European Journal of Neuroscience, Vol. 13, pp. 291–296, 2001

The anabolic-androgenic steroid nandrolone decanoate affects the density of dopamine receptors in the male rat brain

Anna M. S. Kindlundh, Jonas Lindblom,[1] Lena Bergström,[1] Jarl E. S. Wikberg[1] and Fred Nyberg
Department of Pharmaceutical Biosciences, Division of Biological Research on Drug Dependence, Uppsala University, Box 591 Biomedicum, S-751 24 Uppsala, Sweden
[1]Division of Pharmacology, Uppsala University, Uppsala, Sweden

Keywords: autoradiography, dopamine D_1-like receptors, dopamine D_2-like receptors, mesocorticolimibic pathway, nigrostriatal pathway, reward

Abstract

In recent years a male group of anabolic-androgenic steroid misusers has been identified to share socio-demographic and personality related background factors with misusers of psychotropic substances, as well as being involved in habits of multiple drug use. The present study aimed to assess whether anabolic-androgenic steroids (AAS) would affect the density of the dopamine receptors in areas implicated in reward and behaviour in the male rat brain. The effects of 2 weeks of treatment with i.m. injections of nandrolone decanoate (15 mg/kg/day) on the expression of the D_1-like and D_2-like receptors were evaluated by autoradiography. Specific binding of D_1-like receptors was significantly down regulated in the caudate putamen, the nucleus accumbens core and shell. D_2-like receptor densities were down regulated in the nucleus accumbens shell, but up regulated in the caudate putamen, the nucleus accumbens core and the ventral tegmental area. These results are compatible with nandrolone induced neuroadaptive alterations in dopamine circuits associated with motor functions and behavioural paradigms known to be affected following AAS misuse.

Introduction

During the second part of the 20th century, the misuse and abuse of the anabolic-androgenic steroids (AAS), testosterone and its synthetic derivatives, have extended beyond the sphere of the clinics, elite athletes and body builders to also include adolescent males. Adolescents use these agents in order to improve appearance and enhance sports performance, and also for motives such as intoxication, increased bravado and for fun (Yesalis *et al.*, 1993; Nilsson, 1995; Tanner *et al.*, 1995; Yesalis & Bahrke, 1995; Kindlundh *et al.*, 1998; Kindlundh *et al.*, 1999). Male AAS misusers stack their steroids in cycles of 6–12 weeks (Williamson & Young, 1992), are involved in multiple drug use and share socio-demographic and personality factors with the misusers of psychotropic substances, i.e. cannabis, opiates, amphetamine and ecstasy (DuRant *et al.*, 1994, 1995; Yesalis & Bahrke, 1995; Lukas, 1996; Kindlundh *et al.*, 1999, 2000). The AAS misuse has also to some extent been proposed to serve as a gateway for the misuse of other substances (Arvary & Pope, 2000).

The various habits of AAS in misuse and abuse may contribute to the wide spectrum of AAS-induced effects reported on physique and mental health (Kennedy, 1992; Pope & Katz, 1994). Among the psychiatric aspects, pleasurable feelings such as euphoria, enhanced energy and self-esteem, as well as negative effects like irritability, hostility, psychosis, confusion and aggression, have been recorded (Brower *et al.*, 1991; Williamson & Young, 1992; Su *et al.*, 1993; Bahrke *et al.*, 1996; Lukas, 1996). Recent reports indicate that AAS evoke neurobiochemical alterations related to reward and behaviour in rats (Johansson *et al.*, 1997, 2000a, 2000b; Le Grevès *et al.*, 1997; Thiblin *et al.*, 1997, 1999; Hallberg *et al.* 2000; Schlussman *et al.* 2000) and that they may also affect the development of aggression and violent behaviour (Galligani *et al.*, 1996; Thiblin *et al.*, 1997).

The dopaminergic systems play important roles in order to elicit hedonic qualities and cause behavioural changes. The mesocorticolimbic dopamine system seems to play a key role in mediating rewards such as the reinforcing actions of drugs of abuse (Koob, 1992, 1999; Di Chiara, 1998, 1999; Spanagel & Weiss, 1999). Both natural and drug rewards stimulate dopamine transmission in the nucleus accumbens shell (Pontieri *et al.*, 1995; Di Chiara, 1999). The nigrostriatal dopamine pathway seems primarily to be implicated in locomotion (Missale *et al.*, 1998).

The physiological and pharmacological actions of dopamine involve various kinds of neurons and are mediated by the D_1-like receptor subfamily [$D_{1\ and}$ $D_{1b\ (rat)}$/$D_{5\ (human)}$] and D_2-like receptor subtypes (D_2, D_3 and D_4), with opposite biochemical activities and differing distributions (Missale *et al.*, 1998).

The purpose of the present study was to investigate the actions of chronic treatment, with the AAS nandrolone decanoate, upon D_1- and D_2-like receptors in areas of the male rat CNS regulating reward and behaviour.

Correspondence: Anna M.S. Kindlundh, as above.
E-mail: Anna.Kindlundh@farmbio.uu.se

Received 13 July 2000, revised 31 October 2000, accepted 10 November 2000

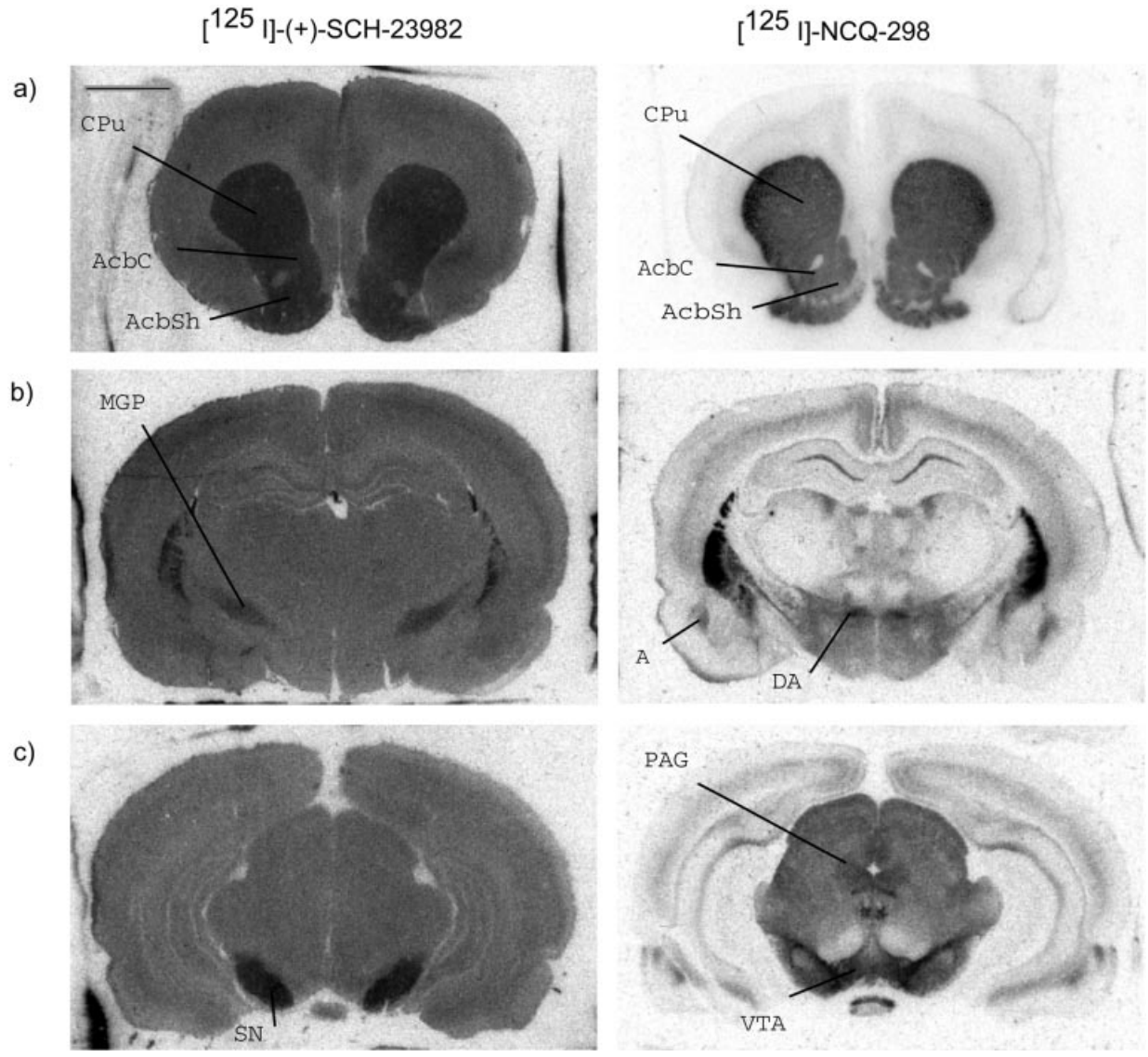


FIG. 1. Representative autoradiograms showing total binding of the D_1-like receptor ligand [^{125}I]-(+)-SCH-23982 and the D_2-like receptor ligand [^{125}I]-NCQ-298 at bregma + 1.6 (a), bregma – 2.8 (b); and between bregma – 5.2 and bregma – 5.6 (c). The brain regions studied were the Cpu, AcbC, AcbSh, MGP, DA, PAG, SN and VTA. Scale bar in the autoradiogram of the D_1-like receptor ligand in (a), 3 mm.

Materials and methods

Animals and tissue preparation

Male Sprague–Dawley rats were purchased from Alab, Sollentuna, Sweden. The animals were housed in groups of four in air-conditioned rooms under an artificial light–dark cycle (lights from 06.00 to 18.00 h) at a temperature of 22–23 °C and a humidity of 55%. The animals had free access to water and food (R36 food pellets, Labfor, Lactimin, Vadstena, Sweden). The rats were randomised into two groups after having been housed for 1 week in order to adapt to the laboratory environment. One group (n = 8) was given intramuscular (i.m.) injections of the AAS nandrolone decanoate (Deca-Durabol®, Organon, Oss, Netherlands) at a daily dose of 15 mg/kg. The other group (n = 8) served as control and was administered daily i.m. injections of the vehicle, sterile arachidis oleum (Apoteket AB, Umeå, Sweden).

The supra-therapeutic dose of AAS used was chosen to mimic the self-administered heavy abuse of AAS (Williamson & Young, 1992). The dose of 15 mg/kg/day has been shown to induce neurobiological changes in previous studies (i.e. Johansson *et al.*, 1997). Body weights were monitored on three different occasions, before the first injection (day 1), after 1 week (day 8) and after 2 weeks (day 15). The rats weighed 340–400 g at the beginning of the treatment.

Experimental procedures for this study were approved by the local ethical committee in Uppsala, Sweden.

The animals were killed by decapitation on day 15. The brains were quickly removed and frozen in 2-methyl butane at –20 °C to –30 °C and stored at –70 °C until used. The frozen brains were sectioned on a cryostat at –19 °C and thaw-mounted on gelatin-coated slides. Coronal sections (12-μm) were collected at three levels; bregma +1.6, –2.8 and –5.3 mm. Sections were dried with a fan for 60 min and stored at –70 °C.

Radioligands

The radioligands [^{125}I]-(+)-SCH 23982 (D_1-like receptor antagonist) and [^{125}I]-NCQ-298 (D_2-like receptor antagonist) were obtained from

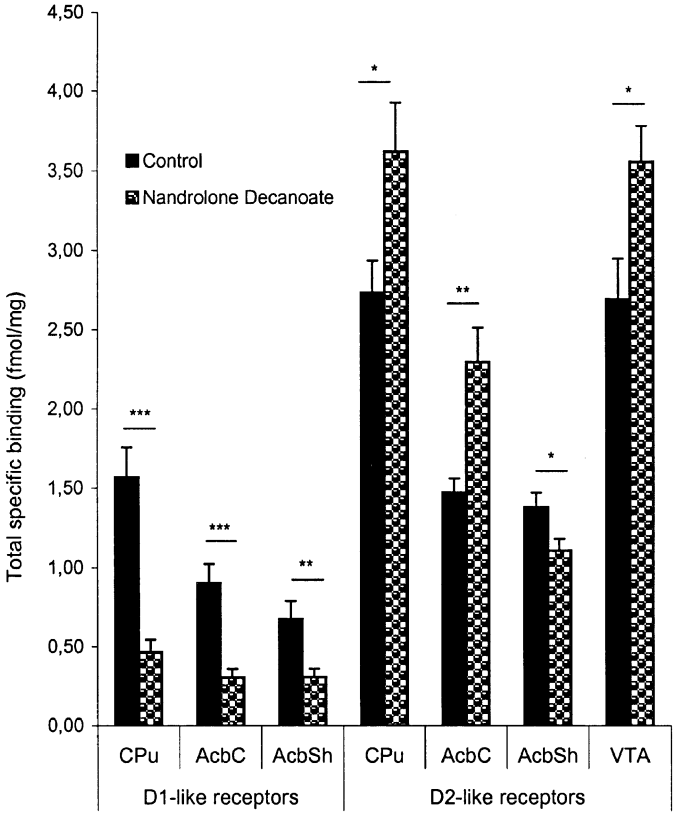


FIG. 2. Total specific binding of the D_1-like receptor ligand [^{125}I]-(+)-SCH-23982 and the D_2-like receptor ligand [^{125}I]-NCQ-298 in the Cpu, AcbC, AcbSh and VTA of male Sprague–Dawley rats after 2 weeks of treatment with the AAS nandrolone decanoate at an i.m. dose of 15 mg/kg/day. The columns and error bars represent mean + SEM in fmol/mg (wet weight). Significance levels are denoted by $***P < 0.001$, $**P < 0.01$ and $*P < 0.05$.

NEN Life Science Products (Boston, USA). Both radioligands had a specific activity of 2200 Ci/mmol.

Autoradiography

The slide-mounted sections were brought to room temperature with a fan for 30 min and encircled with a pap-pen for the autoradiographic study. The sections were preincubated with Tris-HCl (50 mM, pH 7.4) for 30 min, and then incubated in binding buffer (50 mM Tris, 100 mM NaCl, pH 7.4) containing radioligand in the presence (nonspecific binding) or absence (total binding) of 10 μM cis(z)-flupenthixol (Sigma–Aldrich, Saint Louis, USA) for 120 min, washed 2 × 5 min in cold binding buffer, dipped in deionized water and dried with a fan. 100 pM [^{125}I]-(+)-SCH-23982 was used to selectively label D_1-like receptors and 100 pM [^{125}I]-NCQ-298 was used for labelling of D_2-like receptors. Quadruplicates were made for total binding and duplicates for nonspecific binding. Labelled sections and plastic standards (Autoradiographic [^{125}I] Micro-scales, Amersham, Stockholm; 2.2–160 nCi/mg) were placed in X-ray cassettes and exposed to autoradiographic film (Amersham Hyperfilm) at –20 °C.

Development and analysis

After 4 days of exposure, the films were developed manually (Kodak D19, Unifix). The autoradiograms were digitized using a dia-scanner (DuoScan T1200, Agfa), and the optical densities were converted to fmol/mg wet weight based on the coexposed standards using NIH Image software (NIH Image 1.62, NIMH, Bethesda, MD).

The brain regions were identified and selected for measurement according to a rat brain atlas (Paxinos & Watson, 1997). Brain areas were investigated in the following: In Fig. 1a, the caudate putamen (CPu), the nucleus accumbens core (AcbC) and the nucleus accumbens shell (AcbSh); in Fig. 1b, the amygdala (A), the dorsal hypothalamic area (DA) and the medial globus pallidus (MGP) and in Fig. 1c, the periaqueductal grey (PAG), the substantia nigra (SN) (comprising both substantia nigra reticulata (SNR) and substantia nigra compact part dorsal tier (SNCD)), and the ventral tegmental area (VTA). Statistical analyses were performed with the two-tailed Student's *t*-test.

Results

Representative autoradiograms of studied brain regions illustrating total binding of 100 pM [^{125}I]-(+)-SCH-23982 and [^{125}I]-NCQ-298, respectively, are shown in Fig. 1. Total specific binding for the D_1-like receptor [^{125}I]-(+)-SCH-23982 ligand (87–97%) was observed in the Cpu, AcbC, AcbSh, MGP and SN and for the D_2-like receptor [^{125}I]-NCQ-298 ligand (almost 100%) in the CPu, AcbC, AcbSh, A, DA, PAG and the VTA.

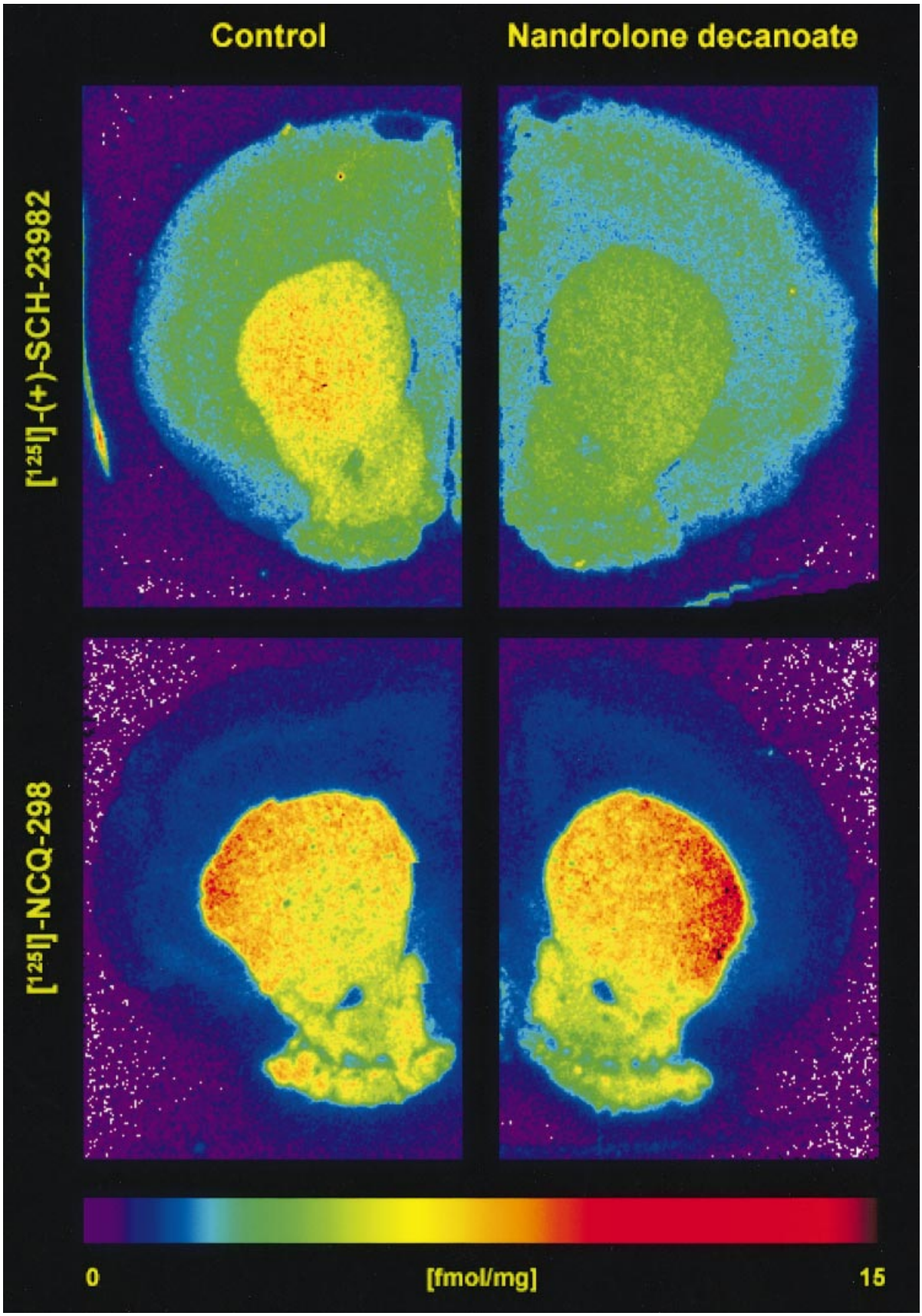
Autoradiographic data of the total specific binding, the B_{max} estimates, of the D_1-like and the D_2-like receptors of the brain regions, which were significantly altered after 2 weeks of treatment with supra-therapeutic doses of nandrolone decanoate, are presented in Fig. 2. The D_1-like receptor density was reduced by 70% in the Cpu ($P < 0.001$), 66% in the AcbC ($P < 0.001$) and 54% in the AcbSh ($P < 0.01$). The D_2-like receptor density showed an increase in the Cpu ($P < 0.05$), the AcbC ($P < 0.01$) and the VTA ($P < 0.05$) and a decrease in the AcbSh ($P < 0.05$).

In Fig. 3, colour-coded autoradiograms of sections from control and AAS-treated animals, respectively, are shown for the brain regions which were significantly affected at bregma + 1.6. The reduction of the B_{max} estimates (mean ± SEM) of the [^{125}I]-(+)-SCH-23982 labelled D_1-like receptors from 1.57 ± 0.19 to 0.47 ± 0.08 fmol/mg in CPu, from 0.90 ± 0.12 to 0.31 ± 0.05 fmol/mg in AcbC, and from 0.68 ± 0.11 to 0.31 ± 0.05 fmol/mg in the AcbSh are indicated by changes in colour from red to yellow to green. The increased B_{max} estimates of the [^{125}I]-NCQ-298 labelled D_2-like receptors in CPu and AcbC were altered from 2.74 ± 0.20 and 1.47 ± 0.09 to 3.62 ± 0.31 and 2.30 ± 0.22 fmol/mg, respectively, which is indicated by the higher density of the red colour in the autoradiogram of the AAS-treated animal compared to the section of the control. The decrease of the B_{max} estimate from 1.38 ± 0.09 to 1.11 ± 0.07 fmol/mg in the AcbSh, is visualized through the slight increase of green in this region.

Discussion

In the present study, chronic treatment with a supra-therapeutic dose of the AAS nandrolone decanoate, was shown to affect dopamine receptors in terminal regions of the mesocorticolimbic and nigrostriatal projections of the male rat brain. Autoradiographic analysis indicated that labelling of D_1-like receptors was decreased in the caudate putamen, the nucleus accumbens core and the nucleus accumbens shell. Labelling of D_2-like binding sites was increased in the caudate putamen, the nucleus accumbens core and the ventral tegmental area, but decreased in the nucleus accumbens shell.

AAS misusers report euphoria, increased energy and sexual arousal (Su *et al.*, 1993). Such positive hedonic properties are usually associated with an enhanced hyperactivity of the dopamine system. The effects observed following the abuse of psychomotor stimulant



drugs such as amphetamine and cocaine (Koob, 1999) share many similarities with the rewarding qualities, as well as the psychiatric side-effects, associated with AAS misuse. Reinforcing properties of drugs of abuse have been shown to involve both D_1 and D_2 receptor components. The D_2-receptor subtype has been suggested to mediate the positive reinforcement, while the D_1-receptor subtype would be critical for the acquisition of this effect (Missale *et al.*, 1998). We suggest that the alterations of the dopamine receptor densities in the mesocorticolimbic system reflect neuroadaptive responses to prior enhanced dopaminergic activity in this area. The induction of these neuroadaptive responses is probably caused by various feedback mechanisms. The restrictions of this study are that the observed changes in dopamine receptor densities do not discriminate between the different receptor subtypes within each subfamily, nor does it give any information on the regulation of the mRNA levels. However, with respect to the down regulation of the D_1-like receptors, it is noteworthy that enhanced synaptic levels of dopamine in the nucleus accumbens have been shown to activate the long negative feedback loop projecting to the ventral tegmental area (Rahman & McBride, 2000), which mainly includes neurons expressing mRNA for the D_1 receptor (Lu *et al.*, 1998). Further, within the nucleus accumbens, D_2 receptors act not only as autoreceptors (Missale *et al.*, 1998), they are also postsynaptically located on GABAergic neurons projecting to the ventral pallidum (Lu *et al.*, 1998) and cholinergic neurons (MacLennan *et al*., 1994), which in turn seem to have potential roles in negative feedback mechanisms (Rahman & McBride, 2000) The reason why the D_2-like receptors were down regulated in the nucleus accumbens shell, whereas they were upregulated in both the core and the caudate putamen, may be that the dorso-lateral core of the nucleus accumbens is related to the striatopallidal complex that projects to the substantia nigra pars compacta and is associated with motor functions, whereas the ventro-medial shell has projections to the ventral tegmental area and is connected to the extended amygdala (Heimer *et al.*, 1991; Di Chiara, 1999). The hypothesis that the changes in dopamine receptor densities are due to an enhanced dopaminergic activity in the mesolimbic pathway is further supported by the observed up regulation of the D_2-like receptors in ventral tegmental area, which may be involved in a short negative feedback loop mediated by D_2 autoreceptors at the somatodendritic position (Kalivas & Duffy, 1991; Kohl *et al.*, 1998). Perfusion of the ventral tegmental area with a D_2 agonist has been shown to decrease the extracellular levels of dopamine in the nucleus accumbens (Kohl *et al.*, 1998).

The suggested elevated dopamine levels in the nucleus accumbens could either be directly or indirectly induced by AAS. In previous studies, an observed increase of the β-endorphin levels in the ventral tegmental area (Johansson *et al.*, 1997) and altered levels of melanocortin and opioid receptors (unpublished data) in animals treated with nandrolone decanoate, indicate that the enhanced dopamine activity, at least to some extent, may be indirectly mediated by proopiomelanocortin expressing neurons.

A recent behavioural study suggests nandrolone reduces locomotion and rearing in rats (Johansson *et al.* 2000b). Although comparisons between measurements of mRNA and its translated protein should be performed with caution (Nicholas *et al.*, 1996), the effects on dopamine receptor densities of the caudate putamen in the present study, closely resembles the alterations in dopamine receptor mRNA levels in a model for Parkinson's disease, where the nigrostriatal neurons were depleted (Gerfen *et al.*, 1990). In striatum, D_1 receptors are postsynaptically located on striatonigral GABAergic neurons and D_2 receptors are postsynaptically located on the striatopallidal GABAergic neurons and both are coordinated to induce opposite feedback mechanisms in the substantia nigra pars reticulata and the entopeduncular nucleus regions (Gerfen *et al.*, 1990). In accordance with the study by Gerfen *et al* (1990) the down regulation of the D_1-like receptors and the up regulation of D_2-like receptors in the present study might reflect a synergistic neuroadaptation in order to diminish the motoric behaviour. As Parkinson's disease is associated with a depressed transmission of dopamine, these similarities may indicate that the effects of AAS on dopamine receptor levels reflect a hypodopaminergic state. It is tempting to speculate that this depressed dopaminergic activity of the nigrostriatal pathway, might reflect a permanent condition of the neuroadaptive force established in order to reduce a prior enhanced dopaminergic activity following chronic exposure to 40–50 times the therapeutic dose of AAS. The suggested enhanced metabolism in striatum (Thiblin *et al.*, 1997), could be due to a different dose regimen and accordingly still be in line with the results of the present study. In agreement with this theory, it has been postulated that between-system adaptations in response to repeated drug use would evoke a neurobiochemical system, which when activated would oppose the primary reinforcing effects of the drug (Koob, 1996).

Furthermore, chronic AAS treatment is shown to affect both substance P and dynorphin levels in striatum (Hallberg *et al.* 2000; Johansson *et al.* 2000a). Dopamine receptor activity mediated by the nigrostriatal pathway affects the striatonigral substance P system (Hanson *et al.*, 1981). It has been postulated that the stimulation of the dynorphin system may elicit the dysphoric syndrome connected to cocaine dependence and provide feedback to decrease dopamine release (Koob, 1996). This is interesting, since D_1 receptors are coexpressed with substance P and preprodynorphin in the striatonigral GABAergic neurons (Hanson *et al.*, 1981; Jones *et al.*, 1999) and D_2 receptors are coexpressed with preproenkephalin in the striatopallidal GABAergic neurons (Missale *et al.*, 1998; Jones *et al.*, 1999).

In summary, chronic treatment with high doses of the AAS nandrolone induces altered expression of D_1- and D_2-like receptors, possibly reflecting an altered dopaminergic activity. These neurobiochemical alterations are suggested to be associated with the reported mood and motoric changes following AAS abuse.

Acknowledgements

This study was supported by the Swedish Medical Research Council (Grant 04X-09459 and 04X-05957). We thank Dr Allen E. Johnson at Karolinska Institutet, Stockholm, for his generous donation of [^{125}I]-NCQ-298.

Abbreviations

A, amygdala; AAS, anabolic-androgenic steroids; AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; Cpu, caudate putamen; DA, dorsal hypothalamic area; MGP, medial globus pallidus; PAG, periaqueductal grey; SN, substantia nigra; VTA, ventral tegmental area.

FIG. 3. Colour-coded autoradiograms of sections from control and nandrolone decanoate-treated animals in brain regions which were significantly affected at bregma +1.6 for both the [^{125}I]-(+)-SCH-23982 labelled D_1-like receptors and [^{125}I]-NCQ-298 labelled D_2-like receptors. The colour scale (nonlinear) indicates the binding site densities in fmol/mg (wet weight). The numerical B_{max} estimates of the illustrated regions ranged between purple (0 fmol/mg) and black–red (15 fmol/mg) are presented in Fig. 2.

References

Arvary, D. & Pope, H.G.J. (2000) Anabolic-androgenic steroids as a gateway to opioid dependence. *N. Engl. J. Med.*, **342**, 1532.

Bahrke, M.S., Yesalis, C.E. & 3rd, & Wright, J.E. (1996) Psychological and behavioural effects of endogenous testosterone and anabolic-androgenic steroids. An update. *Sports Med.*, **22**, 367–390.

Brower, K.J., Blow, F.C., Young, J.P. & Hill, E.M. (1991) Symptoms and correlates of anabolic-androgenic steroid dependence. *Br. J. Addict*, **86**, 759–768.

Di Chiara, G. (1998) A motivational learning hypothesis of the role of dopamine in compulsive drug use. *J. Psychopharmacol.*, **12**, 54–67.

Di Chiara, G. (1999) Drug addiction as dopamine-dependent associative learning disorder. *Eur. J. Pharmacol.*, **375**, 13–30.

DuRant, R.H., Ashworth, C.S., Newman, C. & Rickert, V.I. (1994) Stability of the relationships between anabolic steroid use and multiple substance use among adolescents. *J. Adolesc. Health*, **15**, 111–116.

DuRant, R.H., Escobedo, L.G. & Heath, G.W. (1995) Anabolic-steroid use, strength training, and multiple drug use among adolescents in the United States. *Pediatrics*, **96**, 23–28.

Galligani, N., Renck, A. & Hansen, S. (1996) Personality profile of men using anabolic androgenic steroids. *Horm. Behav.*, **30**, 170–175.

Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma, F.J. Jr & Sibley, D.R. (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, **250**, 1429–1432.

Hallberg, M., Johansson, P., Kindlundh, A.M. & Nyberg, F. (2000) Anabolic-androgenic steroids affect the content of substance P and substance P (1–7) in the rat brain. *Peptides*, **21**, 845–852.

Hanson, G., Alphs, L., Pradhan, S. & Lovenberg, W. (1981) Response of striatonigral substance P systems to a dopamine receptor agonist and antagonist. *Neuropharmacology*, **20**, 541–548.

Heimer, L., Zahm, D.S., Churchill, L., Kalivas, P.W. & Wohltmann, C. (1991) Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience*, **41**, 89–125.

Johansson, P., Hallberg, M., Kindlundh, A. & Nyberg, F. (2000a) The effect on opioid peptides in the rat brain, after chronic treatment with the anabolic androgenic steroid, nandrolone decanoate. *Brain Res. Bull.*, **51**, 413–418.

Johansson, P., Lindquist, A.-S., Nyberg, F. & Fahlke, C. (2000b) The anabolic androgenic steroid nandrolone decanoate affects alcohol intake, defensive behaviors and brain opioid peptides in the rat. *Pharmacol. Biochem. Behav.*, **67**, 1–9.

Johansson, P., Ray, A., Zhou, Q., Huang, W., Karlsson, K. & Nyberg, F. (1997) Anabolic androgenic steroids increase beta-endorphin levels in the ventral tegmental area in the male rat brain. *Neurosci. Res.*, **27**, 185–189.

Jones, E.A., Wang, J.Q., Mayer, D.C. & McGinty, J.F. (1999) The role of dorsal striatal GABA (A) receptors in dopamine agonist- induced behavior and neuropeptide gene expression. *Brain Res.*, **836**, 99–109.

Kalivas, P.W. & Duffy, P. (1991) A comparison of axonal and somatodendritic dopamine release using in vivo dialysis. *J. Neurochem.*, **56**, 961–967.

Kennedy, M.C. (1992) Anabolic steroid abuse and toxicology. *Aust. NZ J. Med.*, **22**, 374–381.

Kindlundh, A.M.S., Hagekull, B., Isacson, D.G.L. & Nyberg, F. (2000) Adolescent use of anabolic-androgenic steroids and relations to self reports of social, personality, and health aspects. *Eur. J. Public Health.* In press.

Kindlundh, A.M.S., Isacson, D.G.L., Berglund, L. & Nyberg, F. (1998) Doping among high school students in Uppsala, Sweden: a presentation of the attitudes, distribution, side effects, and extent of use. *Scand. J. Soc. Med.*, **26**, 71–74.

Kindlundh, A.M.S., Isacson, D.G.L., Berglund, L. & Nyberg, F. (1999) Factors associated with adolescent use of doping agents: anabolic-androgenic steroids. *Addiction*, **94**, 543–553.

Kohl, R.R., Katner, J.S., Chernet, E. & McBride, W.J. (1998) Ethanol and negative feedback regulation of mesolimbic dopamine release in rats. *Psychopharmacology*, **139**, 79–85.

Koob, G.F. (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.*, **13**, 177–184.

Koob, G.F. (1996) Drug addiction: the yin and yang of hedonic homeostasis. *Neuron*, **16**, 893–896.

Koob, G.F. (1999) The role of the striatopallidal and extended amygdala systems in drug addiction. *Ann. NY Acad. Sci.*, **877**, 445–460.

Le Grevès, P., Huang, W., Johansson, P., Thörnwall, M., Zhou, Q. & Nyberg, F. (1997) Effects of an anabolic-androgenic steroid on the regulation of the NMDA receptor NR1, NR2A and NR2B subunit mRNAs in brain regions of the male rat. *Neurosci. Lett.*, **226**, 61–64.

Lu, X.Y., Ghasemzadeh, M.B. & Kalivas, P.W. (1998) Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience*, **82**, 767–780.

Lukas, S.E. (1996) CNS effects and abuse liability of anabolic-androgenic steroids. *Annu. Rev. Pharmacol. Toxicol.*, **36**, 333–357.

MacLennan, A.J., Lee, N., Vincent, S.R. & Walker, D.W. (1994) D2 dopamine receptor mRNA distribution in cholinergic and somatostatinergic cells of the rat caudate-putamen and nucleus accumbens. *Neurosci. Lett.*, **180**, 214–218.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M. & Caron, M.G. (1998) Dopamine receptors: from structure to function. *Physiol. Rev.*, **78**, 189–225.

Nicholas, A.P., Hökfelt, T. & Pieribone, V.A. (1996) The distribution and significance of CNS adrenoceptors examined with in situ hybridization. *Trends Pharmacol. Sci.*, **17**, 245–255.

Nilsson, S. (1995) Androgenic anabolic steroid use among male adolescents in Falkenberg. *Eur. J. Clin. Pharmacol.*, **48**, 9–11.

Paxinos, G. & Watson, C. (1997). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego.

Pontieri, F.E., Tanda, G. & Di Chiara, G. (1995) Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the 'shell' as compared with the 'core' of the rat nucleus accumbens. *Proc. Natl Acad. Sci. USA*, **92**, 12304–12308.

Pope, H.G. Jr & Katz, D.L. (1994) Psychiatric and medical effects of anabolic-androgenic steroid use. A controlled study of 160 athletes. *Arch. Gen. Psychiatry*, **51**, 375–382.

Rahman, S. & McBride, W.J. (2000) Feedback control of mesolimbic somatodendritic dopamine release in rat brain. *J. Neurochem.*, **74**, 684–692.

Schlussman, S.D., Zhou, Y., Johansson, P., Kiuru, A., Ho, A., Nyberg, F. & Kreek, M.J. (2000) Effects of the androgenic anabolic steroid, nandrolone decanoate, on adrenocorticotropin hormone, corticosterone and proopiomelanocortin, corticotropin releasing factor (CRF) and CRF receptor1 mRNA levels in the hypothalamus, pituitary and amygdala of the rat. *Neurosci. Lett.*, **284**, 190–194.

Spanagel, R. & Weiss, F. (1999) The dopamine hypothesis of reward: past and current status. *Trends Neurosci.*, **22**, 521–527.

Su, T.-P., Pagliaro, M., Schmidt, P.J., Pickar, D., Wolkowitz, O. & Rubinow, D.R. (1993) Neuropsychiatric effects of anabolic steroids in male normal volunteers. *JAMA*, **269**, 2760–2764.

Tanner, S.M., Miller, D.W. & Alongi, C. (1995) Anabolic steroid use by adolescents: prevalence, motives, and knowledge of risks. *Clin. J. Sport Med.*, **5**, 108–115.

Thiblin, I., Finn, A., Ross, S.B. & 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.

Thiblin, I., Kristiansson, M. & Rajs, J. (1997) Anabolic androgenic steroids and behavioural patterns among violent offenders. *J. Forensic Psych.*, **8**, 299–310.

Williamson, D.J. & Young, A.H. (1992) Psychiatric effects of androgenic and anabolic-androgenic steroid abuse in men: a brief review of the literature. *J. Psychopharmacol.*, **6**, 20–26.

Yesalis, C.E. & Bahrke, M.S. (1995) Anabolic-androgenic steroids. Current issues. *Sports Med.*, **19**, 326–340.

Yesalis, C.E., Kennedy, N.J., Kopstein, A.N. & Bahrke, M.S. (1993) Anabolic-androgenic steroid use in the United States. *JAMA*, **270**, 1217–1221.