significant. However, a statistically significant increase of HVA concentrations was measured in this structure ($P=0.035$; $F=5.876$), resulting in a higher HVA/DA ratio ($P=0.048$; $F=5.734$) than in controls. In both the hippocampus and striatum, DHEAS treatment also markedly decreased serotonin (5HT) levels, although the effect was statistically significant only in the striatum (for 5HT levels $P=0.019$; $F=8.058$; for 5HIAA/5HT $P=0.018$; $F=8.741$).

Androsterone treatment had no significant effect on DA levels or its turnover in the hippocampus or striatum. However, in the hippocampus it significantly increased the levels of noradrenaline (NE) ($P=0.019$; $F=8.552$), as well as 5HT turnover (for 5HIAA/5HT, $P=0.025$; $F=7.570$; Table I). No significant effect of

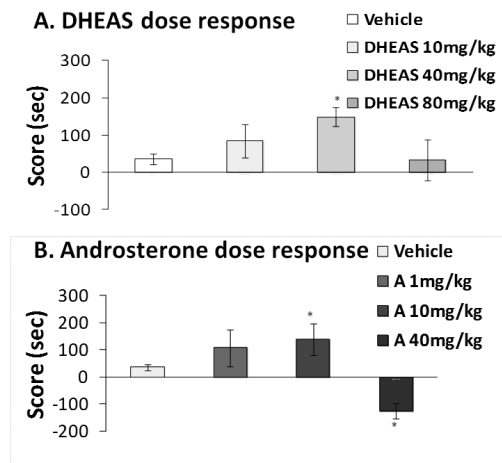


Fig. 4. Dose-response effect of DHEAS and androsterone on behavior in the conditioned place preference test. Both steroids show rewarding properties at doses: 40 mg/kg for DHEAS and 10 mg/kg for androsterone. The effect is lost in higher doses and in case of androsterone the effect of 40 mg/kg is clearly aversive. (A) DHEAS effects; (B) Androsterone effects. Asterisks denote a statistically significant difference ($P<0.05$) compared to control. Data are means \pm SEM; $n=10-13$

Table II

| Effect of peripheral administration of DHEAS and androsterone on the levels of different neurosteroids in brain tissue homogenates | | | | | | | | | | |
|--|---------------|-------------|---------------|-----------------|---------------|-------------|----------------|------------------|--|--|
| nmol/g tissue | Hippocampus | | | | | Striatum | | | | |
| | Saline | HP-β-CD | DHEAS | Androsterone | Saline | HP-β-CD | DHEAS | Androsterone | | |
| Pregnenolone | 1.31 ± 0.28 | 1.93 ± 1.78 | 5.94* ± 3.86 | 1.10 ± 0.34 | 1.32 ± 0.25 | 1.06 ± 0.81 | 7.67* ± 4.90 | 1.32 ± 0.58 | | |
| DHEA | 0.23 ± 0.17 | 0.16 ± 0.07 | 19.83* ± 0.89 | 1.46 ± 0.94 | 0.18 ± 0.13 | 0.14 ± 0.09 | 26.35* ± 12.13 | 1.75 ± 0.89 | | |
| Androstendiol | 0.98 ± 0.70 | 0.71 ± 0.46 | 2.69* ± 0.26 | 0.86 ± 0.49 | 1.10 ± 0.70 | 0.61 ± 0.60 | 3.26* ± 1.38 | 1.01 ± 0.42 | | |
| Androstentriol | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.31* ± 0.01 | 0.07 ± 0.13 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.31* ± 0.25 | 0.12 ± 0.19 | | |
| Allopregnanolone | 0.23 ± 0.21 | 0.17 ± 0.13 | 0.34 ± 0.21 | 0.29 ± 0.26 | 0.26 ± 0.22 | 0.16 ± 0.15 | 0.46 ± 0.20 | 0.40 ± 0.26 | | |
| Isopregnenolone | 0.42 ± 0.30 | 0.31 ± 0.23 | 0.58 ± 0.53 | 0.35 ± 0.26 | 0.46 ± 0.35 | 0.29 ± 0.20 | 0.71 ± 0.40 | 0.48 ± 0.29 | | |
| 5α-dihydrotestosterone | 0.47 ± 0.11 | 0.29 ± 0.14 | 0.46 ± 0.14 | 4.05* ± 2.10 | 0.65 ± 0.21 | 0.35 ± 0.18 | 0.63 ± 0.24 | 6.62* ± 2.29 | | |
| Androsterone | 27.76 ± 37.38 | 1.84 ± 2.48 | 0.62 ± 0.13 | 113.22* ± 43.76 | 10.35 ± 14.52 | 0.53 ± 0.46 | 11.84 ± 20.57 | 240.82* ± 180.81 | | |
| Epandrosterone | 0.16 ± 0.21 | 0.03 ± 0.03 | 0.58 ± 0.12 | 12.71* ± 3.74 | 0.04 ± 0.04 | 0.04 ± 0.03 | 0.86 ± 0.44 | 20.79* ± 5.62 | | |
| Androstandiol | 0.52 ± 0.34 | 0.32 ± 0.10 | 0.23 ± 0.11 | 1.62* ± 0.62 | 0.46 ± 0.27 | 0.28 ± 0.09 | 0.29 ± 0.14 | 2.67* ± 0.85 | | |

DHEAS at dose 80 mg/kg and androsterone at dose 40 mg/kg were injected ip. As vehicle for androsterone hydroxypropyl-β-cyclodextrin 20% was used. Asterisks denote a statistically significant difference ($P<0.05$) compared to control. Data are means ± SEM; $n=5-6$. (HP-β-CD) (hydroxypropyl-β-cyclodextrin; (DHEAS) dehydroepiandrosterone sulphate; (DHEA) dehydroepiandrosterone.

androsterone on monoamine levels and turnover was detected in the striatum.

Brain steroid levels

To assess changes in brain steroid levels of rats treated with DHEAS or androsterone, the concentrations of different steroids were measured in homogenates from the hippocampus and striatum of these animals. DHEAS (80 mg/kg) and androsterone (40 mg/kg) were injected i.p. 30 min before sacrificing animals for tissue harvesting. Steroids were analyzed by the GC-MS method. As anticipated, DHEAS markedly augmented levels of DHEA in the hippocampus ($P<0.001$; $F=47.687$) and the striatum ($P<0.001$; $F=83.848$; Table II). It also statistically significantly increased concentrations of pregnenolone in these tissues ($P<0.001$; $F=10.688$ in the hippocampus; $P<0.001$; $F=7.556$ in the striatum), androstendiol ($P<0.001$; $F=10.688$ in the hippocampus; $P<0.001$; $F=10.769$ in the striatum), and androstentriol ($P<0.001$; $F=73.819$ in the hippocampus; $P<0.001$; $F=34.265$ in the striatum).

Peripheral administration of androsterone increased the level of this steroid in the brain ($P=0.001$; $F=10.983$ for the hippocampus; $P<0.001$; $F=40.474$ for the striatum; Table II), as well as epiandrosterone ($P<0.001$; $F=90.209$ for the hippocampus; $P<0.001$; $F=58.637$ for the striatum), androstandiol ($P<0.001$; $F=15.596$ for the hippocampus; $P<0.001$; $F=30.071$ for the striatum), and 5α -dihydrotestosterone ($P<0.001$; $F=10.767$ for the hippocampus; $P<0.001$; $F=39.450$ for the striatum; Table II). Scheme on Figure 5 illustrates statistically significant changes in brain levels of different steroids in rats, which received ip injections of DHEAS or androsterone, on the background of steroid metabolic pathways.

DISCUSSION

This study examined the behavioral effects of two metabolically linked, but pharmacologically distinct neurosteroids, DHEAS and androsterone, correlating them with changes in metabolism of biogenic amines and steroid metabolism in the brain. The dose ranges of both steroids administered to experimental rats were chosen to cover the physiological and pharmacological spectra. The results show that psychobehavioral and neurochemical effects of both these steroids differ in some aspects, but are similar in others.

In the open field test, acute administration of DHEAS at doses 10 to 80 mg/kg to experimentally naïve rats did not produce consistent statistically significant changes in general ambulation and central field exploration (measuring level of anxiety), although a tendency for an anxiogenic effect was noted in animals treated with DHEAS doses 10 and 40 mg/kg. In contrast, acutely administered androsterone had a robust dose-dependent, biphasic effect on rat ambulation. It significantly stimulated locomotion at a dose of 10 mg/kg, but this effect was lost at the higher dose (40 mg/kg). At doses 1 and 10 mg/kg androsterone also markedly augmented exploration in the central field, suggesting anxiolytic action, which disappeared at the highest dose (40 mg/kg), probably indicating the emergence of sedative effects.

Both steroids also had a biphasic dose-dependent effect on reward, measured in the place preference test. They manifested positive hedonic properties at lower doses (10 and 40 mg/kg for DHEAS and 1 and 10 mg/kg for androsterone), but this effect disappeared for the highest DHEAS dose (80 mg/kg), and became aversive for androsterone dose of 40 mg/kg. These behavioral experiments show that both steroids have rewarding potential and androsterone also manifests anxiolytic properties.

The neurochemical mechanisms underlying the above described behavioral effects of both neurosteroids appear to be complex. Anxiolytic features of androsterone are most likely mediated via activation of the GABA_A receptors, as this steroid, like other $3\alpha,5\alpha$ -reduced steroids (e.g. allopregnanolone), is a potent allosteric agonist of GABA_A receptors (Majewska et al. 1986, Harrison et al. 1987). Indeed, the anxiolytic activity of androsterone has been previously described (Majewska 1992, Frye et al. 2008). The biphasic influence of androsterone on locomotion – stimulant at low doses and inhibitory at higher – also resembles the action of allosteric agonists of GABA_A receptors, benzodiazepines. The locomotor stimulant activity of low doses of GABA_A agonists has been linked to indirect activation of the dopaminergic system controlling motor activity (Widgiz and Beck 1990, Söderpalm et al. 1991). Our analysis of biogenic amines did not reveal a significant effect of high dose of androsterone on levels of DA or its metabolites in the striatum or hippocampus, but showed augmented levels of NA and stimulated turnover of 5HT in the hippocampus. These actions may contribute to ambulatory arousal and

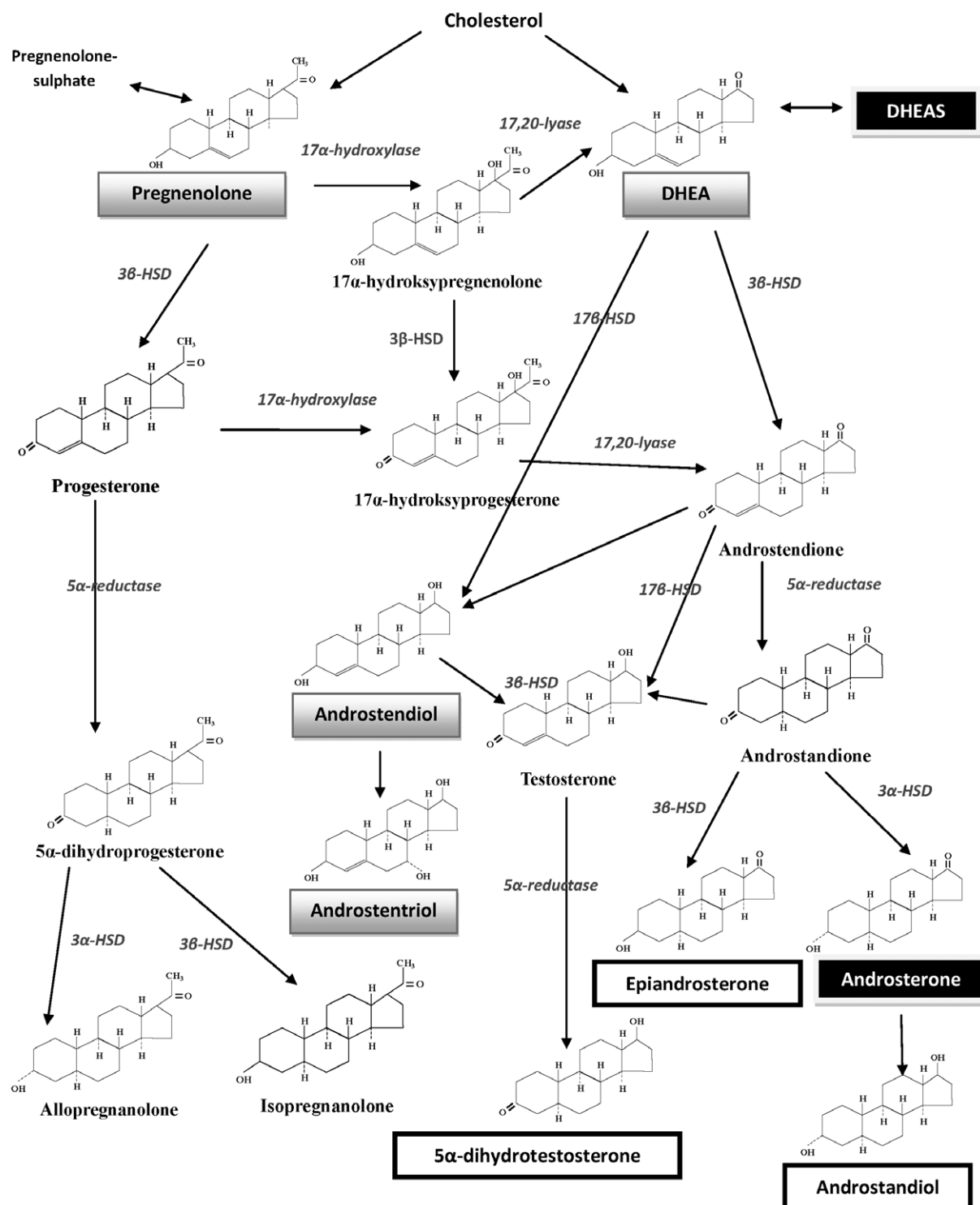


Fig.5. Scheme of neurosteroid synthesis (prepared with help of prof. Bogdan Szukalski). The black frames show neurosteroids injected i.p. (DHEAS – 80 mg/kg and androsterone – 40 mg/kg). Gray filled frames show neurosteroids, the concentrations of which increased after DHEAS injection. Black bold frames show neurosteroids, the concentrations of which increased after androsterone injection.

increased environmental reconnaissance (Flicker and Geyer 1982, Takahashi et al. 2000). Collectively, these data imply that androsterone effect of on anxiety and locomotor activity is, to a significant degree, mediated by its direct interaction with brain GABA_A receptors and an influence on NE and 5HT neurotransmission.

To the best of our knowledge, ours is the first report demonstrating rewarding effects of androsterone and DHEAS in the CPP test, although other androgens (testosterone, 3 α -androstendiol and androstandiol) were previously shown to possess reinforcing features (Packard et al. 1998, Rosellini et al. 2001, Wood 2004, Jorge et al. 2005), whereas estrogens manifested aversive effects in males (de Beun et al. 1991). The hedonic qualities of low to medium doses of androsterone seem analogous to those of benzodiazepines (Spiraki et al. 1985) and could be, to a large degree, mediated by its GABA_A-agonistic activity. Androsterone interaction with the mesolimbic DA system, comparable to that of testosterone (Packard et al. 1998, Wood 2004), does not seem very likely in view of the results of our biochemical tests, although some effect of low doses of androsterone doses cannot be excluded. Nonetheless, because androgens have been shown to augment synthesis of endogenous opioid peptides (Johansson et al. 1997, Stomati et al. 1999), it is possible that rewarding properties of androsterone and DHEAS, are in part also due to their actions on brain opioid systems.

The conditioned place aversion induced by a high dose of androsterone (40 mg/kg) cannot be explained by its GABA-agonistic properties, but could be mediated *via* biogenic amine systems. While administration of this steroid did not alter levels or turnover of monoamines in the striatum, it augmented by nearly 50% the concentration of NA and 5HT turnover in the hippocampus. Microinjection of NA to this brain structure has been previously shown to boost aversion to negative stimuli, while 5HT decreased reactivity to such stimuli (Gage and Springer 1981, Plaznik et al. 1983). Augmented NA neurotransmission in the brain seems also critical for aversion induced by opiate withdrawal (Delfs et al. 2000). These findings suggest that increased activity of NA in the brain may be in part responsible for the aversive action of high doses of androsterone. Such an effect could be analogous to that of amitriptyline (a NA, 5HT-mimetic) (Subhan et al. 2000).

Another mechanism by which androsterone affects reward or aversion may involve pathways activated by

androgen receptors. Our results show that peripheral administration of this steroid altered levels of several androgens in the brain: in addition to augmenting its own concentrations, androsterone increased levels of its isomer epiandrosterone, androstandiol, and 5 α -dihydrotestosterone (an active metabolite of testosterone). While some studies showed rewarding properties of testosterone and androstandiol in the CPP test (Frye et al. 2001, Parrilla-Carrero et al. 2009), the study of King and coworkers (1999) reported a biphasic effect of testosterone – hedonic at low and aversive at high doses – analogous to our findings with androsterone. In fact, dose-dependent biphasic effects on reward are typical for many drugs of abuse. Jointly, these data imply a multifaceted mechanism of androsterone action on the brain reward system, involving interplay between different steroid metabolites and their receptors and interactions with several neurotransmitter systems.

DHEAS has a distinct pharmacological profile from androsterone. It is a negative modulator of the GABA_A receptors and positive modulator of the NMDA and sigma receptors. In our behavioral tests, DHEAS did not alter spontaneous ambulatory activity in the open field test. This effect differs from that reported by Nguyen and others (1999) of reduced locomotion after injection of DHEA in Zucker rats. DHEAS also did not manifest clear effect on anxiety, although a trend for reduced activity in the central field was noted for doses 10 and 40 mg/kg, suggesting mild anxiogenic action. Though some investigators described its anxiogenic qualities (Frye and Lacey 1999, Jacobs et al. 1999), others reported its anxiolytic action and simultaneously capacity to reduce the anxiolytic effect of ethanol (Melchior and Ritzman 1994). Despite ample biochemical and electrophysiological evidence of antagonistic activity of DHEAS on GABA_A receptors, the prevailing view in the literature is that in most behavioral tests it does not show clear anxiogenic effects. The ambiguous psycho-behavioral actions of DHEAS may result from its mixed pharmacological profile and metabolism to other neurosteroids, which may work in opposite direction.

Our findings showing rewarding properties of DHEAS in the place preference test appear contrary to those of Romieu and colleagues (2003), who did not observe such effects for DHEA alone.

However, it is important to underline that the pharmacological profile of DHEA is not identical with that of DHEAS (although they are interconvertible in the body) and that the steroid doses used by these investigators were much lower than those used in our experiments. The hedonic property of DHEAS is nonetheless analogous to that of other androgens (King et al. 1999, Romieu et al. 2003). It could be mediated by several neurotransmitter systems. On the one hand, it could be due to its mild antagonistic action on GABA_A receptors, as the rewarding effect was shown for low dose of pentylenetetrazole – a classical antagonist of these receptors (Gauvin et al. 1991). On the other hand, DHEAS rewarding actions may be due to its influence on brain monoamines. Acute administration of this hormone did not produce significant change in DA concentration in the striatum, but increased levels of metabolites, suggesting augmented synaptic DA activity. In the hippocampus of DHEAS injected rats, DA levels were reduced without changes in its metabolites. DHEAS administration also diminished concentrations of 5HT in the striatum and hippocampus without increasing levels of its metabolite (5HIAA), suggesting reduced synthesis of 5HT. Our data differ from those of Perez-Neri and coauthors (2008a) reporting reduced turnover of DA, but increased turnover of 5HT in the striatum of DHEA-treated rats. These differences, which may be due to pharmacodynamic distinctions between DHEA and DHEAS, point to intricacies of neurosteroid actions on neurotransmission. Overall, our data suggest that relatively rapid effect of peripherally administered DHEAS on central monoamines, particularly stimulation of striatal DA activity, might contribute to the rewarding qualities of this steroid.

Acute peripheral administration of DHEAS also altered the levels of several neuroactive steroids in the hippocampus and striatum in a manner distinct from that of androsterone. In addition to the expected large increase of DHEA concentration, augmented levels of androstendiol, androstentriol, and pregnenolone were observed, which may independently contribute to the rewarding actions of DHEAS. Their impact on behavior in conditioned place preference may be enhanced by effects on learning and memory (Monnet et al. 1995, Majewska 1995, Chen et al. 2006), which play a critical role in conditioning (Ma et al. 2011).

CONCLUSION

The present study documents the dose-dependent rewarding properties of systemically administered DHEAS and androsterone at certain dose ranges. Only androsterone stimulated exploration and showed anxiolytic features. The behavioral effects of both steroids seem to be mediated *via* their interactions with GABA_A receptors and biogenic amines. Both steroids undergo rapid metabolism in the brain to other steroids, which may add to their overall influence on behavior and affect. As it is known that rewarding and aversive properties of substances play a crucial role in drug addictions, our data suggest biologically plausible mechanisms for how endogenous and exogenous androgenic steroids may affect addiction processes.

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