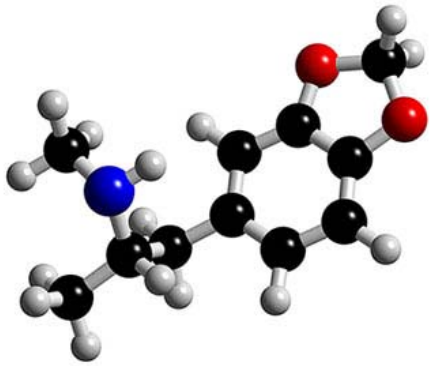
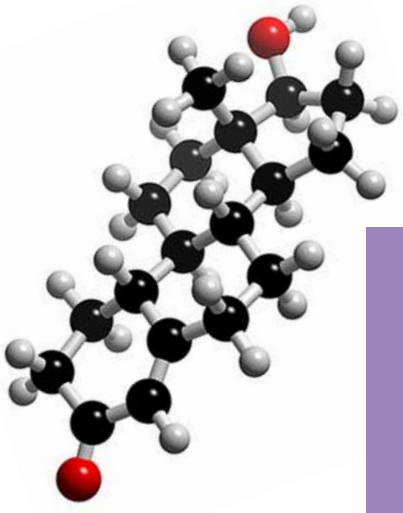
NATIONAL INSTITUTE
FOR HEALTH AND WELFARE

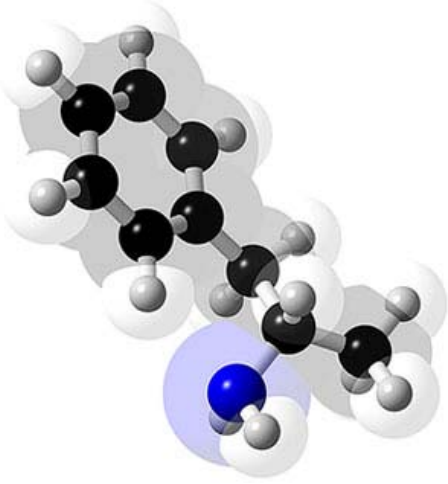


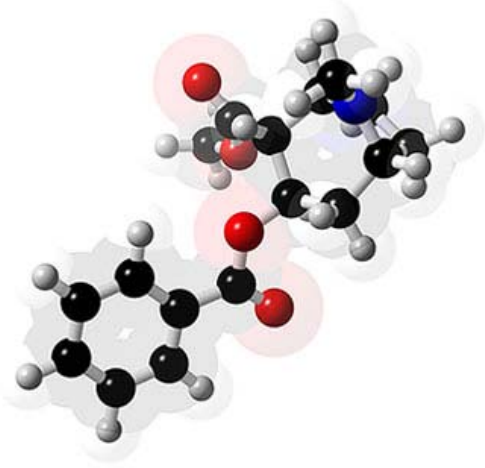


RESEARCH

Sanna Kailanto

Interactions of Nandrolone and Psychostimulant Drugs on Central Monoaminergic Systems





Sanna Kailanto

Interactions of Nandrolone and Psychostimulant Drugs on Central Monoaminergic Systems

ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Biological and Environmental Sciences, University of Helsinki, for public examination in the Arppeanum auditorium, Helsinki University Museum, Snellmaninkatu 3, Helsinki, on April 29nd, at 12 o'clock noon.

Department of Alcohol, Drugs and Addiction,
National Institute for Health and Welfare,
Helsinki, Finland

and

Department of Biosciences
Faculty of Biological and Environmental Sciences,
University of Helsinki, Finland

RESEARCH 30

Helsinki 2010

Cover picture: Karl Harrison
Layout: Christine Strid

ISBN 978-952-245-258-0 (printed)
ISSN 1798-0054 (printed)
ISBN 978-952-245-259-7 (pdf)
ISSN 1798-0062 (pdf)

Helsinki University Print
Helsinki, Finland 2010

Supervised by

Docent Timo Seppälä, MD
Department of Alcohol, Drugs and Addiction
National Institute for Health and Welfare

Reviewed by

Professor Markku Koulu, MD
Department of Pharmacology and Clinical Pharmacology
Faculty of Medicine
University of Turku

Docent Petteri Piepponen, Ph.D
Division of Pharmacology and Toxicology
Faculty of Pharmacy
University of Helsinki

Opponent

Professor Raimo Tuominen, Ph.D
Division of Pharmacology and Toxicology
Faculty of Pharmacy
University of Helsinki

Abstract

Sanna Kailanto, Interactions of nandrolone and psychostimulant drugs on central monoaminergic systems. National Institute for Health and Welfare (THL), Research 30. pp. 121. Helsinki, Finland 2010.
ISBN 978-952-245-258-0 (printed), ISBN 978-952-245-259-7 (pdf)

It has been hypothesized that abuse of supra-therapeutic doses of anabolic androgenic steroids (AASs) can lead to dependence and function as a gateway to abuse of other drugs. This is supported by behavioral studies on animal models and psychiatric evaluations of human subjects, although their neurochemical effects remain largely unknown. A large body of evidence suggests that the ability of the drugs to induce a strong elevation of extracellular dopamine (DA) levels in the nucleus accumbens (NAc), especially, plays a crucial role in their reinforcing effects.

This study had four main aims. The first was to explore the effects of nandrolone decanoate on dopaminergic and serotonergic activities in the brains of rats, at doses which trigger the peripheral anabolic effects. The second aim was to assess whether or not nandrolone pre-exposure modulates the acute neurochemical and behavioral effects of psychostimulant drugs (amphetamine, MDMA and cocaine) in experimental animals. The third was to investigate if the AAS-pre-treatment induced changes in brain reward circuitry are reversible. And the fourth main goal was to evaluate the role of androgen and estrogen receptors in the modulation of the dopaminergic and serotonergic effects of acute injections of stimulant drugs by sub-chronic nandrolone treatment. The extracellular concentrations of the monoamine transmitters were monitored using *in vivo* microdialysis technique.

The results showed that nandrolone decanoate at doses, high enough to induce erythropoiesis, significantly increased the levels of DOPAC and 5-HT in the cerebral cortex. Co-administration of AAS and psychostimulant drugs showed that the increase in extracellular DA and 5-HT concentration evoked by amphetamine, MDMA and cocaine in the NAc was attenuated dose-dependently by pretreatment with nandrolone. Nandrolone pre-exposure also attenuated the ability of stimulants to cause increased stereotyped behavior and locomotor activity. The results demonstrated also that despite the significant decrease in nandrolone concentration in blood, the attenuation of cocaine's effects remained unchanged after a fairly long period without nandrolone, suggesting that nandrolone effects could be long lasting. Blockade of androgen receptors (ARs) with flutamide abolished the attenuating effect of nandrolone pretreatment on amphetamine-induced elevation of extracellular DA concentration, while blockade of estrogen receptors (ERs) with clomiphene reduced the attenuating effect of nandrolone to a lesser extent.

In conclusion, the results of this study show that AAS-pretreatment is able to inhibit the reward-related neurochemical and behavioral effects of amphetamine, MDMA and cocaine in experimental animals. Given that accumbal outflow of DA and 5-HT, as well as LMA and stereotyped behavior are all related to reward, this study suggests that nandrolone, at the doses tested, has a significant effect on the rewarding properties of stimulant drugs. Furthermore, it seems that these effects could be long lasting and it appears that the ability of nandrolone to modulate reward-related effects of stimulants is dependent on activation of AR or ER.

Keywords: amphetamine, anabolic androgenic steroid, cocaine, dopamine, MDMA, nandrolone, nucleus accumbens, 5-HT

Abstract in Finnish

Sanna Kailanto, Interactions of nandrolone and psychostimulant drugs on central monoaminergic systems [Nandrolonin ja piristävien huumausaineiden yhteiskäytön vaikutukset keskushermoston monoamiinijärjestelmään]. Terveyden ja hyvinvoinnin laitos (THL), Tutkimus 30. 121 sivua. Helsinki 2010.
ISBN 978-952-245-258-0 (painettu), ISBN 978-952-245-259-7 (pdf)

Anabolis-androgeenisten steroidien (AAS) väärinkäytön on todettu voivan johtaa riippuvuuteen kyseisistä aineista ja mahdollisesti edistävän myös muiden päihteiden käyttöä. Vaikka koe-eläimillä tehdyt käyttäytymistutkimukset samoin kuin ihmisten psykiatriset arvioinnit tukevat tätä näkemystä, taustalla olevista hermokemiallisista tapahtumista ei kuitenkaan ole kattavaa tietoa. Nykyisten tutkimustulosten perusteella voidaan olettaa, että olennaista päihteiden palkitsevuudessa on niiden kyky nostaa solunulkoisen dopamiinin pitoisuutta, erityisesti accumbens-tumakkeessa.

Tämän tutkimuksen tarkoituksena oli tutkia nandroloni dekanoaatin vaikutuksia rotan aivojen dopaminergiseen ja serotonergiseen hermojärjestelmään nandrolonin annoksilla, jotka aiheuttavat yleisesti tavoiteltuja perifeerisiä muutoksia, kuten punasolujen lisääntynyttä synteesiä. Lisäksi tutkittiin, muuttaako nandrolonin annostelu amfetamiinin, MDMA:n eli ekstaasin ja kokaiinin välittömiä hermokemiallisia ja käytöksellisiä vaikutuksia koe-eläimissä. Tutkimuksessa selvitettiin myös, välittyvätkö nandrolonin dopaminergisiä ja serotonergisiä vaikutuksia muuttavat ominaisuudet androgeeni- tai estrogeenireseptorien kautta. Välittäjäaineiden solunulkoiset pitoisuudet mitattiin *in vivo* mikrodialyysimenetelmällä.

Tulokset osoittavat, että nandroloni lisäsi merkittävästi DOPAC:n ja 5-HT:n (serotoniinin) pitoisuutta rotan aivokuorella, annoksina, joiden havaittiin edistävän myös punasolujen synteesiä. Nandrolonin ja piristävien huumausaineiden samanaikainen annostelu osoitti, että amfetamiinin, MDMA:n sekä kokaiinin aiheuttama dopamiinin vapautuminen accumbens-tumakkeesta väheni merkittävästi nandrolonin esiannostelun seurauksena. Dopamiinin vapautuminen oli riippuvaista nandrolonin annoksesta. Nandrolonin esiannostelun havaittiin vähentävän myös näiden stimuloivien huumausaineiden kykyä lisätä eläinten kaavamaista käytöstä, kuten myös liikeaktiivisuutta. Tulokset osoittavat lisäksi, että nandrolonin ehkäisevä vaikutus kokaiinin vaikutuksille saattaa olla pidempikestoista. Vaikka nandrolonin pitoisuuksien havaittiin laskeneen tai jopa hävinneen rotan verestä, kokaiinin vaikutukset olivat edelleen lievempiä. Androgeenireseptorien salpaus flutamidilla palautti amfetamiinin kyvyn nostaa solun ulkoista dopamiinipitoisuutta accumbens-tumakkeessa nandrolonin esiannostelusta huolimatta. Estrogeenireseptorien salpaus klomifeenillä palautti osittain kokaiinin kyvyn nostaa

solun ulkoista dopamiinipitoisuutta accumbens-tumakkeessa nandrolonin esiannostelusta huolimatta.

Yhteenvetona voidaan todeta, että anabolis-androgeenisen yhdisteen, nandrolonin, havaittiin ehkäisevän amfetamiinin, MDMA:n sekä kokaiinin palkitsevia vaikutuksia, kun mitattiin accumbens-tumakkeen solun ulkoisen dopamiinin pitoisuuden sekä käyttäytymisen muutoksia koe-eläimillä. Ottaen huomioon, että dopamiinin ja serotoniinin vapautuminen accumbens-tumakkeessa, kuten myös liikeaktiivisuuden sekä kaavamaisen käytöksen lisääntyminen, liitetään voimakkaasti huumausaineiden palkitsevuuteen, tulosten perusteella voidaan esittää, että nandroloni tässä testattuina annoksina vähentää piristävien huumausaineiden palkitsevuutta. Lisäksi näyttää siltä, että nämä muutokset saattavat olla pidempiaikaisia ja että nandrolonin vaikutukset välittyvät voimakkaasti juuri androgeeni- ja osittain estrogeenireseptorien välityksellä.

Avainsanat: accumbens-tumake, amfetamiini, anabolinen steroidi, dopamiini, ekstaasi, kokaiini, nandroloni, serotoniini

Contents

Abbreviations

3-MT	3-methoxytyramine
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
AADC	Aromatic amino acid decarboxylase
AAS	Anabolic androgenic steroid
ALAT	Alanine aminotransferase
ANOVA	Analysis of variance
ASAT	Aspartate aminotransferase
AR	Androgen receptor
AUC	Area under the curve
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CPP	Conditioned place preference
DA	Dopamine
DAT	Dopamine transporter
DOPAC	3,4-dihydroxyphenylacetic acid
EDTA	Ethylenediamine tetraacetic acid
ER	Estrogen receptor
GABA	γ-Aminobutyric acid
GC/MS	Gas chromatograph/mass spectrometer
HPLC	High-performance liquid chromatography
HVA	Homovanillic acid
i.c.v.	Intracerebroventricularly
i.g.	Intragastric
i.m.	Intramuscular
i.p.	Intraperitoneal
i.v.	Intravenously
MAO	Monoamine oxidase
MDMA	3,4-methylenedioxymethamphetamine
NAc	Nucleus accumbens
s.c.	Subcutaneous
SEM	Standard error of the mean
SERT	Serotonin transporter
TH	Tyrosine hydroxylase
TPH	Tryptophan hydroxylase
VMAT	Vesicular monoamine transporter
VTA	Ventral tegmental area

List of original publications

This thesis is based on the following original articles referred to in the text by their Roman numerals:

I Kurling S, Kankaanpää A, Ellermaa S, Karila T, Seppälä T. The effect of sub-chronic nandrolone decanoate treatment on dopaminergic and serotonergic neuronal systems in the brains of rats. Brain Research 2005;1044:67–75. [a]

II Kurling S, Kankaanpää A, Seppälä T. Sub-chronic nandrolone treatment modifies neurochemical and behavioral effects of amphetamine and 3,4-methylenedioxy-methamphetamine (MDMA) in rats. Behavioral Brain Research 2008; 189:191–201.[a]

III Kurling-Kailanto S, Kankaanpää A, Seppälä T. Sub-chronic nandrolone administration reduces cocaine-induced dopamine and 5-hydroxytryptamine outflow in the rat nucleus accumbens. Psychopharmacology 2010, Published online 26 February. [b]

IV Kurling-Kailanto S, Kankaanpää A, Hautaniemi J, Seppälä T. Blockade of androgen or estrogen receptors reduces nandrolone's ability to modulate acute reward-related neurochemical effects of amphetamine in rat brain. Pharmacology, Biochemistry and Behavior, *In Press*.

[a] Reprinted with the kind permission of Elsevier B.V.
[b] Reprinted with the kind permission of Springer Science and Business Media.

1 Introduction

A large number of young adults abuse anabolic androgenic steroids (AASs). This type of use probably involves more than a desire to enhance the users' appearance or sports performance, and appears to have much in common with the use of alcohol and tobacco (Bahrke et al., 2000; Kindlundh et al., 1999). AASs have been proposed to be addictive in humans and to serve as a gateway to abuse of other illicit drugs (Arvary and Pope, 2000; Kanayama et al., 2003; Skarberg et al., 2008). Loss of impulse control, often reported among AAS abusers, not only underlies aggression, but could also contribute to the initiation of drug abuse as well as its development into drug addiction (Kreek et al., 2005; Wood, 2004). Since the use of both psychostimulant drugs and anabolic steroids has increased over the past 20 years among young people, the question is raised what happens if these drugs are used simultaneously. Indeed, frequency of AAS use is significantly associated with the frequency of using psychotropic drugs such as cocaine and amphetamine (Thevis et al., 2008). While the side effects of both anabolic steroids and psychostimulant drugs have been well documented, very few studies have been performed to assess the potential effects resulting from the concomitant use of these drugs. It has been hypothesized that steroid hormones are important determinants of stimulant's effects on behavior by influencing neuronal activity and plasticity (Hruska and Pitman, 1982; Perrotti et al., 2000; Quinones-Jenab et al., 2001).

The question whether AASs create dependence or not is without a definite answer. Several studies have shown that chronic AAS use may cause dependence (Brower et al., 1989; Brower et al., 1991; Clark et al., 1996), but the underlying biochemical mechanisms of AASs are still poorly understood. The mesocorticolimbic dopaminergic pathway is considered to play an important role in the reward circuitry of the brain (Di Chiara, 1995; Koob, 1992; Tomkins and Sellers, 2001), and a connection between AASs and central dopaminergic activity has been reported in animal studies (Kindlundh et al., 2001b; Thiblin et al., 1999; Vermes et al., 1979). Moreover, it is suggested that the serotonergic system is involved in the rewarding effects of various drugs (see review Leshner and Koob, 1999).

Given that AASs modulate functions of the brain reward systems, it is likely that in addition to a desire to enhance one's exterior features or sporting performance there are also neurochemical adaptations behind prolonged misuse of AASs. Despite increased awareness of both the public and scientific communities of the profound neural changes AASs are able to induce, experimental research on their neurobiological basis is limited.

2 Review of the literature

2.1 Anabolic androgenic steroids (AASs)

In 1889, C.E. Brown-Séquard injected on himself an extract isolated from dog and guinea pig testicles, and described it as a "rejuvenating elixir" much before the recognition of testosterone (Brown-Séquard, 1889). A small volume of testosterone itself was isolated from enormous amount of bovine testicles in 1927. Testosterone was later reported to restore masculine characteristics in several species after castration (Koch, 1938).

Anabolic androgenic steroids (AASs) are defined as synthetic derivatives of the endogenous sex hormone testosterone. The largest amounts of testosterone are produced in the testes in men. In women it is also synthesized in far smaller quantities in the ovaries, as well as in the adrenal cortex in both sexes. In general, androgens promote protein synthesis and growth of those tissues with androgen receptors. Testosterone is responsible for the anabolic effects including growth of muscle mass and strength and androgenic effects including maturation of male secondary sex characteristics. The effects of testosterone in humans and other vertebrates occur by two main mechanisms: by activation of the androgen receptors (ARs), and by conversion to estradiol and activation of certain estrogen receptors (ERs). The brain is one of the important tissues in humans where the primary effect of testosterone is mediated via aromatization to estradiol.

Testosterone was synthesized by two groups independently, and this earned the group leaders Butenandt and Ruzicka the joint Nobel Prize in Chemistry in 1939 (Hoberman and Yesalis, 1995). The development of synthetic steroids started from this point. Testosterone and its synthetic derivates 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 made these substances attractive also outside clinical use. The first reports of athletes using AASs appeared in the literature during the 1950s (Hoberman and Yesalis, 1995), and even though sports organizations have banned these substances and perform tests regularly, doping scandals in sport are still a common experience.

Known modifications of testosterone molecule include alkylation at the 17α-position and/or modification of the steroid ring structure. The goal of these modifications is the production of derivates that are more anabolic and less androgenic than the parent molecule. The esterification of the 17β-hydroxyl group also increases the steroid activity due to the prolongation of the action, as the steroid gets lipophilic properties and the capability of retaining in fat tissue (Fragkaki et al., 2009). Table 1 summary the different kind of AASs.

TABLE 1. Commonly abused anabolic-androgenic steroids.

Orally used 17α-alkylated testosterone derivatives	**Intramuscularly used 17β-esterificated testosterone derivatives**
methandienone methyltestosterone oxandrolone oxymetolone stanozol ethylestrenol fluoxymesterone danatsol mesterolone metenolone trenbolone	Testosterone esters: cypionate propionate enantate undecanoate Nandrolone esters: decanoate phenylpropionate undecanoate laurate Other: methenolone trenbolone boldenone stanotzol drostanolone

2.1.1 Nandrolone Decanoate

TABLE 2. Nandrolone decanoate.

Nandrolone decanoate (19-nortestosterone decanoate)
Mol wt 428.65 Solubility insol in water, freely sol in ethanol, ether, acetone, chloroform, oils. Half-life 4.3 h Half-life for the release of the ester from the i.m. depot Rat: 4.3–5.4 days / Human 6–8 days Metabolites: 19-norandrosterone, 19-noretiocolanolone, 19-norepiandrosterone Receptors: Androgen, Estrogen, Glucocorticoid, Mineralocorticoid.

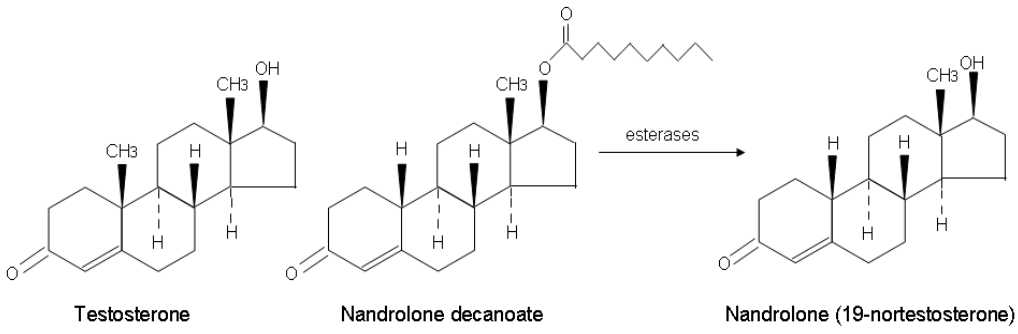


FIGURE 1. Structural formula of testosterone, nandrolone and nandrolone decanoate. Nandrolone decanoate is hydrolysed by esterases to nandrolone after systemic administration.

This thesis is focused on nandrolone; one of the most commonly abused AAS (Brower, 2002; Eklof et al., 2003). Nandrolone is a synthetic modification of the testosterone molecule and lacks a methyl (CH_3) group at the C_{19} position (Fig. 1). The nandrolone molecule is made suitable for injection by conjugation with decanoic acid, creating nandrolone decanoate (Fig.1). Nandrolone has a long half-life in the depot form, but after injection nandrolone decanoate is converted to nandrolone when it is slowly released from the depot into the blood.

2.1.2 Mechanism of action

Anabolic steroids pass through the cell membrane and bind to the cytoplasmic androgen receptor (AR). The androgen receptor is a member of the nuclear receptor superfamily. When the receptor is occupied by a ligand, it is classically translocated to the cell nucleus where it functions as a transcription factor and modulates gene expression, which in turn results in many physical changes. Each AAS has a different binding affinity for the AR, and it is believed that this varying receptor affinity explains the varying effects produced by different AASs (Pereira de Jesus-Tran et al., 2006). Nandrolone, the AAS examined in this thesis, binds the AR with less affinity than the testosterone metabolite, dihydrotestosterone but with a higher affinity than testosterone (Roselli, 1998; Thompson and Siiteri, 1974).

More recent studies in rats have also demonstrated "non-classical" rapid cellular effects of androgens in brain regions that possess few classical receptors (Mermelstein et al., 1996). These steroid actions are thought to be mediated membrane steroid receptors (Sato et al., 2008). Whereas the distribution of classical steroid receptors in the rodent brain is relatively limited, the potential brain targets for membrane androgen action are much broader. The simultaneous modification

of both "classical" and "non-classical" target sites may account for the complex AAS abuse syndrome.

The CNS actions of AASs are complex and the response depends not only on their interaction with ARs in the brain, but also on their ability to regulate and/or act as substrate for aromatase. Aromatase is an enzyme that is responsible for a key step in the biosynthesis of estrogens (Fig 2). Aromatase transforms androgens to estrogens through oxidation and subsequent elimination of a methyl group (Schade and Schubert, 1979). Increased circulating levels of estrogen are shown to be present after the administration of AASs (Lukas, 1993) and the estrogenic activity of nandrolone has been established (Ryan, 1959).

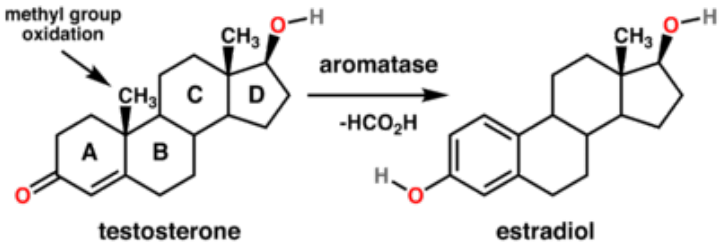


FIGURE 2. Aromatase converts testosterone to estradiol.

2.1.3 Abuse of AAS

Apart from elite athletes aiming for increased performance during competition, both professional and amateur bodybuilders administer AASs to gain maximal muscle volume. During the past decade their use has spread to colleges and high schools as well (Kochakian, 1990; Lukas, 1993). Over the last years, several studies examining substance use-patterns have shown an increase in anabolic-androgenic steroids (AASs) in all user groups, not only in athletes (Graham et al., 2008; Parkinson and Evans, 2006). The use of AASs is no longer limited to professional power training athletes and body builders, but a large number of young adolescents take AASs in high doses with the intention of improving their physical fitness, appearance, increasing of confidence levels, and even pleasure (Faigenbaum et al., 1998; Kindlundh et al., 2001a; Kindlundh et al., 1998; Kindlundh et al., 1999; Yesalis and Bahrke, 1995).

However, this misuse probably involves more than a desire to enhance the user's appearance or sporting performance: it also appears to have much in common with the use of alcohol and tobacco (Bahrke et al., 2000; Kindlundh et al., 1999). 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). One explanation for the popularity of stimulant drugs among steroid users could be the fact that they increase fat burning and induce a sensation of an "energy burst" that makes the user train even harder (Brower, 2002). This trend is alarming because, while the side effects of both anabolic steroids and stimulants have been well documented, very few studies have been performed to assess the potential effects resulting from the concomitant use of these drugs. For example, it has been hypothesized that steroid hormones are important determinants of cocaine's effects on behavior by influencing neuronal activity and plasticity (Hruska and Pitman, 1982; Perrotti et al., 2000; Quinones-Jenab et al., 2001).

2.1.4 Physical and mental side effects

To reduce many of the mental side effects of AAS, it is desirable to promote the anabolic rather than the androgenic effects. However, pure anabolic steroids do not yet exist. Although most of the synthetic AASs exhibit less androgenic activity than testosterone, they induce a variety of physical side effects (e.g. Bahrke and Yesalis, 2004; Wollina et al., 2007). In brief, gynecomastia, baldness, testes and prostate atrophy have been reported in men, while deepening of the voice, menstrual irregularities, increased growth of facial hair and enlargement of clitoris are commonly reported side effects in women after prolonged AAS use (see review Bahrke and Yesalis, 2004). Increased acne, liver dysfunction, as well as increased risk of cardiovascular diseases (for example altered serum lipoproteins, stroke, enlarged heart) are found in both sexes (Karila et al., 2003; Sader et al., 2001).

The mental side effects associated with AASs have been observed more frequently as the use of AASs has become more widespread. Changes in mood, euphoria, delusions, depression and violent behavior, are reported to be associated with the prolonged use of AASs (Bahrke et al., 2000; Burnett and Kleiman, 1994; Choi and Pope, 1994; Copeland et al., 2000; Gruber and Pope, 2000; Parrott et al., 1994; Parssinen et al., 2000; Perry et al., 2003). The neurochemical mechanisms behind AAS-induced mental side effects are not clear. Nevertheless, on the basis of animal studies, it has been suggested that AAS abuse may constitute a risk factor in aggressive behavior, partly by affecting the serotonergic system (Lindqvist et al., 2002; Miczek et al., 1994) and drug dependence by affecting the dopaminergic system in the brain (Kindlundh et al., 2001b; Thiblin et al., 1999; Vermes et al., 1979).

2.1.5 AAS Dependence

No cases of abuse or dependence have been described in men or women who received or self-administered therapeutic doses of AASs for medical indications. However, when used for enhancing physical appearance, AASs can lead to abuse and dependence in both men and women (Brower et al., 1991; Brower et al., 1990; Kashkin and Kleber, 1989). Brower (2002) has proposed a two-stage model of AAS dependence. According to this hypothesis, anabolic effects on muscle growth account for the fist stage of steroid use. However, with chronic exposure, users can develop physical and psychological dependence on AASs, as defined by DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders, 1987). Regarding the background literature AASs abusers share personality factors with the abusers of psychotropic substances, such as cannabis, amphetamine and cocaine (Arvary and Pope, 2000; Kanayama et al., 2003; Lukas, 1996; Skarberg et al., 2008).

2.1.5.1 Animal models

While it is clear that AASs are abused, defining the potential for addiction in humans has been problematic because it is difficult to separate direct psychoactive effects the drugs in humans from the user's psychological dependence on the anabolic effects of AASs. Thus, studies in laboratory animals are a useful tool to explore androgen reward. Several experimental models have been developed to study reward in laboratory animals (Koob, 2006), where conditioned place preference (CPP) and self-administration are the most important methodologies. Testosterone, like many drugs of abuse, will increase rates of bar pressing for electrical brain stimulation, which is considered an indication of a drug's rewarding effects (Caggiula, 1970; Campbell, 1970; Olds, 1958). Male hamsters preferentially self-administer testosterone orally (Johnson and Wood, 2001; Wood, 2002), and in many studies the testosterone induces conditioned place preference (Alexander et al., 1994; de Beun et al., 1992; Kashkin and Kleber, 1989). To the author's knowledge, there is only one CPP-study carried out with nandrolone, and it shows that nandrolone is able to induce CPP in mice (Parrilla-Carrero et al., 2009). Both Syrian hamsters and Sprague-Dawley rats self administer testosterone intravenously (i.v.) (Wood et al., 2004). Syrian hamsters have also been shown to self-admister nandrolone (Ballard and Wood, 2005). As seen, AASs are self-administered by rodents, although the effect is known to be rather modest. Still, it is comparable with other mild reinforcers such as benzodiazepines (Naruse and Asami, 1987) and nicotine (Manzardo et al., 2002).

Pre-exposure to AASs has been demonstrated to affect 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.,

2006; Celerier et al., 2003). Male adult rats have been shown to increase voluntary alcohol intake after cessation of AAS-administration (Johansson et al., 2000b).

2.1.5.2 Neurotransmitters

One way to investigate the abuse potential and risk of development of dependence after use of AASs is to study the steroid's effect on neurotransmitters known to regulate the rewarding effects of other drugs of abuse as well. Animal studies have indicated that AASs are able to evoke biochemical changes in dopaminergic and serotonergic neuronal systems related to reward, a key phenomenon in drug dependence, as well as numerous other behavioral responses in rats (Bitar et al., 1991; Thiblin et al., 1999; Vermes et al., 1979). Testosterone-induced CPP can be blocked by the mixed dopamine D_1/D_2 receptor antagonist, flupenthixol, administered both systemically (Schroeder and Packard, 2000) or directly into the NAc (Packard et al., 1998) in doses which do not impair motor behavior. I.p. injections of the specific dopamine D_1 antagonist SCH23390 or D_2 antagonist sulpiride can also block testosterone induced CPP, indicating involvement of both dopamine D_1 and D_2 receptors (Schroeder and Packard, 2000).

2.2 Neuropharmacology of brain reward system

It is generally believed that most drugs of abuse, if not all, act through a so-called brain reward system, a general name for the mechanism involving the brain neurotransmitter DA and the neural system that it regulates. The mesocorticolimbic dopaminergic pathway plays an important role in the reward circuitry of the brain. A large body of literature is devoted also to the influence of serotonergic systems in mediating physiological responses and behaviours to addictive substances. The brain reward system is activated by natural stimuli essential for survival and reproduction, such as eating and sexual activity, but addictive drug activates this circuitry even more strongly than these natural stimulants (Di Chiara, 1999).

2.2.1 DA

2.2.1.1 The dopaminergic system

The cell bodies of dopaminergic neurons projecting to the forebrain are located in the brain stem, more specifically in the substantia nigra (A9), ventral tegmental (A10; VTA) and retrorubral areas (A8). Mesolimbic and mesocorticolimbic

dopaminergic pathways originate mainly in the VTA, from where they project to the nucleus accumbens, olfactory tubercle, medial caudate putamen, septum, amygdala, hippocampus, limbic cortex and other brain areas, fig 3 (Björklund, 1984; Dahlström and Fuxe, 1964).

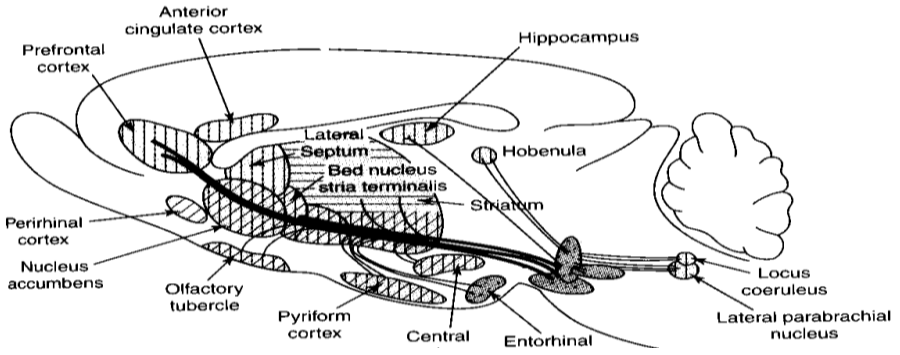


FIGURE 3. Schematic diagram illustrating the distribution of the main central neuronal pathways containing dopamine, modified by Cooper (2003).

Catecholamine neurotransmitters are synthesized from the dietary amino acid L-tyrosine. The biosynthesis of DA is presented in figure 4 (next page). The first reaction, where tyrosine is converted to L-3,4,-dihydroxyphenylalanine (L-DOPA), is catalyzed by tyrosine hydroxylase (TH), and is the rate-limiting step (e.g. Cooper, 2003). L-DOPA is subsequently converted to DA by the enzyme aromatic L-amino acid decarboxylase (AADC).

Newly synthesized DA is transported into synaptic vesicles by the vesicular monoamine transporter 2 (VMAT2), and stored in these vesicles awaiting release, which occurs after action potential or other depolarizing stimulus by exocytosis of a Ca^{2+} ion dependent manner into the synaptic cleft (Raiteri et al., 1979).

DA released into the synaptic cleft binds to receptors that belong in two families: D_1 and D_2. D_1-like receptors include D_1 and D_5 types, whereas D_2-like receptors include D_2, D_3 and D_4 types. The receptors can be differentiated on the basis of biochemical function, cellular location and distribution profile over the brain (Cooper, 2003; Waxham, 1999). All of these receptors are known to belong to a family of guanine-nucleotide binding protein (G-protein) coupled transmembrane receptors, but there are two sub-families that have opposite effects at cellular level: action of D_1–like receptors results in stimulation of the enzyme adenylate cyclase, while activation of D_2–like receptors inhibits adenylate cyclase. DA receptors can be located either post- or presynaptically.

Synaptic action of catecholamines is primarily terminated by efficient Na^+ and K^+ dependent transporter-mediated uptake into nerve-endings (uptake 1). There

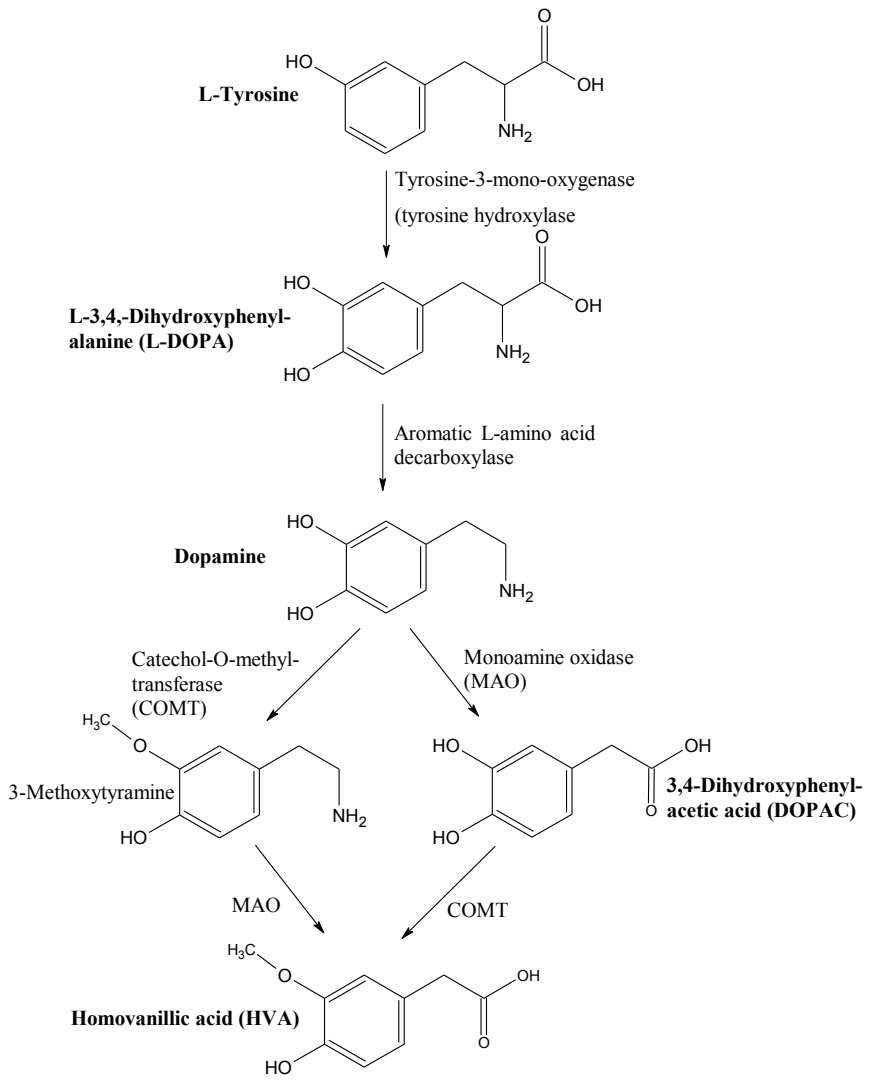


FIGURE 4. Simplified presentation of DA synthesis and metabolisms.

is, however, also some uptake into surrounding tissue (uptake 2). DA homeostasis is maintained through several mechanisms, one being uptake by the plasma membrane bound DA transporter (DAT) and other is synthesis of DA (Jaber et al., 1997). DA metabolism may follow either of two routes, which is determined by whether the first step is catalyzed by monoamine oxidase (MAO) or catechol-*O*-methyltransferase (COMT). MAO and COMT are located in mitochondria of nerve endings and glia cells. MAO metabolizes cytosolic DA into DOPAC, which is the main metabolite in rats (Westerink, 1985). DA is metabolized by COMT and MAO into HVA, which is the major metabolite in humans (fig 4) (Cooper, 2003).

2.2.1.2 Nandrolone decanoate and DA

The impact of AASs, particularly nandrolone, on the brain dopaminergic systems has been studied to some extent in rodents. Two-week dosing of nandrolone decanoate at 15 mg/kg daily decreased density of dopamine D_1-like receptor protein in the caudate putamen and NAc core and shell (Kindlundh et al., 2001b). Similar results were later obtained for mRNA levels (Kindlundh et al., 2003b). On the other hand, the densities of dopamine D_2-like receptors were upregulated in the caudate putamen, VTA and NAc core, while downregulated in the shell (Kindlundh et al., 2001b). Sub-chronic nandrolone administration to rats causes alterations in mRNA expression of DA receptors. An increase was detected in the D_1 –receptor in amygdala and the D_4 in the NAc, and a decrease in D_1 receptor in the hippocampus (Birgner et al., 2008). In these studies, no changes in gene expression of TH or AADC were detected.

The daily dosing of nandrolone at 1, 5 and 15 mg/kg for 14 days increased amounts of DAT in the caudate putamen (Kindlundh et al., 2004) and treatment with nandrolone (15 mg/kg/day for 14 days) caused an up-regulation of the binding potential of the DAT in the striatum (Kindlundh et al., 2002). This could explain some of the alterations seen in DA receptors after nandrolone dosing, since receptor number or activity could be affected by nandrolone's actions on increased DA up-take by DAT.

Nandrolone pre-treatment (15 mg/kg/day for 14 days) has been shown to attenuate amphetamine-induced decreases of the DA metabolite levels (Birgner et al., 2007). Furthermore, nandrolone decanoate pre-treatment is shown to abolish the effect of amphetamine on the hippocampal DOPAC/DA ratio and the hypothalamic DOPAC level and DOPAC/DA ratio (Birgner, 2006). This reduced metabolism might possibly be a compensation for increased DA uptake, since the DA baseline levels remains unchanged. This is in line with earlier findings of DAT up-regulation. Nandrolone has not been shown to change the MAO-A or MAO-B activity in rats (Thiblin et al., 1999).

2.2.2 5-HT

2.2.2.1 The serotonergic system

5-HT-containing neurons are restricted to clusters of cells lying in or near the midline or raphe regions of the pons and upper brain stem (Figure 5). Dahlström and Fuxe described nine 5-HT containing cell groups (B1-B9), which exist in midbrain and brainstem areas of the raphe nuclei and the reticular area (Dahlström and Fuxe, 1964). The more caudal groups project largely to the medulla and spinal cord and the more rostral cell groups (B7-B9) are thought to provide the extensive

5-HT innervations of the telencephalon and diencephalon. The intermediate groups may project into both ascending and descending groups (Cooper, 2003).

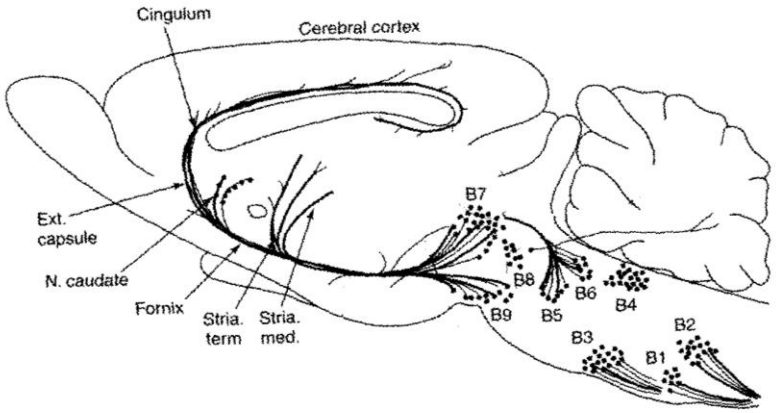


FIGURE 5. Schematic diagram illustrating the distribution of the main serotonin-containing pathways in the rat central nervous system, modified by Cooper (2003).

5-HT is derived from dietary amino acid tryptophan (figure 6). The first step in the synthetic pathway is hydroxylation of tryptophan at the 5 position by tryptophan hydroxylase (TPH) to form 5-hydroxytryptophan (Walther et al., 2003). Once synthesized, 5-hydroxytryptophan is sequentially converted to 5-HT by AADC. Like DA, newly synthesized 5-HT is transported into synaptic vesicles by VMAT2 and stored in, and released from these vesicles (Cooper, 2003).

5-HT released into the synaptic cleft binds to receptors that belong in seven families (5-$HT_{1\text{-}7}$) and several of these are further subdivided (Barnes and Sharp, 1999). In general, 5-HT_1 -family receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}) are negatively coupled to adenylate cyclase and are considered as autoreceptors. 5-HT_1 -family receptors can also exist as heteroreceptors localized on axon terminals of non-serotonergic neurons, postsynaptic to the 5-HT releasing neuron (Zifa and Fillion, 1992). The members of 5-HT_2 –family receptors (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{2D}, 5-HT_{1F}) are postsynaptic receptors and activate phospholipase C (Cooper, 2003). Other positively adenylyl cyclase coupled 5-HT receptors are 5-HT_4, 5-HT_6 and 5-HT_7 subtypes. The 5-HT_5 group (5-HT_{5A}, 5-HT_{5B}) represent a new family of 5-HT receptors that do not resemble the 5-HT_1 or 5-HT_2 families and have a still unknown coupling mechanism, and which seem to be widespread within the CNS (Erlander et al., 1993). In contrast to G-protein coupled receptors, the 5-HT_3 receptors directly activate a 5-HT –gated cation channel.

Synaptic action of 5-HT is primary terminated by efficient Na^+ and K^+ dependent transporter-mediated uptake into nerve-endings. 5-HT homeostasis

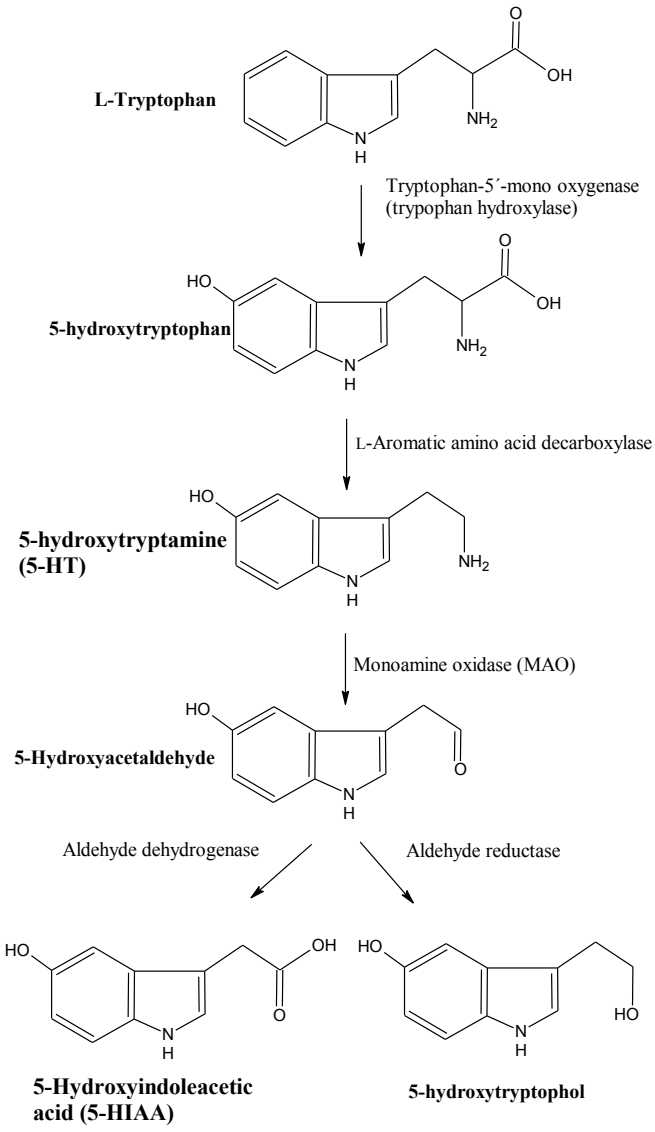


FIGURE 6. 5-HT synthesis and metabolism.

is maintained through uptake by the plasma membrane bound 5-HT transporter (SERT) (Cooper, 2003). After uptake to a nerve-ending 5-HT is recycled or metabolized by monoamine oxidase (MAO) to 5-HIAA (figure 6).

2.2.2.2 Nandrolone decanoate and 5-HT

So far, there are only sparse reports on the ability of nandrolone to affect 5-HT and 5-HIAA synthesis or metabolism, and their reported measured tissue contents after nandrolone dosing are highly diverse. Tamaki et al. (2003) have observed increased levels of 5-HT and 5-HIAA in the cerebral cortex and hypothalamus after nandrolone treatment in the brains of rats, and Thiblin et al. (1999) has earlier documented similar results with oxymethenolone. With the 15 mg/kg does of nandrolone decanoate administered for 14 days to male rats, Lindqvist et al. observed decreased levels of 5-HT in the forebrain and dorsal striatum and decreased levels of 5-HIAA in the striatum (Lindqvist et al., 2002). The tryptophan hydroxylase activity of individual nuclei of the limbic system, hypothalamus, and midbrain was determined by Kritzer et al. after testosterone treatment, and no changes in activity occurred in any of the areas examined (Kritzer, 1997). To author's knowledge, there are no studies 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).

Nandrolone has also been shown to induce alterations in the density of 5-HT receptors: 5-HT_{1B} receptors were down-regulated and 5-HT_{2} receptors up-regulated in rat brain after two weeks of nandrolone dosing (Kindlundh et al., 2003a). With regard to 5-HT_{3} receptors, there are, to the authors knowledge, no studies carried out 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). Hamsters treated with anabolic/androgenic steroids (a mixture of AASs consisting of testosterone cypionate, nortestosterone, and dihydroxytestosterone) showed significant decreases in 5-HT_{1A} receptor-immunoreactive staining and protein levels in the anterior hypothalamus with no concomitant decrease in the number of 5-HT_{1A} receptor-expressing neurons (Ricci et al., 2006). Testosterone is shown to increase SERT binding site densities in male rat brain (McQueen et al., 1999), and therefore these alterations seen in 5-HT receptors after nandrolone dosing could be a result of nandrolone's actions on increased DA up-take by SERT.

2.2.3 Additional transmitters

In addition to dopamine and 5-HT, other systems like GABA and opioid –system contribute to modulation of brain reward mechanisms.

Some behavioral evidence supporting for the regulatory role of the GABA-ergic system in psychomotor stimulant-reward have been obtained. Benzodiazepines, alprazolam and chlordiazepoxide, have been shown to attenuate cocaine self-administration (Goeders et al., 1993; Goeders et al., 1989), and GABA transaminase

inhibitor has been shown to attenuate elevation in accumbal DA levels induced by cocaine (Morgan and Dewey, 1998).

There is significant experimental evidence implicating that also the endogenous opioid system processes of reward and reinforcement. Indeed, many behaviors associated with reward and reinforcement, for example feeding behavior, are controlled by distinct components of the endogenous opioid system located in relevant brain regions (Gianoulakis, 2009). The main effect of opioid receptor stimulation seems to be inhibition of GABA-ergic interneurons, which in turn leads to disinhibition of the DA containing output neurons, activation of mesolimbic neural transmission and increased release of DA in the terminal area NAc (Di Chiara and North, 1992).

2.3 Psychostimulant drugs

The term "psychostimulant" generally refers to drugs that produce a spectrum of effects in humans which includes increased energy, cardiovascular stimulation, elevated mood, and a decreased need for sleep. At higher doses, or after longer periods of use, psychostimulant drugs can produce a range of disordered thought processes, including severe psychotic episodes (e.g. Le Moal and Koob, 2007). In animals, psychostimulants increase locomotor activity and are readily self-administered due to their powerful reinforcing properties. Most psychostimulant drugs are known to interact with monoamine neurons in the CNS. One of the major brain areas involved in the rewarding properties of cocaine and particularly of amphetamine appears to be the NAc (Bardo, 1998; McBride et al., 1999).

In general, drugs that target transporter proteins, as psychostimulants do, can be divided into two classes based on their precise mechanism of action: reuptake inhibitors and substrate-type releasers (Rothman and Baumann, 2003). Reuptake inhibitors bind to transporter proteins, but are not themselves transported into the cells. They elevate extracellular transmitter concentrations by blocking transport-mediated reuptake of transmitters from the synapse. Substrate-type releasers bind to transporter proteins, and these drugs are subsequently transported into the cytoplasm of nerve terminals. Releasers elevate transmitter concentration by promoting efflux of transmitters by a process of transporter-mediated exchange and disrupting storage of transmitters in vesicles (Rudnick and Clark, 1993). This latter action increases the pool of neurotransmitter available for release.

2.3.1 Amphetamine and MDMA

Amphetamine is a synthetic compound that was developed in the late 1880's and it was medically used in the treatment of narcolepsy and a variety of other disorders from the 1936 to mid-1940s. Amphetamines were originally synthesized as potential alternative drugs for the treatment of asthma. Nowadays amphetamine is one of the standard treatments for ADHD, as well as narcolepsy and other sleeping disorders. After the 1930's, when the CNS stimulating effect of amphetamine was discovered, it became a popular stimulant drug among university students in the United States. For military purposes amphetamine was first used in the Spanish Civil War, and at the end of the World War II there was a real epidemic of stimulant abuse (Angrist, 1978). Since the 1960s, amphetamine has been popular with many youth subcultures as a recreational drug.

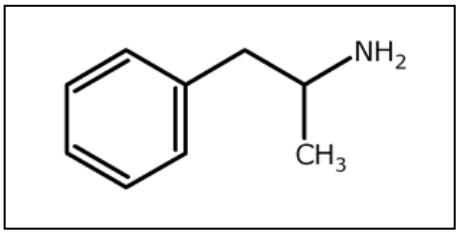


FIGURE 7. Molecular structure of amphetamine

3,4-Methylenedioxy-*N*-methylamphetamine (MDMA) was first synthesized in 1912, but was largely forgotten over the following 65 years (Bernschneider-Reif et al., 2006). MDMA first appeared as a street drug in the early 1970s after its analogue, 3,4-methylenedioxyamphetamine (MDA), became criminalized in the United States. In the late 1980s MDMA marketed as "Ecstasy" began to be widely used, becoming an integral element of rave culture and other psychedelic and dance floor-influenced music scenes. Spreading along with rave culture, illicit MDMA use became increasingly widespread among young adults in universities and later in high schools.

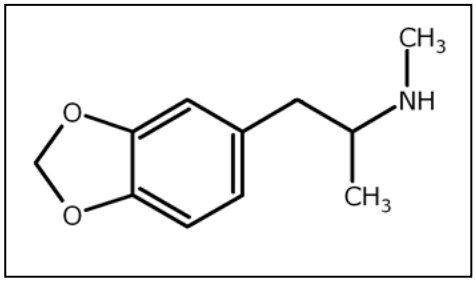


FIGURE 8. Molecular structure of MDMA

In rats acute behavioral effects of amphetamine and MDMA include strong locomotor activation, and with higher doses of drugs, stereotyped behavior characterized by intense sniffing and head bobbing, which have shown to be of dopaminergic origin (Kulmala et al., 1987; Randrup and Munkvad, 1967; Taylor and Snyder, 1971). Both of these amines increase synaptic concentrations of DA by multiple mechanisms; by drifting into the nerve endings and displacing DA (and norepinephrine) from storage vesicles into the cytoplasmic pool from where it is released via reversal of the transporter, by inhibiting DA (and norepinephrine) reuptake, and by inhibiting MAO (Blaschko, 1952; Ferris et al., 1972; Glowinski and Axelrod, 1965; Johnson et al., 1986). As measured using *in vivo* microdialysis, amphetamine elevates strongly and MDMA to a slightly lesser extent, the extracellular DA levels in the NAc and striatum (Hernandez et al., 1987; Hiramatsu et al., 1991; Nash, 1990; Zetterstrom et al., 1983), which are implicated in locomotor and stereotyped activity, respectively. Amphetamine and MDMA decrease extracellular concentrations of the DA metabolites DOPAC and HVA (Gough et al., 1991; Hernandez et al., 1987; Hiramatsu et al., 1991; Slikker et al., 1988; Zetterstrom et al., 1983), since amphetamine is known to be a competitive and reversible inhibitor of MAO (Filinger and Stefano, 1982). Another reason could be that the majority of DOPAC is presumably derived from metabolism of intraneuronal DA and the cytoplasmic DA is partially depleted as a result of DA release (Kuczenski, 1980; Zetterstrom et al., 1986). HVA is a secondary metabolite of DOPAC, and thus changes in its concentrations should presumably parallel those of DOPAC. The effects of amphetamine (Kuczenski, 1980) and MDMA (Gough et al., 1991) on HVA indeed parallel the effects on DOPAC with small to moderate doses, but diverge at higher doses, which is suggested to result from DA metabolism shifting to an 3-methoxytyramine (3-MT) pathway.

Extracellular 5-HT levels are elevated by higher doses of amphetamine, however with less efficiency than those of DA (Hernandez et al., 1987), whereas MDMA has been shown to affect 5-HT release and uptake more than those of DA (Johnson et al., 1986; Nichols et al., 1982; Schmidt et al., 1987). Elevation of extracellular 5-HT levels, induced by amphetamine and MDMA, is accompanied by a decrease in 5-HIAA levels. This can be assumed to result from depletion of cytoplasmic 5-HT, because 5-HIAA mirrors the metabolism of intraneuronal 5-HT (Grahame-Smith, 1971). In addition, MDMA inhibits MAO-A (Leonardi and Azmitia, 1994), which may also result in decrease in extracellular 5-HIAA concentrations. Consistent with the neurochemical effects of MDMA, acute injection induces behavioral effects indicative of the so-called 5-HT syndrome, which in rats includes Straub tail and splaying of hind limbs (Slikker et al., 1989; Spanos and Yamamoto, 1989). The MDMA induced increase in locomotor activity is also 5-HT dependent (Callaway et al., 1991). Damage to 5-HT terminals following MDMA dosing is well documented, and results have shown that MDMA is more toxic to 5-HT terminals than some other stimulants (eg.. Baumann et al., 2007). It

seems that the 5-HT transporter may play a central role in MDMA toxicity, because it has been shown that exposure to methamphetamine which is structurally related to MDMA, only degenerates 5-HT transporter containing axons in the NAc, while the axons lacking the transporters are spared (Brown and Molliver, 2000).

2.3.2 Cocaine

Cocaine (benzoylmethylecgonine) is a crystalline tropane alkaloid that is obtained from the leaves of the coca plant (Erythroxylon coca). For over a thousand years South American indigenous peoples have chewed the coca leaf. The history of cocaine use is thoroughly described in the literature (Gay et al., 1975). The cocaine alkaloid was first isolated by Albert Niemann in 1860. Chemically, cocaine is a benzoylecgonine methylester, which is an optically active crystalline base (figure 9). In the 1880's Sigmund Freud published a series of writings praising its effects, whilst his friend and colleague Karl Koller discovered its usefulness as a local anesthetic. The coca extract containing soft drink named Coca-Cola® appeared on the market in 1888, and it became one of the most popular soft drinks. In the 1890's the disadvantages of cocaine use – development of dependence and toxic effects – were noticed, and in the year 1906 cocaine use became restricted in the United States. Since 1914 possession, selling and delivery of coca-products has been illegal, however cocaine is still the most widely abused psychostimulant drug of abuse today.

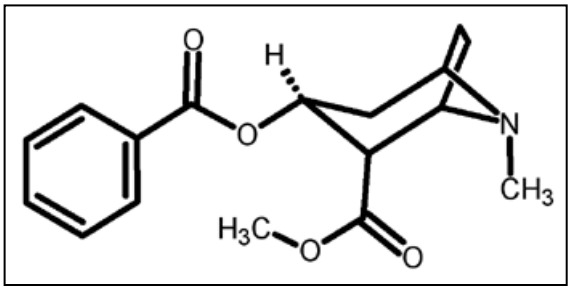


FIGURE 9. Molecular structure of cocaine

In rats the acute behavioral effects of cocaine include those typical to psychostimulants: increased locomotor activity, and with higher doses of the drug stereotyped behavior, both of which have been shown to be dependent on the mesocorticolimbic dopaminergic network (Kelly and Iversen, 1976). Cocaine blocks the reuptake of DA, 5-HT and norepinephrine, thus increasing their synaptic concentration, without inducing transmitter release (Heikkila et al., 1975; Koe,

1976). As measured by *in vivo* microdialysis, cocaine strongly elevates extracellular levels of DA and 5-HT (and norepinephrine) at least in the NAc, striatum, prefrontal cortex and VTA, without any marked effect on neurotransmitter metabolites (Carboni et al., 1989; Hurd and Ungerstedt, 1989a; Hurd and Ungerstedt, 1989b; Kuczenski et al., 1991; Moghaddam and Bunney, 1989). Since the effect of cocaine on extracellular DA levels is prevented by treatment with γ-butyrolactone and tetrodotoxin, action potential is necessary for elevation of DA concentration (Carboni et al., 1989; Nomikos et al., 1990).

3 Aims of the study

It has been hypothesized that abuse of supra-therapeutic doses of AASs can lead to dependence and function as a gateway to abuse of other illicit drugs. This is supported by behavioral studies in animal models as well as psychiatric evaluations of human subjects. A large body of evidence suggests that a drugs ability to induce a strong elevation of extracellular DA levels in the NAc, in particular, plays a crucial role in its reinforcing effects. Although AASs are abused by humans, their neurochemical effects remain largely unknown. Alterations of both DA and 5-HT functions are indicated as a possible neurochemical basis for these behavioral effects. However, the effects of supra-therapeutic doses of AASs on the dopaminergic and serotonergic systems are not yet fully evaluated. In order to understand the characteristics of addictive behavior and provide novel pharmacological treatment strategies for addiction diseases, it is essential to understand neurochemical mechanisms mediating the rewarding properties of drugs of abuse.

The specific aims of this series of studies were:

1) To evaluate the neurochemical effects of the AAS, nandrolone decanoate, on dopaminergic and serotonergic activities in the brains of rats, at doses that can cause physical effects.
2) To assess whether or not nandrolone pre-exposure modulates the acute neurochemical effects of amphetamine, MDMA and cocaine, and behavioral effects of amphetamine and MDMA in rats.
3) To investigate if the AAS-pre-treatment induced changes in brain reward circuitry, measured as altered response to stimulant drug of abuse, are reversible.
4) To examine, using androgen receptor antagonist and estrogen receptor antagonist, whether or not these hormonal systems are involved in nandrolone's ability to modulate the dopaminergic and serotonergic effects of acute injection of amphetamine.

4 Material and methods

4.1 Animals

Studies were performed on adult male Wistar rats, weighing 290-380 g and they were delivered from Harlan Netherlands B.V., (Netherlands) at least 1 week prior to the experiments. In paper I the animals were housed individually in Macrolon III-type cages (20 x 36,5 x 18,5 = 730 cm^3) and in papers II, III and IV they were housed in groups of three in Techniplast Eurostandard type IV cage (595 x 380 x 200 mm, floor area 1820 cm^2), except after surgery, when the rats were housed individually. The temperature- and humidity were controlled in the room (21 ± 2 °C) with a 12-h light cycle, 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. The animal experiments of papers I and II were approved by the local institutional committee for animal care and use and the chief veterinary surgeon of the county administrative board and those of papers III and IV were approved by the State Provincial Office of Southern Finland Animal Experiment Board. All studies were conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

4.2 Drugs and doses

4.2.1 Nandrolone

There are considerable differences, regarding experimental design, between existing neurochemical studies of supra-therapeutic AAS exposure in dose (1–45 mg/kg), dose interval (1–7 days), duration of administration (1 day–15 weeks) and route of administration (i.m. or s.c). We chose the doses of 5 mg/kg and 20 mg/kg nandrolone decanoate (Deca-Durabolin®, N.V. Organon, the Netherlands) / body weight, calculated as free base, according to neurochemical and behavioral studies done with rats. We chose those doses also to correspond, at least in some level, to one cycle of use during early and experienced AAS abuse respectively, based on a one year follow-up study and a survey study of 500 AAS abusers (Fudala et al., 2003; Parkinson and Evans, 2006).

In papers I, II and III, both doses were used, and in paper IV was used only the larger nandrolone dose was used. In paper I the animals received one i.m. injection daily, 5 days per week during a two-week period (10 injections per animal). In rest

of the papers (II, III and IV) we injected nandrolone every other day during a 10-day period (total of 5 injections per animal), based on our preliminary experiment which showed that combined the half-life (ester hydrolysis, distribution, and elimination) of nandrolone decanoate is 4.3 days, and on the study of van der Vies, who demonstrated that nandrolone depot has a half-life of 5.4 days in rat and 6 days in human (van der Vies, 1985). The injections were given in the left and right hind leg alternately. The matching vehicle for Deca-Durabolin®, a mixture of arachinoid oil and benzylalcohol, was prepared by the University Pharmacy (Helsinki, Finland) for control purposes.

4.2.2 Stimulant drugs

3,4-Methylenedioxymethamphetamine hydrochloride (National Institute on Drug Abuse; NIDA, Bethesda, MD USA) and amphetamine sulfate (Sigma Chemical co, St Louis, MO, USA) were dissolved in saline (0.9 % NaCl) at concentrations of 5 mg/kg (MDMA) and 1 mg/kg (amphetamine), calculated as free base. Both drugs were racemic mixtures. The drugs were injected intraperitoneally (i.p.) in a volume of 1 ml/kg of body weight during the microdialysis experiment. The normal clinical oral dose of amphetamine is 0.07–0.2 mg/kg (Baselt, 2004), while addicts may self-administer doses as high as 0.7–4.3 mg/kg (Gustavsen et al., 2006). Typical amounts of MDMA abused by humans are one or two tablets of 80–100 mg orally, which corresponds to 1–3 mg/kg (Green et al., 2003). In addition to comparability to human doses, experiences from our earlier animal studies were taken into account: a 1 mg/kg dose of amphetamine and a 5 mg/kg dose of MDMA both produce robust and reproducible neurochemical and behavioral effects without any observable adverse effects on animal welfare.

Cocaine hydrochloride (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was dissolved in saline (0.9 % NaCl) at a concentration of 20 mg/kg (calculated as free base). The drug was a racemic mixture. The drug was injected i.p. at a volume of 1 ml/kg of body weight during the microdialysis experiment, on the 6^{th} or 28^{th} day from the last nandrolone injection. The cocaine dose chosen represents the minimum dose that produced strong stereotyped behaviours in rats and sufficient DA release from the NAc in without any observable adverse effects on animal welfare.

4.2.3 Receptor antagonists

Flutamide (2-methyl-*N*-[4-nitro-3-(trifluoromethyl) phenyl] –propanamide; 50 mg/kg (Sigma Chemical Co., St Louis, MO, USA), clomiphene (2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy] -N,N-diethyl-ethanamine; 20 mg/kg (Sigma

Chemical Co., St Louis, MO, USA) or water were administered intragastricly (i.g.) on days 1, 3, 5, 8 and 10 ca. 6 hours before nandrolone dosing, and continued also on days 12 and 15 from beginning of the experiment. Clomiphene was administered as a 10 mg/ml water solution and flutamide as 25 mg/ml water and Tween® 20 suspension (Fluka Sigma-Aldrich Chemie GmbH, CH-9471 Buchs, Germany).

4.3 Neuro- and biochemical experiments

4.3.1 Sample collection (paper I)

On day 1 and day 8 the rats were lightly anaesthetized with halothane gas (Trothane, I.S.C Chemicals Ltd., England) and a 0.5 ml blood sample was collected from the *vena caudalis*. The blood was collected in a tube containing 25 µl of Na_2EDTA-UH-Q water solution (0.16 mM). Half of each sample was centrifuged at +4°C (1800 x g,) and the resulting plasma was stored at –20°C, while the other half was kept as whole blood at room temperature, from which the blood count was performed within 24 hours. The animals were rendered unconscious with CO_2 and decapitated on day 17 (3 days from the last injection) and trunk blood was collected and processed as described above. The brains were rapidly removed and the brain regions (olfactory bulb, prefrontal cortex, cerebral cortex, striatum, nucleus accumbens, hippocampus, hypothalamus, as well as pons and medulla) were dissected (Paxinos and Watson, 1986) and immediately frozen and stored at –70 °C until assayed.

4.3.2 Surgery and microdialysis (papers II, III and IV)

The rats were anesthetized using halothane gas (Halothane Liquid BP; Rhodia Organique Fine Ltd., Bristol, UK) and mounted in a stereotactic frame on the first day after the last nandrolone dose (papers II and IV) For paper III half of the animals were taken into operation at the same stage as described above for papers II and IV, while the other half were taken into operation after a recovery period; on the 15th day after the last nandrolone dose. 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). 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 5 days.

One day before the dialysis experiment, the rats were allowed to habituate to test cages and a microdialysis probe (CMA/12, membrane length 2 mm; CMA Microdialysis, Solna, Sweden) was inserted through the guide cannula into the NAc. On the day of the experiment, the animals were placed in test cages and 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, 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 aqueous antioxidant solution (1.0 mM oxalic acid, 3.0 mM L-cysteine, 0.1 mM Acetic acid) was added to each vial before collecting the dialysate samples (Kankaanpää et al., 2001). The perfusate from the first 60 min was discarded, after which the samples were collected at 20 min intervals. Amphetamine, MDMA, cocaine or saline was injected i.p. after collection of four basal samples. At the end of the experiment the animals were anesthetized with halothane gas, and blood samples were drawn using cardiac puncture (papers II and III). 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.

4.3.3 Chemical analyses

4.3.3.1 DA, 5-HT and their metabolites in brain tissue

The concentration of DA, 5-HT and their metabolites (DOPAC, HVA, and 5-HIAA) in brain tissues were determined using high performance liquid chromatography (HPLC) (ESA; ESA Inc., Chelmsford, MA USA) with electrochemical detection and an Insertil ODS-3V 5 µm (250 mm x 4.6 mm ID) reverse-phase column (GL-Sciences Inc., Tokyo Japan) as described earlier (Kankaanpää et al., 2001), with necessary modifications for processing brain tissue samples. The frozen samples were homogenized in a 21-fold quantity of anti-oxidative solution (1.0 mM oxalic acid, 3.0 mM L-cysteine and 0.1 M acetic acid), using a Vibra-Cell VC 600 high intensity ultrasonic processor (Sonics and Materials Inc., Danbury, CT, USA) equipped with a tapered microtip. The processing time was 1.5 s and the amplitude was set at 40% of the maximum value. After a 10-minute centrifugation (5500 x g) at +4°C, the supernatants were filtered through a 0,45 µm Bulk Acrodisc LC 13 mm filter (Pall Gelman Laboratory, USA). The sample volume to injector was 10 µl and the flow rate was 1.2 ml/min and potentials for detector electrodes were -175 mV and +250 mV (cell 5014B).

4.3.3.2 Selected blood parameters

The blood count was performed with an automated system composed of a Sysmex F-800 (Toa Medical Electronics Co., Japan) and a Sysmex Auto Diluter AD-260 (Toa Electronics, German). The following assays were carried: hemoglobin, red and white blood cells, hematocrit, MCV (mean corpuscular volume), MCH (mean corpus hemoglobin) and MCHC (mean corpus hemoglobin concentration). Reticulocytes were counted manually from a smear glass. Aspartate aminotransferase and alanine aminotransferase levels were determined from plasma samples according to the ECCL (European Committee for Clinical Laboratory) standards and creatinine (fS-Krea) by Jaffe´s reaction.

4.3.3.3 DA, 5-HT and their metabolites in dialysate samples

In order to determine DA, 5-HT and their metabolites DOPAC, HVA and 5-HIAA, 20-µl aliquots of dialysate samples were injected into an ESA (ESA Inc. Chelmsford, MA, USA) high performance liquid chromatography (HPLC) apparatus equipped with an Inertsil ODS-3V 5 µm (4.6 X 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_2EDTA, 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 a 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 mV and +250 mV.

4.3.3.4 Amphetamine, MDMA and cocaine in blood or brain tissue

Amphetamine and MDMA concentrations were analyzed with gas chromatography – mass spectrometry (GC-MS) by using a method described earlier (Kankaanpää et al., 2004). Briefly, amphetamine and MDMA were extracted from blood, and derivatized with heptafluorobutyric anhydride (HFBA) as follows: 200 µl of blood was mixed with 50 µl of alkaline buffer and 500 µl of extraction–derivatization reagent (toluene + HFBA + internal standard), centrifuged, and injected into a GC-MS apparatus. When analyzing brain tissue samples, the procedure was as follows: The deep frozen rat brains were dissected sagittally along the fissura longitudinalis cerebri, the left hemispheres were weighed and homogenized in 4 ml of 0.1 M $HClO_4$. After centrifugation, 200-µl aliquots of the supernatants were transferred to clean test tubes and the extraction-derivatization was performed according to the procedure described for blood samples.

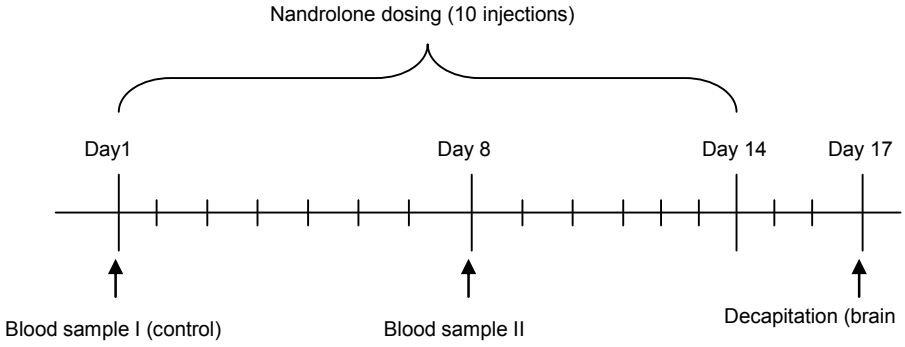
Trunk blood concentrations of cocaine and its metabolites benzoylecgonine and ecgonine methyl ester were measured using GC-MS. Briefly, cocaine and the metabolites were extracted from 1 ml of whole blood by solid phase extraction as follows: first, blood samples were acidified with sodium acetate buffer (pH 4) containing the internal standard benzoylecgonine-d3 (IS). 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 (PFPOH) and pentafluoropropionic anhydride (PFPA) and incubated. The excess derivatization reagents were evaporated and the residues were re-suspended in ethyl acetate. Then, a 2-µl aliquot of the sample was injected into the GC-MS. The analysis was performed with an Agilent (Agilent Technologies, Palo Alto, CA, USA) 6890N gas chromatograph, 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 of length 30 m, internal diameter 0.32 mm and film thickness 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 selected ion monitoring (SIM) mode. The limit of quantitation was 10 ng/ml for all the analyte.

4.3.3.5 Nandrolone in blood samples

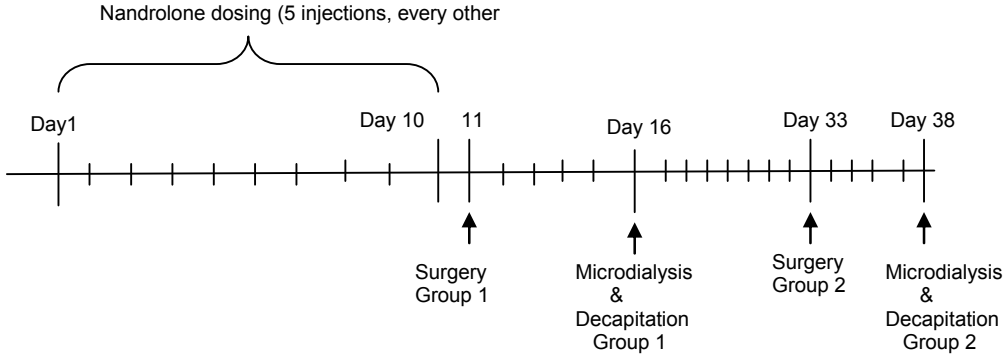
Blood nandrolone concentrations were measured with GC-MS as derivatives formed with N-methyl-n-(trimethylsilyl)trifluoroacetamide (MSTFA; Sigma Aldrich Co Ltd, Dorset, UK). The internal standard (IS), isotope labeled tetrahydrocannabinol (THC-D3) was from Cerilliant (Round Rock, TX, USA). Nandrolone was extracted from blood samples as follows: 5 ml of toluene containing IS 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 MSTFA. 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 320 µm and film thickness 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 quantitation was set at 0.5 µg/l.

4.3.4 Timelines for dosing and experiments

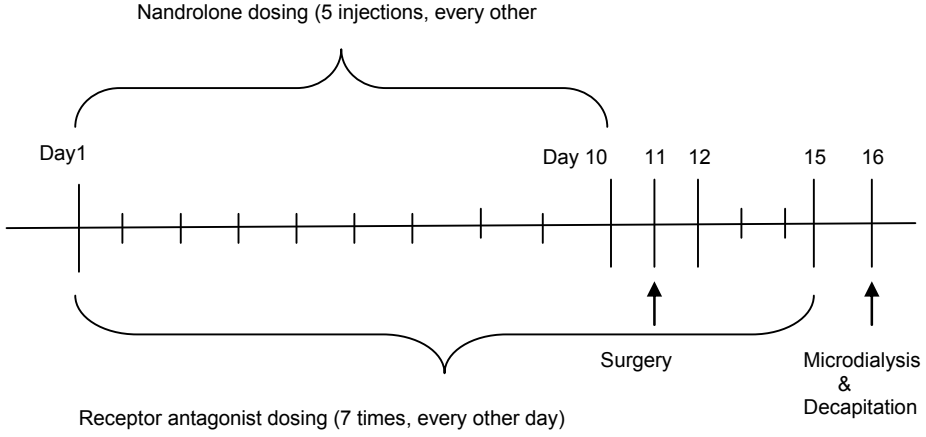
Paper I



Paper II & III



Paper IV



4.4 Behavioral experiments

4.4.1 Locomotor activity measurement

In papers II and III the behaviors of the rats during the microdialysis experiments 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. Locomotor activity of the animals was estimated from the number of complete passes across midline of the test cage at intervals of 20 min (corresponding to the sampling interval in the microdialysis experiments).

4.4.2 Characterization of drug-induced behavioral changes

In addition, a more detailed behavioral analysis was performed at the same time intervals. The beginning, frequency, and/or duration of different behavior was monitored visually for 1 min every 5th min. The behavioral patterns were scored according to a rating scale modified from previous studies described by Kankaanpää et al. (2002) and Chin et al. (2002). The rats were given a single behavioral score per each 1 min observation point and mean values were calculated for each 20-min sampling interval. Behavioral scores are seen in Table 3.

TABLE 3. Description of the stereotype behavior and related scores (per 1 min observation point).

Scoring (points)	Behavioral pattern
0:	Passive motionlessness; animal is stationary, lying or sleeping
1:	Active motionlessness; animal is lying, but alert
2:	Active motionlessness with occasional movements
3:	Sniffing, grooming, occasional locomotor activity
4:	Locomotor activity with burst of rearings and slender agitation
5:	Stereotyped behavior; compulsive-like, rapid, and repetitive purposeless behavior, such as intensive sniffing (e.g. during a prolonged rearing), head or body weaving or head bobbing
6:	Intense stereotyped behavior
7:	Ataxia; impairment in the ability of the animal to execute coordinated movements (Ataxia was defined as impairment in the ability of the animal to execute coordinated motor responses leading, in the extreme, to incapacitation*)

* According to Sturgeon et. al. (1979).

4.5 Statistical analysis

The body weights measured and blood samples collected from the animals before the treatments were considered to represent basal levels from which relative changes after treatments were calculated. The statistical comparisons of body weights and blood counts were performed with ANOVA for repeated measurements and pair wise comparisons were made using Bonferroni´s test. Neurotransmitter levels and plasma ASAT, ALAT and creatinine concentrations were analyzed by one-way ANOVA followed by Bonferroni´s test. In the microdialysis experiments the mean of the four samples before the drug treatments was considered as basal release (100 %) of neurotransmitters, according to which relative changes after the injections were calculated. The absolute basal releases were calculated from the measured values. In the locomotor activity -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 (AUC) with the trapezoidal method, after which the data was then subjected to one-way analysis of variance (ANOVA) followed by Tukey´s test. The results are presented as means ± SEM (standard error of the mean) and the level of statistical significance was set at $p \leq 0.05$. 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 MDMA blood concentrations were analyzed with one-way ANOVA followed by Tukey´s test. The correlation between neurochemical and behavioral data were analyzed with linear Pearson correlation method. The results are presented as means ± SEM (standard error of the mean) and the level of statistical significance was set at $p \leq 0.05$. SPSS Statistics 16 was used as statistical program.

5 Results

5.1 The effects of nandrolone in brain regions regulating motivation and reward-related associative learning

5.1.1 Neurochemical measurements

The data from study I suggests that treatment with supra-therapeutical doses of the AAS nandrolone decanoate induces changes in the dopaminergic and serotonergic neuronal systems in the brains of rats. As evident from Table 4, both doses of nandrolone significantly increased the levels of DOPAC in the cerebral cortex, and 20 mg/kg of nandrolone increased the concentrations of 5-HT in the cerebral cortex.

For the higher dose the 5-HT levels in the hypothalamus and in the hippocampus were higher when compared to the lower nandrolone dose, but not so much when compared to the control group. The 5-HIAA levels in the prefrontal cortex had increased in rats treated with nandrolone of 5 mg/kg compared with the control animals. Nandrolone (5mg/kg) significantly increased the DOPAC/DA ratio in the hypothalamus when compared with the control group.

TABLE 4. Summary of the effects of two different nandrolone treatments on DA, DOPAC, HVA, 5-HT and 5-HIAA concentrations and ratios of DOPAC/DA, HVA/DA and 5-HIAA/5-HT in brains of rats. Only the results been found statistically significant ($p < 0.05$) in the original papers are presented here.

	Control Arach. oil	5 mg/kg nandrolone	20 mg/kg nandrolone
Cerebral cortex			
DOPAC	67 ± 5.6	94 ± 8.3*	113 ± 5.6**
5-HT	163 ± 10.7	150 ± 8.9	203 ± 9.1*
Prefrontal cortex			
5-HIAA	510 ± 22.4	618 ± 33.5*	473 ± 30.2
Hippocampus			
5-HT	110 ± 6.5	83 ± 8.5	115 ± 8.2#
Hypotalamus			
5-HT	385 ±25.7	329 ± 21.2	453 ± 27.9##
DOPAC/DA	1.21 ± 0.15	1.65 ± 0.43#	1.17 ± 0.09

* $P < 0.05$, ** $P < 0.01$ significant changes from the control group.
# $P < 0.05$, ## $P < 0.01$ significant changes from the other nandrolone group.
Data is expressed as mean ng amine/g tissue ± s.e.m. n = 10–12.

5.1.2 Blood parameters

Figure 10 summarizes the effects of nandrolone (5 and 20 mg/kg) on the blood count. The effects of nandrolone on erythrocyte, hemoglobin and reticulocyte concentrations were statistically significant. In addition, after nandrolone treatment the levels of hemoglobin and erythrocytes increased, and reticulocyte levels decreased. The change in concentrations of hemoglobin, erythrocytes and reticulocytes, when compared with the control group, was significant only after the lower dose. The dose of 20 mg/kg nandrolone reduced plasma creatinine concentrations.

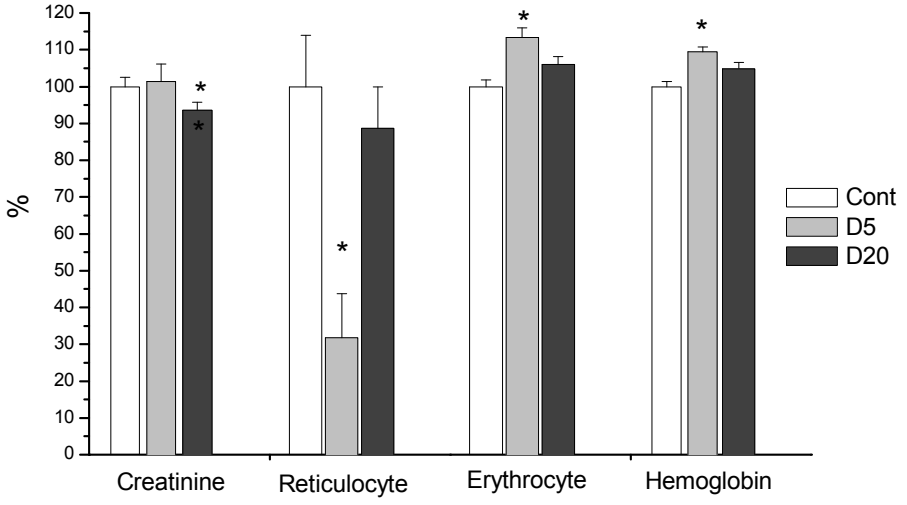


Figure 10. Comparison of the two different doses of nandrolone decanoate and arachinoid oil (control) on different blood parameters. Samples were collected after decapitation. Data expressed as percentages of basal level (control group = 100 %), and are given as means ± SEM. *P < 0.05, compared to control group, Bonferroni's test (N = 6). Only the results which have been found statistically significant (p < 0.05) in the original papers are presented here.
Cont = Control/vehicle, D5 = nandrolone decanoate 5 mg/kg, D20 = nandrolone decanoate 20 mg/kg.

5.2 The combined effects of nandrolone and stimulant drugs

The pre-treatment with nandrolone did not affect spontaneous release of the transmitters or their metabolites. The basal levels also remained unaltered after acute saline injection during the microdialysis experiments (see for original publications).

The increase in extracellular DA concentration induced by amphetamine, MDMA and cocaine in the NAc was attenuated by sub-chronic treatment with the AAS, nandrolone. The magnitude of changes depended on the dose of nandrolone administered. Both doses of nandrolone also inhibited cocaine induced 5-HT outflow in the NAc dose-dependently. However, only the lower dose of nandrolone attenuated MDMA-induced increase in 5-HT-levels, while the higher dose potentiated it.

Table 5 summarizes the effects of nandrolone co-treatment with amphetamine, MDMA and cocaine on extracellular concentrations of DA, 5-HT and their metabolites in the NAc.

TABLE 5. Summary of the acute effects of psychostimulant drugs alone and co-treated with nandrolone decanoate on the extracellular concentrations of DA, 5-HT and their metabolites in the NAc.

Treatment	DA	DOPAC	HVA	5-HT	5-HIAA
Amphetamine	↑↑↑↑↑	↓↓	↓	-	-
Nandro 5 + Amphetamine	↑↑↑↑	↓	↓	-	-
Nandro 20 + Amphetamine	↑↑↑	↓	↓	-	-
MDMA	↑↑↑↑	↓↓	↓↓	↑↑↑↑↑	↓
Nandro 5 + MDMA	↑↑↑	↓	↓	↑↑↑↑	↓
Nandro 20 + MDMA	↑↑	↓	↓	↑↑↑↑↑↑	↓↓
Cocaine	↑↑↑↑	↓	↓	↑↑↑	-
Nandro 5 + Cocaine	↑↑↑	↓	↓	↑↑	-
Nandro 20 + Cocaine	↑↑	↓	↓	↑	-

The arrows show the change induced by drug treatments: ↑ maximal increase to more than 100 % of the basal output, ↑↑ max increase to more than 200 %, ↑↑↑ max increase to more than 300 %, ↑↑↑↑ max increase to more than 600 %, ↑↑↑↑↑ max increase to more than 1000 %, ↑↑↑↑↑↑ max increase to more than 2000 %, ↓ max decrease to less than 80 %, ↓↓ max decrease to less than 50 %. All results presented here with arrows have been found statistically significant ($p < 0.05$) as evaluated by statistical tests in the original papers; (-) indicates no significant change.

5.2.1 Behavior

Amphetamine, MDMA and cocaine produced a rapid and long lasting increase in locomotor activity and stereotypic behavior (Figure 11). Nandrolone pre-treatment *per se* or acute saline injections did not alter the behavior of the rats. Analysis of the behavioral data suggests that effects of amphetamine, MDMA and cocaine are attenuated by AAS-treatment, paralleling observed monoamine results. After the stimulant injection, the behavior of the nandrolone-pretreated animals was less stereotyped than those observed animals pre-treated with vehicle-oil. Nandrolone pre-exposure also modified the ability of amphetamine, MDMA and cocaine to cause increased locomotor activity. Both nandrolone doses decreased stimulant

drugs induced locomotor activity, although the difference to when compared to vehicle treated animals was statistically significant only with the higher nandrolone dose with amphetamine and MDMA. The lack of statistical significance seems to be due to a higher variation among 5 mg/kg group.

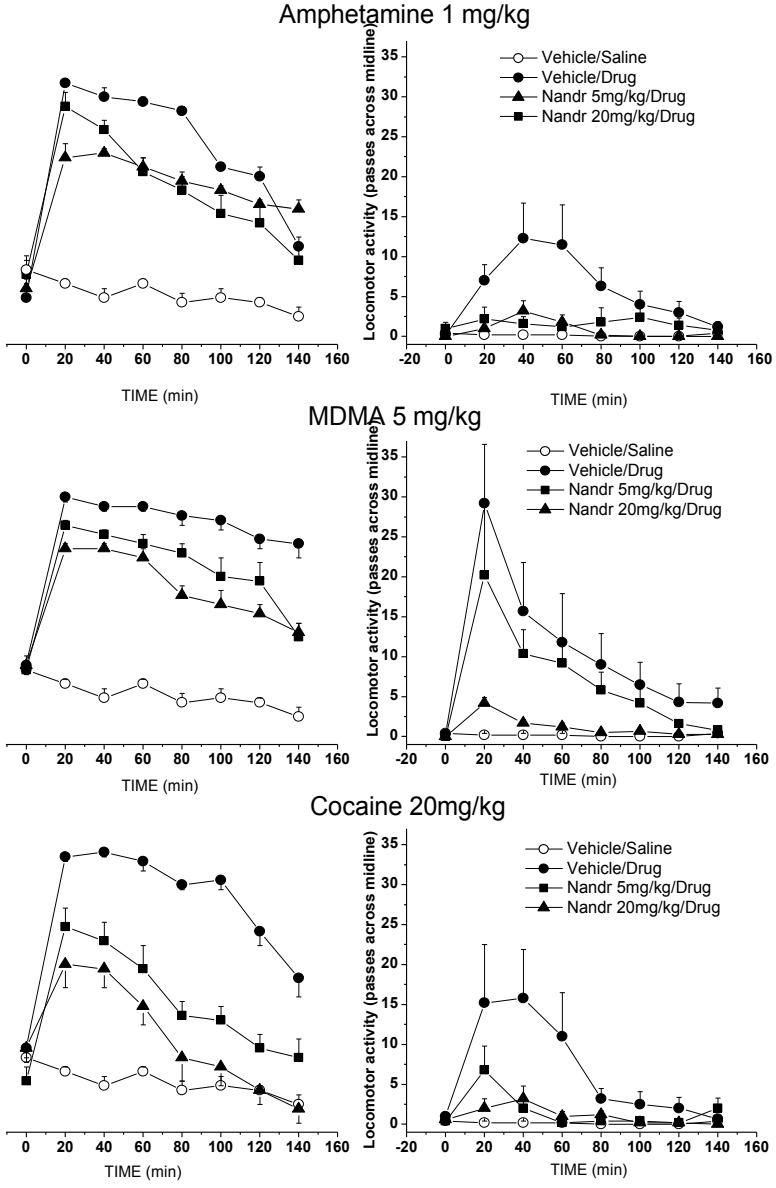


FIGURE 11. The behavioral effects of amphetamine, MDMA and cocaine together with nandrolone pre-treatment at doses of 5 and 20 mg/kg. Stimulant drugs were administered at 0 min. Data are given as means ± SEM (n = 6).

5.2.2 Blood drug concentrations

According to our preliminary study of one brain sample per treatment group, subchronic i.m. pre-treatment with nandrolone at doses of 5 and 20 mg/kg caused only negligible changes in brain tissue amphetamine concentration: results for all treatment groups were between 3.0 and 4.0 µg/g, with the lowest concentration measured for the group that received no nandrolone. In the case of MDMA (n = 2), however, the preliminary study showed more variable results: the MDMA brain concentrations in animals treated with arachinoid oil, nandrolone 5 mg/kg, and nandrolone 20 mg/kg were 5.2, 1.7, and 2.0 µg/g respectively. Therefore, MDMA was also measured from blood samples from 19 animals that were included in the neurochemical and behavioral study. The means of the MDMA blood concentrations in all treatment groups were 0.10–0.11 µg/ml. The concentrations of cocaine, benzoylecgonine or ecgonine methyl ester did not statistically differ between treatment groups.

5.2.3 Neurochemical and behavioral correlation

Correlation analysis between neurochemical and behavioral data was carried out. DA, its metabolites and 5-HT concentrations correlate strongly with locomotor activity and stereotyped behaviour after stimulant dosing (Table 6).

TABLE 6. Correlation between neurochemical and behavioral data.

Correlation between	DA	DOPAC	HVA	5-HT	5-HIAA
All animals:					
Behavior	.890***	-.636**	.468**	.871**	-.261
Locomotor activity	.721***	-.521**	.414**	.701**	-.273

**$p \leq 0.05$ ***$p \leq 0.001$. Data shown as correlation coefficient.

5.3 The effect of recovery period

5.3.1 Blood concentrations

Blood concentrations of nandrolone are show in figure 12. Animals treated with nandrolone decanoate differed from vehicle treated animals, as expected. The only exception was the 5 mg/kg -group after 28 recovery days, which did not differ statistically from the control groups. Every group after 28 recovery days differed statistically from the corresponding group after 6 recovery days in nandrolone concentration.

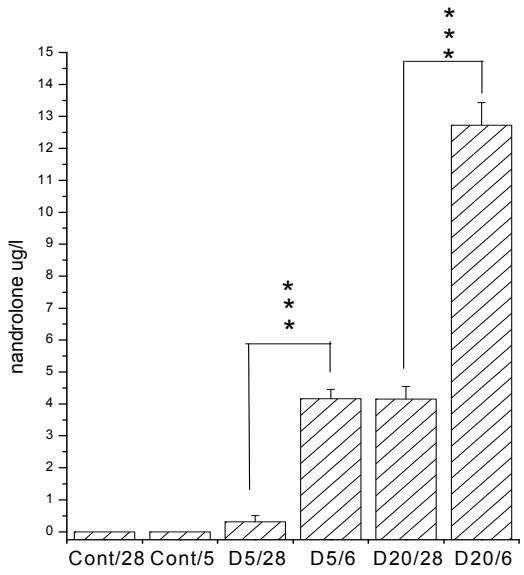


FIGURE 12. The effects of sub-chronic nandrolone i.m. injections on blood concentration of nandrolone (µg/l) in time of decapitation.

The values are expressed as mean ± SEM, ***$p \leq 0.001$
Cont/28 = Control 28 day recovery period, Cont/6 = Control 6 day recovery period, D5/28 = nandrolone 5 mg/kg 28 day recovery period, D5/6 = nandrolone 5 mg/kg 6 day recovery period, D20/28 = nandrolone 20 mg/kg 28 day recovery period, D20/6 = nandrolone 20 mg/kg 6 day recovery period.

5.3.2 Monoamines

The temporal profiles of the effects of nandrolone pre-treatments and cocaine injections on extracellular DA and 5-HT levels after 6- and 28-day recovery periods are shown in figure 13. Nandrolone pre-treatment decreased dose-dependently the cocaine-induced elevation of extracellular DA- and 5-HT-levels as compared to the vehicle. The length of the recovery period (6 fc. 28 days) had no effect on cocaine's action per se. The attenuating effect of nandrolone pre-treatment on cocaine-induced elevation of extracellular DA and 5-HT remained persistent over the 28-day recovery period after the last nandrolone dose. The absolute basal accumbal extracellular concentrations of DA and 5-HT did not differ significantly between the treatment groups. The levels remained unaltered in groups injected with saline after basal samples.

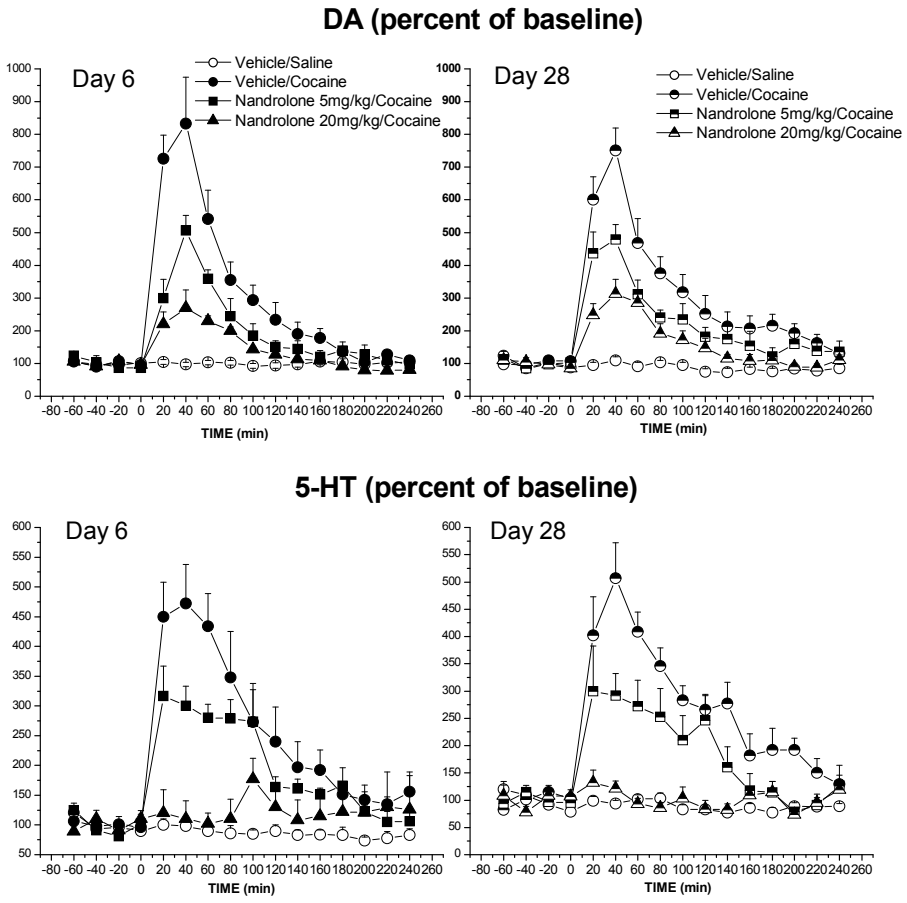


FIGURE 13. The effect of acute cocaine (20 mg/kg) on extracellular DA and 5-HT levels in the NAc after 6 days or 28 days from the last nandrolone dose. Cocaine was administered at 0 min. Data expressed as percentages of basal release are given as means ± S.E.M. (n = 6). All results presented here have been found statistically significant ($p < 0.05$) as evaluated by statistical tests in the original papers.

5.3.3 Behavior

Nandrolone pre-exposure modified the ability of cocaine to increase locomotor activity and stereotyped behavior. The effect of nandrolone dosing on cocaine-induced behavioral changes remained persistent over the 28-day recovery period when compared to the 6-day recovery period as seen in figure 14. The only exception was the nandrolone 5 mg/kg 28-day recovery -group in which the dose failed to attenuate statistically the locomotor activity induced by cocaine. The length of the recovery period (6 *vs*. 28 days) had no effect on cocaine's action *per se*.

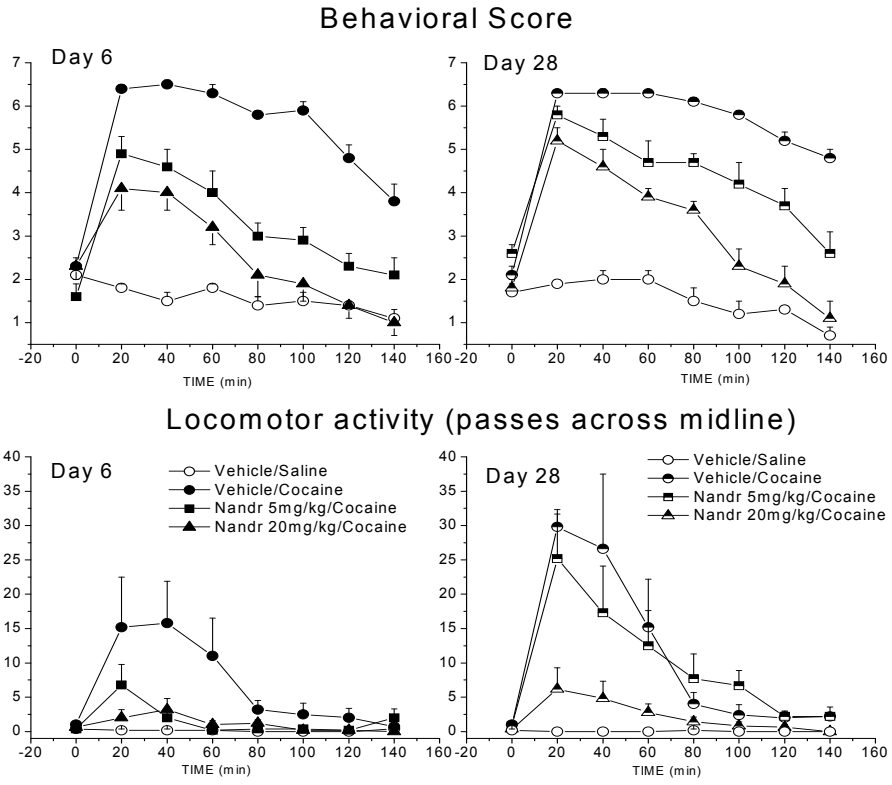


FIGURE 14. The effect of acute cocaine (20 mg/kg) injection on stereotyped behavior and locomotor activity after 6 days or after 28 days from the last nandrolone injection. Cocaine was administered at 0 min. (n = 6).

5.4 The effects of androgen and estrogen receptor blockade on nandrolone's attenuating effect on psychostimulant-induced neurochemical changes

The pretreatment with nandrolone (20 mg/kg) decreased the amphetamine-induced elevation of extracellular DA levels and increased the DOPAC and HVA levels compared to vehicle-oil treatment in the NAc.

Administration (i.g), of androgen and estrogen receptor antagonists, prevented nandrolone's attenuating effect on amphetamine-evoked monoamine release in the NAc as seen in figure 15. The dose of 50 mg/kg of flutamide, administered before nandrolone injection, restored the DA releasing effect of amphetamine. Flutamide compensated the modifying effects of nandrolone after amphetamine injection

also on extracellular DOPAC and HVA levels. The dose of 20 mg/kg of clomiphene (i.g.), administered before nandrolone injection, restored in part the DA releasing effect of amphetamine and also compensated the modifying effects on extracellular HVA levels. Pre-treatment with clomiphene seemed to have the same kind of effect on DOPAC, but without the statistical significance. Pre-treatment with nandrolone and flutamide or clomiphene did not affect spontaneous release of the transmitters or their metabolites.

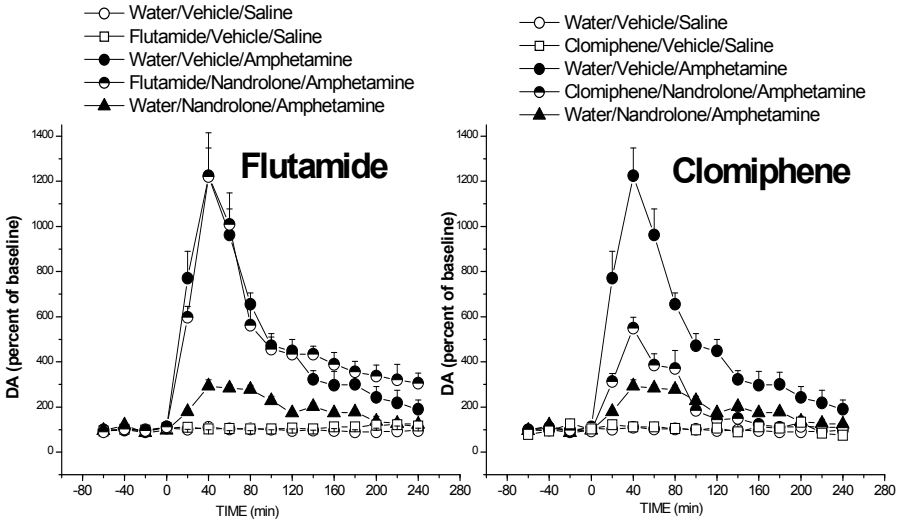


FIGURE 15. The effects of pre-treatment of flutamide (50 mg/kg) or clomiphene (20 mg/kg), and nandrolone (20mg/kg) together with acute amphetamine (1 mg/kg) injections (at 0 min) on extracellular DA and 5-HT levels in the NAc. Data expressed as percentages of basal release are given as means± S.E.M. (n = 5-6). All results presented here have been found statistically significant ($p < 0.05$) as evaluated by statistical tests in the original papers.

6 Discussion

6.1 The effects of nandrolone in brain regions regulating motivation and reward-related associative learning

In this study (paper I), increased levels of DOPAC as well as 5-HT were seen in the cerebral cortex after nandrolone dosing. Also, the lower dose of nandrolone significantly increased the DOPAC/DA ratio in the hypothalamus and 5-HIAA levels in the prefrontal cortex. DOPAC/DA ratio increased quite also in prefrontal cortex, however without statistical significance. Even though the few results of AASs effects on brain monoamine systems seen are diverse, our findings in the cerebral cortex are in line with previous studies indicating dopaminergic and serotonergic dysregulation in the rat brain following administration of nandrolone decanoate.

The drawback of the studies made is that findings on AAS induced changes in monoaminergic systems are not explicit. Aggregated data suggests that testosterone ultimately alters DA activity in the NAc (Thiblin et al., 1999) and chronic exposure to nandrolone affects the levels of DA receptors (Kindlundh et al., 2001b; Kindlundh et al., 2003b) and increase of DAT protein observed by binding studies done with in vitro autoradiography and PET technology (Kindlundh et al., 2002; Kindlundh et al., 2004) in rat brain. It has also been difficult to establish a uniform pattern of AASs' effects on rat brain 5-HT, possibly due to differences in study designs. There are results where AASs have no effect on 5-HT concentration in rat brain (Vermes et al., 1979), results where AAS treatment lowered the level of 5-HT (Lindqvist et al., 2002) and contrary results where Tamaki et al. (2003) recently observed increased levels of 5-HT and 5-HIAA in the cerebral cortex and hypothalamus after nandrolone treatment (more details are given in the section Review of the Literature). These inconsistent findings demonstrate that nandrolone does not have a uniform effect in all brain regions, all cell types in the same region, nor at all receptor types on the same neuron.

When speculating on the possible mechanisms that mediate nandrolone's action on monoamine metabolism, the possible activation of MAO is excluded, since it would have increased the metabolism of both 5-HT and DA, but in this experiment only DA metabolism was increased. This is in concert with an earlier study where nandrolone propionate had no effect on MAO-A activity (Thiblin et al., 1999). Our results here are limited by difficulty in determining whether the changes in 5-HT concentration correspond to an increased secretion or synthesis, or both, of 5-HT. However, on the basis of the sub-chronic nature of the treatment,

we believe that the altered levels observed in this study correspond principally to an increased biosynthesis, because an increased release is more likely to be an acute effect. There is a need for future studies to investigate the impact of varied AAS treatments on the mediating brain reward system.

It is tempting to speculate whether chronic treatment with supra-therapeutical doses of nandrolone mainly reflects a stimulatory influence on the mesolimbic DA system rather than the nigro-striatal DA system. Stimulation of the mesolimbic DA system is known to be related to reward (Koob, 1992). Taken together, these data indicate that an increase in dopaminergic neuronal activity might account for some of the positive effects, e.g. euphoria and increased self-esteem and confidence, which frequently appear as early effects following the administration of AASs to humans (Corrigan, 1996; Su et al., 1993).

Sub-chronic administration of nandrolone increased erythrocytes and hemoglobin concentrations, but reduced the growth of body mass and reduced levels of creatinine. The results are in line with earlier reports showing that use of AASs induces the synthesis of erythrocytes (Wu, 1997), increases hemoglobin concentrations (Besa, 1994; Shahidi, 1973) and decreases weight gain (Johansson et al., 2000a; Johansson et al., 2000b; Lindqvist et al., 2002). Decreased weight gain may be a consequence of reduced food intake, reported after nandrolone administration (Lindblom et al., 2003; Yu-Yahiro et al., 1989), but food consumption was not recorded in our study.

However, the concentrations of reticulocytes were unexpectedly reduced. This inverse effect may be explained by the rapid increase in red-cell mass. Although the administration of AASs to humans and laboratory animals has been associated with increased erythropoietin activity and an increased pool of erythropoietin responsive stem cells, this mechanism has not yet been entirely clarified.

Somewhat surprisingly, the effects of the lower dose appeared to be more pronounced than the effects of the higher dose, although the effects of AASs are generally considered to be dose related (Shahidi, 2001). Given that nandrolone has been shown to have a higher androgen receptor binding affinity than testosterone (Kumar et al., 1999; Roselli, 1998), and dosing in this study was highly supra-therapeutical, it can be speculated whether that saturation of androgenic receptors occurs already at the lower nandrolone dose. The apparently more pronounced effects of the lower dose could reflect the situation where this nandrolone dose has anabolic effects, while the extreme dose may cause spill over on other steroid receptors, e.g. the corticoid receptors, thereby triggering catabolic processes counteracting the androgen receptor mediated effects. Such a mechanism (catabolism) is in line with the significant loss of growth and reduced levels of creatinine displayed by the higher nandrolone treated animals.

Taken together, it appears that nandrolone at doses, high enough to induce erythropoiesis, induces changes in the dopaminergic and serotonergic neuronal systems in the brains of rats. These phenomena may account for some of the

observed central stimulatory properties that have been reported following AAS abuse.

6.2 The combined effects of nandrolone and stimulant drugs

The main findings of the present series of experiments are that sub-chronic pre-treatment with nandrolone decanoate attenuates dose-dependently the reward-related neurochemical and behavioral effects of acute amphetamine, MDMA and cocaine administration. Our findings are in line with the reported ability of nandrolone to decrease morphine-reward (Celerier et al., 2003) as well as preliminary findings (Mcallister and Compton, 1995) indicating that chronic administration of AASs blunts the subjective effects of cocaine in rats. The increase in extracellular DA concentration induced by amphetamine, MDMA and cocaine in the NAc was attenuated by sub-chronic treatment with the two different nandrolone doses, and both nandrolone doses also evoked changes in extracellular levels of 5-HT after MDMA and cocaine dosing.

The whole cascade of monoamine action, from synthesis to receptor activation, provides several possible mechanisms by which nandrolone might affect the dopaminergic and serotonergic response to stimulants. To exclude the additional possibility that drug pharmacokinetics are a mechanism, which would lead to a misinterpretation of data, we measured the blood MDMA and cocaine concentrations, proving that the ability of nandrolone to modulate the neurochemical and behavioral effects of stimulants is not due to nandrolone-induced changes in pharmacokinetics. As stated in the Review of the literature (Sections 2.2.1.2 and 2.2.2.2), there are only a few reports of the ability of nandrolone to affect DA or 5-HT synthesis or metabolism, and these reports are fairly diverging. Nevertheless, in the studies done here, nandrolone failed to affect the basal levels of DA, 5-HT and their metabolites in extracellular space in the NAc. Thus it seems that the steps of DA and 5-HT transmission (synthesis, storage or release), in all likelihood, are not the primary target of nandrolone action, but are, rather, in some other mechanisms mediated by transporter proteins or postsynaptic receptors. Mechanisms of action of the amphetamines include both DA and 5-HT reverse-transport and uptake inhibition and have markedly complicated the assessment of nandrolone actions on DAT and SERT. However, the dosing of cocaine, which can be considered as a pure uptake inhibitor, supports the hypothesis that nandrolone modifies the dopaminergic response to the psychomotor stimulant drug via DAT. This is supported by studies where chronic nandrolone treatment has been reported to increase both the amount of DAT and its affinity to DA (Kindlundh et al., 2002; Kindlundh et al., 2004). 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 MDMA and cocaine administration. Testosterone has been shown to increase the expression of SERT mRNA and the density of SERT sites in the forebrain (McQueen et al., 1999), but to the author's knowledge, no studies have been carried out with nandrolone. Our interpretation of the data – that upregulation of DAT and SERT sites would lead to reduction in cocaine effects – is based on an assumption that the 20 mg/kg dose of cocaine used in this study, does not saturate the DAT binding sites. There are, indeed, several studies that show that 40 mg/kg dose increases the extracellular concentration of DA and 5-HT in rat brain as compared to 20 mg/kg dose (Chen and Reith, 1994; Martin-Fardon et al., 1996; Sorge et al., 2005; Szumlinski et al., 2000). If the pre-treatment with nandrolone adds up the number of DAT and SERT, as speculated here, you can think that more DA and 5-HT is transported into the cell, because there are now more intact DAT and SERT.

However, the effects of nandrolone on MDMA-induced elevation of 5-HT levels were two sided: the lower dose of nandrolone decreased the effect of MDMA, while the higher dose potentiated it. Damage to 5-HT terminals following high doses of MDMA is well documented (O'Hearn et al., 1988; Ricaurte et al., 1993) and it is preceded by an acute stage in which substantial release of 5-HT leads to exhaustion of the neuronal 5-HT stores. An apparently similar outflow of 5-HT was observed after MDMA injection in rats pre-treated with the higher dose of nandrolone. Therefore, it can be speculated that high-dose sub-chronic nandrolone treatment might increase cell vulnerability to MDMA, leading to effects resembling the acute toxicity at lower concentrations of MDMA. This is supported also by behavioral results were animals locomotor activity were almost completely paralyzed. There is strong possibility that this is a reflection of the so-called 5-HT syndrome in rat (Grahame-Smith, 1971). Whether this phenomenon results from increased vulnerability of the neurons to acute stage of MDMA neurotoxicity or to some other mechanism, remains to be elucidated.

Acute administration of amphetamine, MDMA and cocaine, all induced a rapid and long-lasting increase in locomotor activation and stereotypic behavior. Furthermore, nandrolone pre-treatment attenuated dose-dependently both locomotor activity and stereotyped behavior induced by these stimulant drugs. These results are concordant with earlier studies in which testosterone has been shown to attenuate amphetamine induced locomotor activity and stereotyped behavior (Beatty et al., 1982; Dluzen et al., 1986; Savageau and Beatty, 1981). Correlation with microdialysis results is to be expected, because stimulant-induced hyperlocomotion is predominantly mediated by increased release of DA in the NAc (Bedford et al., 1980; Creese and Iversen, 1974; Wellman et al., 2002). One exception was that even though there was a potent increase in 5-HT concentration after the higher nandrolone dose and MDMA, there was no remarkable increase in

behavioral scores. This could be a result of significantly lowered DA concentration. Indeed, more lateral and vertical repetitive head movements, vigorous sniffing, forepaw treading and head weaving, many of which resemble behaviors characteristic of the so-called 5-HT syndrome in rat (Grahame-Smith, 1971), were seen in this group.

In conclusion, these data show that treatment with supra-therapeutical doses of the AAS, nandrolone decanoate, attenuates reward-related neurochemical effects as well as behavioral effects, induced by amphetamine, MDMA and cocaine dosing.

Correlation analysis between neurochemical and behavioral data was carried out and DA, its metabolites and 5-HT concentrations correlate strongly with locomotor activity and stereotyped behaviour seen after stimulant dosing, which is to be expected since it well established that monoamine concentrations in the NAc are strongly related to activation of locomotor and stereotyped behavior (see Review of the literature 2.3).

6.3 The effect of recovery period

The main finding of the present experiment (paper III) is that nandrolone-induced attenuation of reward-related neurochemical and behavioral effects caused by acute cocaine administration is seen for at least 28 days after the last nandrolone injection, despite the significant decrease in blood nandrolone concentration. The length of the recovery period (6 vs. 28 days) had no effect on cocaine's action *per se*. The extracellular concentrations of DA, and its metabolites, in samples collected from the NAc after the cocaine injection did not differ statistically between the 6-day recovery period group and the 28-day recovery period group. The attenuating effect of nandrolone on cocaine-induced elevation of extracellular DA also remained persistent over the 28-day recovery period. In addition, the effect of nandrolone on cocaine-induced behavioral changes remained persistent over the 28-day recovery period. The only exception was the lower nandrolone dose 28-day recovery group, which failed to attenuate statistically the locomotor activity induced by cocaine. These results are in line with the concept that addictive drugs are hypothesized to produce changes in brain dopaminergic pathways that endure long after the person stops taking the drugs. There are several studies showing that chronic administration of cocaine and methamphetamine is associated with, among other things, a decrease in D_2 receptors and DAT during withdrawal, and changes, when compared to non-users, can last up to 11 months after the last drug dosing (Volkow et al., 2001a; Volkow et al., 2001b; Volkow et al., 1990).

6.4 The effects of androgen and estrogen receptor blockade on nandrolone's attenuating effect on psychostimulant-induced neurochemical changes

The main finding of this series of experiments is that pre-treatment with the androgen receptor antagonist flutamide robustly prevents, and the estrogen receptor antagonist clomiphene partly reduces, the nandrolone's attenuating effect on increased extracellular DA in the NAc after amphetamine dosing.

The literature concerning the role of androgen and estrogen receptors in drug reward is scarce. In the study of Frye, flutamide failed to attenuate conditioned place preference (CPP) induced by a testosterone secondary metabolite, 3α-diol (Frye, 2007). These results, however, do not necessarily conflict with our findings, even though both methods may give an estimate of addictive potential of a drug. In the experimental set-up used by Frye (2007), the receptor blocker flutamide itself was found to cause CPP, which makes the results extremely difficult to interpret.

Intracellular ARs (the so-called "classical" receptors), are found in the NAc, but their density is shown to be relatively low (Balthazart et al., 1998; Stumpf and Sar, 1976). Therefore, it is possible that the site of action could be outside the NAc, although the number of receptors in the brains of the animals may be higher due to the sub-chronic treatment with nandrolone, which has been shown to up-regulate intracellular (nuclear) AR in male rats (Menard and Harlan, 1993; Wesson and McGinnis, 2006). Outside the NAc, steroids may regulate monoamine release indirectly by modulating the activity of ascending inputs from monoaminergic nuclei that project to the NAc: the circuits via which nuclear AR-positive cells in bed nucleus of the stria terminalis and medial preoptic area projecting to the ventral tegmental area (VTA). Androgen sensitive cells in the lateral hypothalamus project to the VTA as well (Sato et al., 2008), and VTA itself contains some androgen receptors (Kritzer, 1997). These projections provide an opportunity for androgens to modify the activity of the mesocorticolimbic DA system. Finally, many of the brain areas that supply afferents to midbrain DA nuclei are themselves rich in classical steroid receptors, and are in a position to provide powerful, albeit indirect, genomic influence over the midbrain system (Kritzer, 1997). Recent studies in rats have also demonstrated rapid cellular effects of androgens in brain regions that possess only relatively few classical receptors (Mermelstein et al., 1996). These faster androgen actions are thought to be mediated by "non-classical" receptors, the membrane steroid receptors (Sato et al., 2008). Whereas the distribution of classical steroid receptors in rodent brain is relatively limited, the potential brain targets for androgen action via membrane receptors are much broader.

Aromatase transforms some portion of the androgens to estrogens through oxidation and subsequent elimination of a methyl group (Schade and Schubert, 1979). Nandrolone is shown to be a substrate for aromatase enzyme (Ryan, 1959) and is shown to stimulate the aromatase activity, which is related to the ability of estrogens of nandrolone origin to bind estrogen receptors in rat brain (Roselli, 1998). This is in line with findings of ours, that blockade of ER also reduces partly the nandrolone's ability to attenuate amphetamine-induced increase in extracellular DA in the NAc. It is suggested that the modifications of both ARs and ERs in the NAc by AASs, and likely AAS metabolites, are responsible for behavioral complexity associated with abuse of anabolic steroids (van de Poll et al., 1986).

Steroid receptors, when occupied by a ligand, act as transcription factors to modulate gene expression, which in turn results in a host of neurobiological changes. Receptor activation could lead to alterations in neuronal excitability and signal transduction in brain regions implicated in addiction, which in turn could change the organism's neurochemical response to stimulant drugs in brain. However, because this study gives little further indication on the mechanism by which these effects are mediated, they are not further discussed here.

Even with evidence as strong as seen here of involvement of the ARs and ERs, it has to be remembered that synthetic AASs and their metabolites do not only bind to ARs and ERs but also, with higher doses, to glucocorticoid and progestin receptors (Janne, 1990). They have also been shown to interact with GABA (Masonis and McCarthy, 1995) and 5-HT receptors (Kindlundh et al., 2003a). Consequently, the effects of AAS are far from purely androgenic and may involve actions at multiple genomic and non-genomic substrates. These other mechanism could explain some the minor peripheral effects seen here, however, if receptor blocking is presumed to be fairly selective, there can be said that the brain effects seems to mediated via ARs and ERs.

7 Summary and conclusions

This thesis reports alterations in the male rat brain dopaminergic and serotonergic systems, induced by subchronic nandrolone decanoate administration and co-administration with psychostimulant drugs. The reduced activation of dopaminergic neurons may correspond to the increased prevalence of illicit drug usage among people who self-administer AASs and abusers of AASs may require larger doses of drugs to achieve the desired effects. However, because pre-treatment with nandrolone substantially decreases the efficacy of stimulant drugs on extracellular DA and 5-HT as well as related behaviors in the present studies, 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.

The main outcomes from the studies included in this thesis are:

1) Nandrolone decanoate at doses, high enough to induce erythropoiesis, induces changes in the dopaminergic and serotonergic system in the in the cerebral cortex, the area that regulates motivation and emotions as well as higher cognitive functions.
2) Amphetamine, MDMA and cocaine induced hedonic effects, as measured by increasing of extracellular DA and 5-HT in NAc and increased locomotor activity, as well as stereotyped behavior are attenuated dose-dependently by sub-chronic treatment with nandrolone. The results suggest that chronic AAS treatment alters neurochemical and behavioural responses related to stimulant addictive properties.
3) Despite the significant decrease in nandrolone concentration in blood, the attenuation of cocaine's effects remained unchanged after a fairly long period without nandrolone (28 days cf. 6 days), suggesting that AAS treatment would produce long-term changes in reward brain circuits.
4) Androgen receptor antagonism attenuated the effects of nandrolone pretreatment on amphetamine-induced elevation of extracellular DA and 5-HT concentrations in NAc suggesting that hormonal systems are involved in AASs ability to modulate the dopaminergic and serotonergic effects of acute injection of amphetamine. So, it seems that the ability of nandrolone to modulate reward-related effects of amphetamine is, at least in part dependent on activation of the ARs or ERs.

8 Acknowledgements

This work was carried out at the Department of Alcohol, Drugs and Addiction, National Institute for Health and Welfare, Helsinki. I thank Professors Pekka Puska, Director General of National Institute for Health and Welfare and Pekka Hakkarainen, Head of the Department of Alcohol, Drugs and Addiction, for the prime operational environment the institute has provided.

The financial support from the Finnish Foundation for Alcohol Research is gratefully acknowledged.

I wish to thank the reviewers of this thesis, Docent Petteri Piepponen and Professor Markku Koulu, for their valuable comments and criticism. I am furthermore grateful to M.Sc Thomas Blencowe for the linguistic revision of the manuscript. All this is manly improved the thesis.

I would express my deepest gratitude to Docent Timo Seppälä, my teacher and adviser, for initiating me into this highly interesting field of steroid research, and for giving me the opportunity to perform this work. His excellent knowledge and guidance throughout this work made the completion of this thesis work possible.

My sincerest gratitude goes to PhD Aino Kankaanpää, for introducing me to the fascinating world of microdialysis and neuropharmacology. I am deeply grateful for all the guidance and support she has given me during the years.

I wish to thank the people at Alcohol and Drug Analytic Unit for providing supportive and friendly atmosphere, in which it is a pleasure to belong.

Last but not least, my warmest thanks go to my parents, my dear sister and my love one, Markus, for their support, understanding and encouragement.

References

Alexander, G. M., Packard, M.G., Hines, M., 1994. Testosterone has rewarding affective properties in male rats: implications for the biological basis of sexual motivation. Behav Neurosci. 108, 424–8.

Angrist, B., Sudilowsky, A., 1978. Central nervous system stimulants: historical aspects and clinical effects. In: Handbook of Psychopharmacology, Vol. 11: Stimulants (Iversen LL, Iversen SD & Snyder SH, eds). Plenum Press, New York.

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

Bahrke, M. S., Yesalis, C.E., Kopstein, A.N., Stephens, J.A., 2000. Risk factors associated with anabolic-androgenic steroid use among adolescents. Sports Med. 29, 397–405.

Bahrke, M. S., Yesalis, C. E., 2004. Abuse of anabolic androgenic steroids and related substances in sport and exercise. Curr Opin Pharmacol. 4, 614–20.

Ballard, C. L., Wood, R. I., 2005. Intracerebroventricular self-administration of commonly abused anabolic-androgenic steroids in male hamsters (Mesocricetus auratus): nandrolone, drostanolone, oxymetholone, and stanozolol. Behav Neurosci. 119, 752–8.

Balthazart, J., Foidart, A., Houbart, M., Prins, G.S., Ball, G.F., 1998. Distribution of androgen receptor-immunoreactive cells in the quail forebrain and their relationship with aromatase immunoreactivity. J Neurobiol. 35, 323–40.

Bardo, M. T., 1998. Neuropharmacological mechanisms of drug reward: beyond dopamine in the nucleus accumbens. Crit Rev Neurobiol. 12, 37–67.

Barnes, N. M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology. 38, 1083–152.

Basaria, S., Wahlström, J.T., Dobs, A.S., 2001. Clinical review 138: Anabolic-androgenic steroid therapy in the treatment of chronic diseases. J Clin Endocrinol and Metab. 86, 5108–17.

Baselt, R., 2004. Disposition of Toxic Drugs and Chemicals in Man. Biomedical Publications, Foster City, California.

Baumann, M. H., Wnag, W., Rothman, R.B., 2007. 3,4-Methylene-dioxymeth-amphetamine (MDMA) neuro-toxicity in rats: a reappraisal of past and present findings. Psychopharmacology (Berl). 189, 407–24.

Beatty, W. W., Dodge, A.M., Traylor, K.L., 1982. Stereotyped behavior elicited by amphetamine in the rat: influences of the testes. Pharmacol Biochem Behav. 16, 565–8.

Bedford, J. A., Borne, R.F., Wilson, M.C., 1980. Comparative behavioral profile of cocaine and norcocaine in rats and monkeys. Pharmacol Biochem Behav. 13, 69–75.

Bernschneider-Reif, S., Oxler, F., Freudenmann, R.W., 2006. The origin of MDMA ("ecstasy") – separating the facts from the myth. Pharmazie. 61, 966–72.

Besa, E. C., 1994. Hematologic effects of androgens revisited: an alternative therapy in various hematologic conditions. Semin Hematol. 31, 134–45.

Birgner, C., Kindlundh-Högberg, A.M.S., Ploj, K., Lindblom, J., Nyberg, F., Bergström, L., 2006. Effects on rat brain dopamine and dopac levels after sub-chronic nandrolone administration followed by an aphetamine challenge. Pharmacolocyonline. 3, 99–108.

Birgner, C., Kindlindh-Högberg, A.M., Nyberg, F., Bergström, L., 2007. Altered extracellular levels of DOPAC and HVA in the rat nucleus accumbens shell in response to sub-chronic nandrolone administration and a subsequent amphetamine challenge. Neurosci Lett. 412, 168–72.

Birgner, C., Kindlindh-Högberg, A.M., Alsio, J., Lindblom, J., Schioth, H.B., Bergström, L., 2008. The anabolic androgenic steroid nandrolone decanoate affects mRNA expression of dopaminergic but not serotonergic receptors. Braia Res. 1240, 221–8.

Bitar, M. S., Ota, M., linnoila, M., Shapido, B.H., 1991. Modification of gonadectomy-induced increases in brain monoamine metabolism by steroid hormones in male and female rats. Psychoneuroendocrinology. 16, 547–57.

Björklund, A., Lindvall, O., Dopamine-containing systems in the CNS. In: Handbook of chemical neuroanatomy:

classical transmitters in the CNS. Elsevier Science BV., Amsterdam, 1984, pp. 55–122.

Blaschko, H., 1952. Amine oxidase and amine metabolism. Pharmacol Rev. 4, 415–58.

Brower, K. J., Blow, F.C., Bedesford, T.P., Fuelling, C., 1989. Anabolic-androgenic steroid dependence. J Clin Psychiatry. 50, 31–3.

Brower, K. J., Eliopulos, G.A., Blow, F.C., Ctlin, D.H., Beresford, T.P., 1990. Evidence for physical and psychological dependence on anabolic androgenic steroids in eight weight lifters. Am J Psychiatry. 147, 510–2.

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, 759–68.

Brower, K. J., 2002. Anabolic steroid abuse and dependence. Curr Psychiatry Rep. 4, 377–87.

Brown-Séquard, C. E., Note on the effects produced on man by subcutaneous injections of a liquid obtained from the testicles of animals. Lancet, 1889, pp. 105-107.

Brown, P., Molliver, M. E., 2000. Dual serotonin (5-HT) projections to the nucleus accumbens core and shell: relation of the 5-HT transporter to amphetamine-induced neurotoxicity. J Neurosci. 20, 1952–63.

Burnett, K. F., Kleiman, M. E., 1994. Psychological characteristics of adolescent steroid users. Adolescence. 29, 81–9.

Caggiula, A. R., 1970. Analysis of the copulation-reward properties of posterior hypothalamic stimulation in male rats. J Comp Physiol Psychol 70, 399–412.

Callaway, C. W., Johnson, M.P., Gold, L.H., Nichols, D.E., Geyer, M.A., 1991. Amphetamine derivatives induce locomotor hyperactivity by acting as indirect serotonin agonists. Psychopharmacology. 104, 293–301.

Campbell, H. J., 1970. The effect of steroid hormones on self-stimulation, central and peripheral. Steroidologia. 1, 8–24.

Carboni, E., Imperato, A., Perezzani, L., Di Chiara, G., 1989. Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience. 28, 653–61.

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

Celerier, E., Ahdepill, T., Wikander, H., Berrendo, F., Nyberg, F., Maldano, R., 2006. Influence of the anabolic-androgenic steroid nandrolone on cannabinoid dependence. Neuropharmacology. 50, 788–806.

Chen, N. H., Reith, M. E., 1994. Autoregulation and monoamine interactions in the ventral tegmental area in the absence and presence of cocaine: a microdialysis study in freely moving rats. J Pharmacol Exp Ther. 271, 1597–610.

Chin, J., Sternin, O., Wu, H.B., Burrell, S., Lu, D., Jenab, S., 2002. Endogenous gonadal hormones modulate behavioral and neurochemical responses to acute and chronic cocaine administration. Brain Res. 945, 123–30.

Choi, P. Y., Pope, H. G., Jr., 1994. Violence toward women and illicit androgenic-anabolic steroid use. Ann Clin Psychiatry. 6, 21–5.

Clark, A. S., Lindenfeld, R.C., Gibbons, C.H., 1996. Anabolic-androgenic steroids and brain reward. Pharmacol Biochem Behav. 53, 741–5.

Cooper, J. R., Bloom, F.E., Roth, H. R., 2003. Dopamine In The Biochemical Basis of Neuropharmacology. Oxford University Press, New York.

Copeland, J., Peters, R., Dillon, P., 2000. Anabolic-androgenic steroid use disorders among a sample of Australian competitive and recreational users. Drug Alcohol Depend. 60, 91–6.

Corrigan, B., 1996. Anabolic steroids and the mind. Med J Aust. 165, 222–6.

Creese, I., Iversen, S. D., 1974. The role of forebrain dopamine systems in amphetamine induced stereotyped behavior in the rat. Psychopharmacologia. 39, 345–57.

Dahlström, A., Fuxe, K., 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration od monoamines in the cell bodies of brain stem neurons. Acta Physiol Scand Suppl SUPPL 232:1–55.

de Beun, R., Jansen, E., Slangen, J.L., Van de Poll, N.E., 1992. Testosterone as appetitive and discriminative stimulus

in rats: sex- and dose-dependent effects. Physiol Behav. 52, 629–34.

Di Chiara, G., North, R. A., 1992. Neurobiology of opiate abuse. Trends Pharmacol Sci. 13, 185–93.

Di Chiara, G., 1995. The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend. 38, 95–137.

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

Dluzen, D. E., Green, M.A., Ramirez, V.D., 1986. The effect of hormonal condition on dose-dependent amphetamine-stimulated behaviors in the male rat. Horm Behav. 20, 1–6.

DuRant, R. H., Escobeno, L.G., Heath, G.W., 1995. Anabolic-steroid use, strength training, and multiple drug use among adolescents in the United States. Pediatrics. 96, 23–8.

Eklof, A. C., Thurelius, A.M., Garle, M., Rane, A., Sjöqvist, F., 2003. The anti-doping hot-line, a means to capture the abuse of doping agents in the Swedish society and a new service function in clinical pharmacology. Eur J Clin Pharmacol.

Erlander, M. G., Lovenberg, T.W., Baron, B.M., de Lecea, L., Danielson, P.E., RAche, M., 1993. Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. Proc Nat Acad Sci U S A. 90, 3452–6.

Faigenbaum, A. D., Zaichkowsky, L.D., Gardner, D.E., Micheli, L.J., 1998. Anabolic steroid use by male and female middle school students. Pediatrics. 101, E6.

Ferris, R. M., Tang, F.L., Maxwell, R.A., 1972. A comparison of the capacities of isomers of amphetamine, deoxypipradrol and methylphenidate to inhibit the uptake of tritiated catecholamines into rat cerebral cortex slices, synaptosomal preparations of rat cerebral cortex, hypothalamus and striatum and into adrenergic nerves of rabbit aorta. J Pharmacol Exp Ther. 181, 407–16.

Filinger, E. J., Stefano, F. J., 1982. Monoamine oxidase inhibition by d-amphetamine in ganglia and nerve endings. Experientia. 38, 844–5.

Fragkaki, A. G., Angelis, Y.S., Koupparis, M., Tsantili-Kakoulugou, A., Kokotos, G., Georgakopoulos, C., 2009. Structural characteristics of anabolic androgenic steroids contributing to binding to the androgen receptor and to their anabolic and androgenic activities. Applied modifications in the steroidal structure. Steroids. 74, 172–97.

Frye, C. A., 2007. Some rewarding effects of androgens may be mediated by actions of its 5alpha-reduced metabolite 3alpha-androstanediol. Pharmacol Biochem Behav. 86, 354–67.

Fudala, P. J., Weinrieb, R.M., Calarco, J.S., KAmpman, K.M., Boardman, C., 2003. An evaluation of anabolic-androgenic steroid abusers over a period of 1 year: seven case studies. Ann Clin Psychiatry. 15, 121–30.

Gay, G. R., Inaba, D.S., Sheppard, C.W., Newmeyer, J.A., 1975. Cocaine: history, epidemiology, human pharmacology, and treatment. a perspective on a new debut for an old girl. Clini Toxicol. 8, 149–78.

Gianoulakis, C., 2009. Endogenous opioids and addiction to alcohol and other drugs of abuse. Curr Top Med Chem. 9, 999–1015.

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, 43–9.

Goeders, N. E., McNulty, M.A., Mirkis, S., McAllister, K.H., 1989. Chlordiazepoxide alters intravenous cocaine self-administration in rats. Pharmacol Biochem Behav. 33, 859–66.

Goeders, N. E., McNulsty, M.A., Guerin, G.F., 1993. Effects of alprazolam on intravenous cocaine self-administration in rats. Pharmacol Biochem Behav. 44, 471–4.

Gough, B., Ali, S.F., Sliker, W., Holson, R.R., 1991. Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoamines in rat caudate. Pharmacol Biochem Behav. 39, 619–23.

Graham, M. R., Davies, B., Grace, F.M., Kicman, A., Baker, J.S., 2008. Anabolic steroid use: patterns of use and detection of doping. Sports Med. 38, 505–25.

Grahame-Smith, D. G., 1971. Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. J Neurochem. 18, 1053–66.

Green, A. R., Mechan, A.O., Elliot, J.M., O´shea, E., Colado, M.I., 2003. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol Rev. 55, 463–508.

Gruber, A. J., Pope, H. G., Jr., 2000. Psychiatric and medical effects of anabolic-androgenic steroid use in women. Psychother Psychosom. 69, 19–26.

Gustavsen, I., Morland, J., Bramness, J.G., 2006. Impairment related to blood amphetamine and/or methamphetamine concentrations in suspected drugged drivers. Accid Anal Prev. 38, 490–5.

Heikkila, R. E., 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, 847–52.

Hernandez, L., Lee, F., Hoebel, B.G., 1987. Simultaneous microdialysis and amphetamine infusion in the nucleus accumbens and striatum of freely moving rats: increase in extracellular dopamine and serotonin. Brain Res Bull. 19, 623–8.

Hiramatsu, M., DiStefano, E., Chang, A.S., Cho, A.K., 1991. A pharmacokinetic analysis of 3,4-methylenedioxymethamphetamine effects on monoamine concentrations in brain dialysates. Eur J Pharmacol. 204, 135–40

Hoberman, J. M., Yesalis, C. E., 1995. The history of synthetic testosterone. Sci Am. 272, 76–81.

Hruska, R. E., Pitman, K. T., 1982. Distribution and localization of estrogen-sensitive dopamine receptors in the rat brain. J Neurochem. 39, 1418–23.

Hurd, Y. L., Ungerstedt, U., 1989a. Cocaine: an in vivo microdialysis evaluation of its acute action on dopamine transmission in rat striatum. Synapse. 3, 48–54.

Hurd, Y. L., Ungerstedt, U., 1989b. In vivo neurochemical profile of dopamine uptake inhibitors and releasers in rat caudate-putamen. Eur J Pharmacol. 66, 251–60.

Jaber, M., Jones, S., Giros, B., Caron, M.G., 1997. The dopamine transporter: a crucial component regulating dopamine transmission. Mov Disord. 12, 629–33.

Janne, O. A., 1990. Androgen interaction through multiple steroid receptors. NIDA Res Monogr. 102, 178–86.

Johansson, P., Hllberg, P., 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, 413–8.

Johansson, P., Lindqvist, A., Nyberg, C., 2000b. Anabolic androgenic steroids affects alcohol intake, defensive behaviors and brain opioid peptides in the rat. Pharmacol Biochem Behav. 67, 271–9.

Johnson, M. P., Hoffman, A.J., Nichols, D.E.,, 1986. Effects of the enantiomers of MDA, MDMA and related analogues on [3H]serotonin and [3H]dopamine release from superfused rat brain slices. Eur J Pharmacol. 132, 269–76.

Johnson, L. R., Wood, R. I., 2001. Oral testosterone self-administration in male hamsters. Neuroendocrinology. 73, 285–92.

Kanayama, G., Cohane, G.H., Weiss, R.D., Pope, H.G., 2003. Past anabolic-androgenic steroid use among men admitted for substance abuse treatment: an underrecognized problem? J Clin Psychiatry. 64, 156–60.

Kankaanpää, A., Meririnne, E., ariniemi, K., Seppälä, T., 2001. Oxalic acid stabilizes dopamine, serotonin, and their metabolites in automated liquid chromatography with electrochemical detection. J Chromatogr B Biomed Sci Appl. 753, 413–9.

Kankaanpää, A., Ellermaa, S., Meririnne, E., Hirsjärvi, P., Seppälä, T., 2002. Acute neurochemical and behavioral effects of stereoisomers of 4-methylaminorex in relation to brain drug concentrations. J Pharmacol Exp Ther. 300, 450–9.

Kankaanpää, A., Gunnar, T., Ariniemi, K., Lillsunde, P., Mykkänen, S., Seppälä, T., 2004. Single-step procedure for gas chromatography-mass spectrometry screening and quantitative determination of amphetamine-type stimulants and related drugs in blood, serum, oral fluid and urine samples. J Chromatogr B Analyt Technol Biomed Life Sci. 810, 57–68.

Karila, T. A., Karjalainen, J.E., Mäntysaari, M.J., Viitasalo, M.T., Seppälä, T.A., 2003. Anabolic androgenic steroids produce dose-dependant increase in left ventricular mass in power atheletes, and this effect is potentiated by concomitant

use of growth hormone. Int J Sports Med 24, 337–43.

Kashkin, K. B., Kleber, H. D., 1989. Hooked on hormones? An anabolic steroid addiction hypothesis. JAMA. 262, 3166–70.

Kelly, P. H., Iversen, S. D., 1976. Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. Eur J Pharmacol. 40, 45–56.

Kindlundh, A. M., Isacson, D.G., 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, 71–4.

Kindlundh, A. M., Isacson, D.G., Berglund, L., Nyberg, F., 1999. Factors associated with adolescent use of doping agents: anabolic-androgenic steroids. Addiction. 94, 543–53.

Kindlundh, A. M., Hagekull, B., Isacson, D.G., 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, 322–8.

Kindlundh, A. M., Lindblom, J., Bergström, L., Wikberg, J.E., Nyberg, F.,, 2001b. The anabolic-androgenic steroid nandrolone decanoate affects the density of dopamine receptors in the male rat brain. Eur J Neurosci. 13, 291–6

Kindlundh, A. M., Bergström, M., Monazzam, A., Hallberg, M., Blomqvist, G., Langström, B., 2002. Dopaminergic effects after chronic treatment with nandrolone visualized in rat brain by positron emission tomography. Prog Neuropsychopharmacol Biol Psychiatry. 26, 1303–8.

Kindlundh, A. M., Lindblom, J., Bergström, 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, 113–20.

Kindlundh, A. M., 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, A. M., Rahman, S., Lindblom, J., Nyberg, F., 2004. Increased dopamine transporter density in the male rat brain following chronic nandrolone decanoate administration. Neurosci Lett. 356, 131–4.

Koch, F. C., 1938. The Chemistry and Biology of Male Sex Hormones. Bull N Y Acad Med. 14, 655–680.

Kochakian, C. D., 1990. History of anabolic-androgenic steroids. NIDA Res Monogr. 102, 29–59.

Koe, B. K., 1976. Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. J Pharmacol Exp Ther. 199, 649–61.

Koob, G. F., 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci. 13, 177–84.

Koob, G. F., Le Moal, M., 2006. Animal Models of Drug Addiction. In Neurobiology of Addiction. Academic press.

Kreek, M. J., Nielsen, D.A., Butelman, E.R., LaForge, K.S., 2005. Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nat Neurosci. 8, 1450–7.

Kritzer, M. F., 1997. Selective colocalization of immunoreactivity for intracellular gonadal hormone receptors and tyrosine hydroxylase in the ventral tegmental area, substantia nigra, and retrorubral fields in the rat. J Comp Neurol. 379, 247–60.

Kuczenski, R., 1980. Amphetamine-haloperidol interactions on striatal and mesolimbic tyrosine hydroxylase activity and dopamine metabolism. J Pharmacol Exp Ther. 215, 135–42.

Kuczenski, R., segal, D.D., Aizenstein, M.L., 1991. Amphetamine, cocaine, and fencamfamine: relationship between locomotor and stereotypy response profiles and caudate and accumbens dopamine dynamics. J Neurosci. 11, 2703–12.

Kulmala, H. K., Boja, J.W., Schechter, M.D.,1987. Behavioral suppression following 3,4-methylenedioxymeth-amphetamine. Life Sci. 41, 1425–9.

Kumar, N., Crozat, A., Li, F., Catterall, J.F., BArdin, C.W., Sundram, K., 1999. 7alpha-methyl-19-nortestosterone, a synthetic androgen with high potency: structure-activity comparisons with

other androgens. J Steroid Biochem Mol Biol. 71, 213–22.

Le Moal, M., Koob, G. F., 2007. Drug addiction: pathways to the disease and pathophysiological perspectives. Eur Neuropsychopharmacol. 17, 377–93.

Leonardi, E. T., Azmitia, E. C., 1994. MDMA (ecstasy) inhibition of MAO type A and type B: comparisons with fenfluramine and fluoxetine (Prozac). Neuropsychopharmacology. 10, 231–8.

Leshner, A. I., Koob, G. F., 1999. Drugs of abuse and the brain. Proc Assoc Am Physicians. 111, 99–108.

Lindblom, J., Kindlundh, A.M., Nyberg, F., Bergström, L., Wikberg, J.E., 2003. Anabolic androgenic steroid nandrolone decanoate reduces hypothalamic proopiomelanocortin mRNA levels. Brain Res. 986, 139–47.

Lindqvist, A. S., Johansson-Steenland, P., Nyberg, F., Fhalke, C., 2002. Anabolic androgenic steroid affects competitive behaviour, behavioural response to ethanol and brain serotonin levels. Behav Brain Res. 133, 21–9.

Lukas, S. E., 1993. Current perspectives on anabolic-androgenic steroid abuse. Trends Pharmacol Sci. 14, 61–8.

Lukas, S. E., 1996. CNS effects and abuse liability of anabolic-androgenic steroids. Annu Rev Pharmacol Toxicol. 36, 333–57.

Manzardo, A. M., Stei, L., Belluzzi, J.D., 2002. Rats prefer cocaine over nicotine in a two-lever self-administration choice test. Brain Res. 924, 10–9.

Martin-Fardon, R., Arnaud, M., Rousseau, E., Kamenka, J.M., Privat, A., Vignon, J.,, 1996. N-[1-(2-Benzo(b)thiophenyl) cyclohexyl]piperidine (BTCP) and cocaine induce similar effects on striatal dopamine: a microdialysis study in freely moving rats. Neurosci Lett. 211, 179–82.

Masonis, A. E., McCarthy, M. P., 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, 35–8.

Mcallister, S., Compton, D., Cocaine-ondiced locomotion: modification by acute or chronic anabolic-androgenic steroid treatment. Presented at 57th Annu Scietif. Meet. Coll. Problems Drug Depend., Scottsdale, AZ, 1995.

McBride, W. J., Murphy, J.M., Ikemoto, S., 1999. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res. 101, 129–52.

McQueen, J. K., Wilson, H., Sumner, B.E., Flink, G., 1999. Serotonin transporter (SERT) mRNA and binding site densities in male rat brain affected by sex steroids. Brain Res Mol Brain Res. 63, 241–7.

Menard, C. S., Harlan, R. E., 1993. Up-regulation of androgen receptor immunoreactivity in the rat brain by androgenic-anabolic steroids. Brain Res. 622, 226–36.

Mendelson, S. D., McEwen, B. S., 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, 339–43.

Mermelstein, P. G., Becker, J.B., Seymeier, D.J., 1996. Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. J Neurosci. 16, 595–604.

Miczek, K. A., Weerts, K.A., Weerts, E., Hanay, M., Tidey, J., 1994. Neurobiological mechanisms controlling aggression: preclinical developments for pharmaco-therapeutic interventions. Neurosci Biobehav Rev. 18, 97–110.

Moghaddam, B., Bunney, B. S., 1989. Differential effect of cocaine on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens: comparison to amphetamine. Synapse. 4, 156–61.

Morgan, A. E., Dewey, S. L., 1998. Effects of pharmacologic increases in brain GABA levels on cocaine-induced changes in extracellular dopamine. Synapse. 28, 60–5.

Naruse, T., Asami, T., 1987. Intravenous self-administration of diazepam in rats. Eur J Pharmacol. 135, 365–73.

Nash, J. F., 1990. Ketanserin pretreatment attenuates MDMA-induced dopamine release in the striatum as measured by in vivo microdialysis. Life Sci. 47, 2401–8.

Nichols, D. E., Lloyd, D.H., Hoffman, A.J., Nichols, M.B., Yim, G.K., 1982. Effects of certain hallucinogenic amphetamine analogues on the release of [3H]

serotonin from rat brain synaptosomes. J Med Chem. 25, 530–5.

Nomikos, G. G., DAmsma, G., Wenkstern, D., Fibiger, H.C., 1990. In vivo characterization of locally applied dopamine uptake inhibitors by striatal microdialysis. Synapse. 6, 106–12.

O'Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J., Molliver, M.E., 1988. Methylene-dioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J Neurosci. 8, 2788–803.

Olds, J., 1958. Effects of hunger and male sex hormone on self-stimulation of the brain. J Comp Physiol Psychol. 51, 320–4.

Packard, M. G., Schroeder, J.P., Alexander, G.M., 1998. Expression of testosterone conditioned place preference is blocked by peripheral or intra-accumbens injection of alpha-flupenthixol. Horm Behav. 34, 39–47.

Parkinson, A. B., Evans, N. A., 2006. Anabolic androgenic steroids: a survey of 500 users. Med Sci Sports and Exerc. 38, 644–51.

Parrilla-Carrero, J., Figueroa, O., Lugo, A., Garcia-Sosa, R., Brito-Vargas, P., Cruz, B., 2009. The anabolic steroids testosterone propionate and nandrolone, but not 17alpha-methyltestosterone, induce conditioned place preference in adult mice. Drug Alcohol Depend. 100, 122–7.

Parrott, A. C., Choi, P.Y., Davies, M., 1994. Anabolic steroid use by amateur athletes: effects upon psychological mood states. J Sports Med Phys Fitness. 34, 292–8.

Parssinen, M., Kujala, U., Vartiainen, E., Sarna, S., Seppälä, T.,, 2000. Increased premature mortality of competitive powerlifters suspected to have used anabolic agents. Int J Sports Med. 21, 225–7.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press Inc.,San Diego.

Pereira de Jesus-Tran, K., Cote, P.L., CAntin, L., Blanchet, J., LAbrie, F., Breton, R., 2006. Comparison of crystal structures of human androgen receptor ligand-binding domain complexed with various agonists reveals molecular determinants responsible for binding affinity. Protein Sci. 15, 987–99.

Perrotti, L. I., Beck, K.D., Luine, V.N., Quinones, V., 2000. Progesterone and cocaine administration affect serotonin in the medial prefrontal cortex of ovariectomized rats. Neurosci Lett. 291, 155–8.

Perry, P. J., Kutcher, E.C., Lund. B.C., Yates, W.R., Holman, T.L., Demers, L., 2003. Measures of aggression and mood changes in male weightlifters with and without androgenic anabolic steroid use. J Forensic Sci. 48, 646-51.

Quinones-Jenab, V., Perrotti, L.I., Fabiam, S.J., Chin, J., Russo, S. J., Jenab, S., 2001. Endocrinological basis of sex differences in cocaine-induced behavioral responses. Ann N Y Acad Sci. 937, 140–71.

Raiteri, M., Cerrito, F., Cervoni, A.M., Levi, G., 1979. Dopamine can be released by two mechanisms differentially affected by the dopamine transport inhibitor nomifensine. J Pharmacol Exp Ther. 208, 195–202.

Randrup, A., Munkvad, I., 1967. Brain dopamine and amphetamine-induced stereotyped behaviour. Acta Pharmacol Toxicol. 25, Suppl 4:62.

Ricaurte, G. A., Markowska, A.L., Wenk, G.L., Hatzidimitriou, G., Wlos, J., Olton, D.S., 1993. 3,4-Methylene-dioxymethamphetamine, serotonin and memory. J Pharmacol Exp Ther. 266, 1097–105.

Ricci, L. A., RAsakham, K., Grimes, J.M., Melloni, R.H., 2006. Serotonin-1A receptor activity and expression modulate adolescent anabolic/androgenic steroid-induced aggression in hamsters. Pharmacol Biochem Behav. 85, 1–11.

Roselli, C. E., 1998. The effect of anabolic-androgenic steroids on aromatase activity and androgen receptor binding in the rat preoptic area. Brain Res. 792, 271–6.

Rothman, R. B., Baumann, M. H., 2003. Monoamine transporters and psychostimulant drugs. Eur J Pharmacol. 479, 23–40.

Rudnick, G., Clark, J., 1993. From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. Biochim Biophys Acta. 1144, 249–63.

Ryan, K. J., 1959. Biological aromatization of steroids. J Biol Chem. 234, 268–72.

Sader, M. A., Griffiths, K.A., McCredie, R.J., Handelsman, D.J., Celermajer, D.S., 2001. Androgenic anabolic steroids and arterial structure and function in male bodybuilders. J Am Coll Cardiol 37, 224–30.

Sato, S. M., Schultz, K.M., Sisk, C.L., Wood, R.I., 2008. Adolescents and androgens, receptors and rewards. Horm Behav. 53, 647–58.

Savageau, M. M., Beatty, W. W., 1981. Gonadectomy and sex differences in the behavioral responses to amphetamine and apomorphine of rats. Pharmacol Biochem Behav. 14, 17–21.

Schade, K., Schubert, K., 1979. Estrogen biosynthesis and its inhibition – a review. Endokrinologie. 74, 90–9.

Schmidt, C. J., Levis, J.A., Lovenberg, W., 1987. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem Pharmacol. 36, 747–55.

Schroeder, J. P., Packard, M. G., 2000. Role of dopamine receptor subtypes in the acquisition of a testosterone conditioned place preference in rats. Neurosci Lett. 282, 17–20.

Shahidi, N. T., 1973. Androgens and erythropoiesis. N Engl J Med. 289, 72–80.

Shahidi, N. T., 2001. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. Clin Ther. 23, 1355–90.

Skarberg, K., Nuberg, F., Engström, I., 2008. The development of multiple drug use among anabolic-androgenic steroid users: six subjective case reports. Subst Abuse Treat Prev Policy. 3, 24.

Slikker, W., Jr., Ali, S.F., Scallet, A.C., Firth, C.H., Newport, G.D., Bailey J.R., 1988. Neurochemical and neurohistological alterations in the rat and monkey produced by orally administered methylenedioxy-methamphetamine (MDMA). Toxicol App Pharmaco. 94, 448–57.

Slikker, W., Jr., Holson, R.R., Ali, S.F., Kolta, M.G., Paule, M.G., Scallet, A.C., 1989. Behavioral and neurochemical effects of orally administered MDMA in the rodent and nonhuman primate. Neurotoxicology. 10, 529–42.

Sorge, R. E., Rajabi, H., Steward, J., 2005. Rats maintained chronically on buprenorphine show reduced heroin and cocaine seeking in tests of extinction and drug-induce reinstatement. Neuropsychopharmacology. 30, 1681–92.

Spanos, L. J., Yamamoto, B. K., 1989. Acute and subchronic effects of methylenedioxymethamphetamine [(+/-)MDMA] on locomotion and serotonin syndrome behavior in the rat. Pharmacol Biochem Behav. 32, 835–40.

Stumpf, W. E., Sar, M., 1976. Steroid hormone target sites in the brain: the differential distribution of estrogin, progestin, androgen and glucocorticosteroid. J Steroid Biochem. 7, 1163–70.

Sturgeon, R. D., Fessler, R.G., Meltzer, H.Y., 1979. Behavioral rating scales for assessing phencyclidineinduced locomotor activity, stereotyped behavior and ataxia in rats. Eur J Pharmacol. 59, 169–79.

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, 2760–4.

Szumlinski, K. K., McCAfferty, C.A., Maisonneuve, I.M., Glick, S.D., 2000. Interactions between 18-methoxy-coronaridine (18-MC) and cocaine: dissociation of behavioural and neurochemical sensitization. Brain Res. 871, 245–58.

Tamaki, T., Shiraishi, T., Takeda, H., MAtsumiya, T, Roy, R.R., Edgerton, V.R., 2003. Nandrolone decanoate enhances hypothalamic biogenic amines in rats. Med Sci Sports Exerc. 35, 32–8.

Taylor, K. M., Snyder, S. H., 1971. Differential effects of D- and L-amphetamine on behavior and on catecholamine disposition in dopamine and norepinephrine containing neurons of rat brain. Brain Res. 28, 295–309.

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, 1059–65.

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, 1301–6.

Thompson, E. A., Jr., Siiteri, P. K., 1974. The involvement of human placental microsomal cytochrome P-450 in aromatization. J Biol Chem. 249, 5373–8.

Tomkins, D. M., Sellers, E. M., 2001. Addiction and the brain: the role of neurotransmitters in the cause and treatment of drug dependence. CMAJ. 164, 817–21.

Walther, D. J., Peter, J.U., Bashammakh, S., Hortnagl, H., Voits, M., Flink, H., 2003. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science. 299, 76.

van de Poll, N. E., van Zanten, S., de Jonge, F.H., 1986. Effects of testosterone, estrogen, and dihydro-testosterone upon aggressive and sexual behavior of female rats. Horm Behav. 20, 418–31.

van der Vies, J., 1985. Implications of basic pharmacology in the therapy with esters of nandrolone. Acta Endocrinol. Suppl. 271, 38–44.

Waxham, M. N., 1999. Neurotransmitter receptors. In Fundamental Neuroscience. Academic Press, London.

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, 117–26.

Vermes, I., Varszegi, I., Toth, E.K., Telegdy, G., 1979. Action of androgenic steroids on brain neurotransmitters in rats. Neuroendocrinology. 28, 386–93.

Wesson, D. W., McGinnis, M. Y., 2006. Stacking anabolic androgenic steroids (AAS) during puberty in rats: a neuroendocrine and behavioral assessment. Pharmacol Biochem Behav. 83, 410–9.

Westerink, B., 1985. Sequence and significance of dopamine metabolism in the rat. Neurochem Int. 7, 221–227.

Volkow, N. D., Fowler, J.S., Wolf, A.P., schlyer, D., Shiue, C.Y., Alpert, R, 1990. Effects of chronic cocaine abuse on postsynaptic dopamine receptors. Am J Psychiatry. 147, 719–24.

Volkow, N. D., Chang, L., Wang, G.J., Fowler, J.S., Ding, Y.S., Sedler, M., 2001a. Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am J Psychiatry. 158, 2015–21.

Volkow, N. D., Chang, L., Wang, G.J., Fowler, J.S., Leonido-Yee, M., Franceschi, D., 2001b. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. Am J Psychiatry. 158, 377–82.

Wollina, U., Pabst, F., Schonlebe, J., Abdel-Naser, M.B., Konrad, H., Gruner, M., 2007. Side-effects of topical androgenic and anabolic substances and steroids. A short review. Acta Dermatovenerol Alp Panonica Adriat. 16, 117–22.

Wood, R. I., 2002. Oral testosterone self-administration in male hamsters: dose-response, voluntary exercise, and individual differences. Horm Behav. 41, 247–58.

Wood, R. I., 2004. Reinforcing aspects of androgens. Physiol Behav. 83, 279–89.

Wood, R. I., Johnson, L.R., Chu, L., Schad, C., 2004. Testosterone reinforcement: intravenous and intracerebro-ventricular self-administration in male rats and hamsters. Psychopharmacology. 171, 298–305.

Wu, F. C., 1997. Endocrine aspects of anabolic steroids. Clin Chem. 43, 1289–92.

Yesalis, C. E., Bahrke, M. S., 1995. Anabolic-androgenic steroids. Current issues. Sports Med. 19, 326–40.

Yu-Yahiro, J. A.,Michael, R.H., Nasrallah, D.V., Schofield, B., 1989. Morphologic and histologic abnormalities in female and male rats treated with anabolic steroids. Am J Sports Med. 17, 686–9.

Zetterstrom, T., Sharo, T., Marsden, C.A., Ungerstedt, U., 1983. In vivo measurement of dopamine and its metabolites by intracerebral dialysis: changes after d-amphetamine. J Neurochem. 41, 1769–73.

Zetterstrom, T., Sharp, T., Ungerstedt, U., 1986. Effect of dopamine D-1 and D-2 receptor selective drugs on dopamine release and metabolism in rat striatum in vivo. Naunyn Schmiedebergs Arch Pharmacol. 334, 117–24.

Zifa, E., Fillion, G., 1992. 5-Hydroxytryptamine receptors. Pharmacol Rev. 44, 401–58.