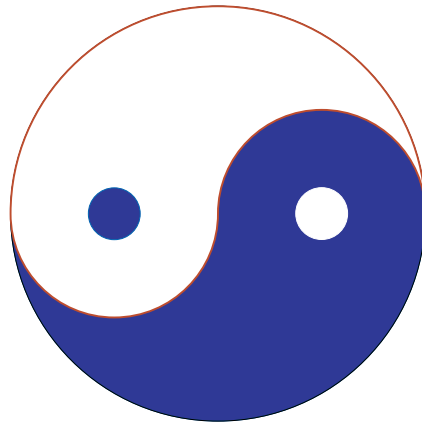


Epidemiological and Neurobiological Evidence for Misuse of Anabolic Androgenic Steroids

BY

ANNA MS KINDLUNDH



UPPSALA
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Epidemiological and neurobiological
evidence for misuse of
anabolic-androgenic steroids

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ANNA MS KINDLUNDH



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ABSTRACT

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Misuse of anabolic-androgenic steroids (AAS), is attributed to elite athletes and body builders. The attentive involvement of AAS in acts of violence seen in society has raised interest to evaluate the importance of social, psychological and neurobiological mechanisms that underlie the psychiatric states associated with onset of controlled misuse, its maintenance, and *via* abuse its transition to addiction. The objective of this thesis is to examine whether misuse of AAS shares mechanisms with epidemiological and neurobiological models of psychotropic substances.

Epidemiological studies through a survey conducted in Uppsala, Sweden, suggest that misuse of doping agents, specifically AAS, has extended also to include adolescent males taking these agents in order to improve muscle mass, enhance sports performance, become intoxicated, braver, and because it is fun to try. Intake of AAS is in a subgroup highly connected to misuse of psychotropic substances. The adolescent AAS profile is highlighted in a multivariate model positing the factors high immigrant status, perceived average/bad school achievement, truancy, average/low self-esteem, strength training, heavy alcohol consumption and use of prescription tranquillisers to be independently associated with lifetime misuse.

Neurobiological studies indicate that chronic treatment with supra-therapeutic doses of the AAS nandrolone, significantly affects dopamine receptor density in the male rat brain and the corresponding gene transcripts in the mesocorticolimbic and nigrostriatal dopamine systems, in brain areas of importance for hedonia, reward-related learning, incentives and motoric behaviours. Identical treatment regimen affects the density of serotonin receptors in regions regulating anxiety, aggression, cognitive functions, impulsivity and its associated loss of inhibitory control. These alterations may reflect aversive conditions that could be linked to severe alleostatic states of addiction following chronic continuous "binge" intoxications of addictive drugs.

Thus, the AAS profile of misuse shares similarities with mechanisms of psychotropic substances regarding psychological and social models of onset and maintenance and with respect to AAS-induced neurobiological changes in the brain. This trend is alarming, strengthening the need of prevention and treatment programs targeting the specific subgroups of misusers.

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To my parents
Sten, and the memory of my mother Ulla

“The goal of life is living in agreement with nature”

Zeno (335 BC - 263 BC)

ORIGINAL PAPERS

This thesis is the first multidisciplinary thesis to provide epidemiological and neurobiological evidence for misuse of anabolic-androgenic steroids.

- I Kindlundh, A.M.S., 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.
Scandinavian Journal of Social Medicine, 26, 71-74.
- II Kindlundh, A.M.S., Isacson, D.G., Berglund, L. & Nyberg, F. (1999) Factors associated with adolescent use of doping agents: anabolic-androgenic steroids.
Addiction, 94, 543-553.
- III Kindlundh, A.M.S., Hagekull, B., Isacson, D.G.L. & Nyberg, F. (2001) Adolescent use of anabolic-androgenic steroids and relations to self reports of social, personality, and health aspects.
European Journal of Public Health, 11, 322-328.
- IV Kindlundh, A.M.S., Lindblom, J., Bergström L., Wikberg, J.E.S & Nyberg, F. (2001) The anabolic-androgenic steroid nandrolone decanoate affects the density of dopamine receptors in the male rat brain.
European Journal of Neuroscience, 13, 291-296.
- V Kindlundh, A.M.S., Bergström, M., Monazzam, A., Hallberg, M., Blomqvist, G., Långström, B. & Nyberg, F. (2002) Dopaminergic effects after chronic treatment with nandrolone visualised in rat brain by PET.
Neuro-Psychopharmacology & Biological Psychiatry, in press.
- VI Kindlundh, A.M.S., Lindblom, J., Hallberg, M., Frändberg, P-A., LeGrevès, P., Zhou, Q. & Nyberg, F. (2002) Regulation of mRNAs for tyrosine hydroxylase, dopa decarboxylase, dopamine D₁- and D₂-receptor subunits after chronic treatment with nandrolone. *Manuscript*.
- VII Kindlundh, A.M.S., Lindblom, J., Bergström, L. & Nyberg, F. (2002) Nandrolone decanoate induces alterations in the density of serotonergic 5HT_{1B} and 5HT₂ receptors in the male rat brain.
Manuscript, submitted.

ABBREVIATIONS

AADC	L-aromatic aminoacid decarboxylase
AAS	Anabolic-androgenic steroids
Acb	Nucleus Accumbens
AcbC	Nucleus accumbens core
AcbSh	Nucleus accumbens shell
cAMP	Cyclic adenosine 3', 5'-monophosphate
CNS	Central nervous system
COMT	Catechol-o-metyltransferase
DAT	Dopamine transporter
DOPAC	Dihydroxyphenylacetic acid
DRN	Dorsal raphe nucleus
DS	Dorsal subiculum
DSM IV	Diagnostic and statistical manual of mental disorders IV
Dx	Dopamine receptor subtype
GABA	Gamma amino butyric acid
GP	Globus pallidus
HVA	Homovanillic acid
L-DOPA	L-dihydroxyphenylalanine
LSD	Lysergic acid diethylamide
MAO-A	Monoaminoxidase A
MAO-B	Monoaminoxidase B
MDMA	Methylenedioxydiethylamine
MRN	Medial raphe nucleus
PAG	Periaqueductal gray
PFC	Prefrontal cortex
SN	Substantia nigra
TH	Tyrosine hydroxylase
TPH	Tryptophane hydroxylase
VP	Ventral pallidum
VTA	Ventral tegmental area
5-HIAA	5-hydroxyindole acetic acid
5HT	5-hydroxytryptamine, serotonin
5HTx	Serotonin receptor subtype

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Chapter 1

HISTORY

From Ancient Times to Modern Society

The fascination and admiration of the body image, the desire to enhance muscular strength and the use of drugs dates back to ancient history. In the Epic of Gilgamesh, which dates to the Akkadian Empire 3000 years B.C., Gilgamesh is presented as superhuman, so powerful that the gods create a counterpart, Enkidu, to moderate and balance his desires and actions. In ancient Greece one of the symbols of remarkable strength was Hercules. The Oracle in Delphi gave him the assignment to fulfil twelve master deeds that required superhuman strength and are depicted in the freeze of the Zeus Temple in Olympia. Male statues like Myron's Discobolus and Polyclitus' Doryphorus embody concepts of invulnerable masculine power and beauty. In the Old Testament, (Judges chapter 13-16) a character of muscular strength is Samson, one of the judges in Israel, who destroyed the city gates of Gaza. Nordic mythology also tells stories of characters with extraordinary strength and tasks. In *Brennu-Njáls tale, chapter XIX*, Gunnarr á Hlíðarenda is introduced. He is ascribed all physical abilities in growth, strength, agility, ability to swim, archery and is besides all this beautiful. Further, the ancient Greeks used hallucinogenic mushrooms and sesame seeds to enhance performance, the gladiators in the Roman Coliseum used stimulants to overcome fatigue, and berserks of Nordic mythology used bufotonin for stimulating effects [45,364]. Saharan African Cafre ate bread that they dipped into a special alcoholic liquor to become intoxicated. The term doping is derived from the Boer-Dutch (Africaans) *dopen* related to this religious act. A late expression for the cult of strength and beauty is Leni Riefenstahl's movie about the Olympic games in Berlin, which is initiated with a scene where the statue Discobolus of ancient Greece becomes alive and is transformed into a discus thrower of the modern Olympic games. The connection backwards to the athletics of the antiquity was a recurrent theme for the Olympics of 1936 in Berlin. Today much of the cult of strength and beauty has incorporated into popular culture, movies, sports, cartoon characters and commercials. In modern society, anabolic-androgenic steroids have been misused by countless elite athletes and body builders for their attributed effects of improved appearance and athletic performance. The discovery of these agents could however be linked to medical purposes on the 18th Century when the Scottish medical doctor John Hunter (1728-1793), recognized as a well-known anatomist and surgeon, developed methods for testicular transplantation experiments. Inspired by Hunter, the German Professor Berthold of Göttingen discovered in 1849 that castration of roosters induced decline in sexual behaviour and atrophy of the combs, and further

that these effects promptly were restored when testicular tissue was grafted to the intestines (replacement therapy) [28]. The importance of a substance secreted from the testis into the blood-stream was highlighted 40 years later. In June 1889, the French physiologist Charles Édouard Brown-Séquard announced at the Société de Biologie in Paris that he had devised an excellent therapy for his body and mind. He injected himself liquid extracts from the testicles of dogs and guinea pigs that increased his physical strength and intellectual energy, relived his constipation and lengthened the arc of his urine [52]. Crude bioactive extracts of gonadal, pituitary, and placental protein hormones were prepared during the 1920s and 1930s. In 1931, Adolf Butenandt isolated 15 mg of androsterone, a non-testicular male hormone, from 15,000 litres of policemen's urine. Two major independent research teams contributed to the successful identification of the primary male hormone testosterone in 1935 [56,283]. For this discovery, Butenandt and Ruzicka were awarded the Nobel Prize for Chemistry in 1939. In the late 1940s and early 1950s attempts were initiated to synthesise steroid compounds that would be anabolic without exhibiting androgenic effects. This successfully resulted in the synthesis of testosterone derivatives, which together with testosterone itself, gave rise to the anabolic-androgenic steroids. The clinical use of anabolic-androgenic steroids has historically involved treatment of wasting conditions associated with chronic debilitating effects (e.g. initially victims in concentrations camps), trauma, burns, surgery, radiation therapy, anaemia, hypogonadism, depression, melancholia, and psychosis. The clinical use has been restrictive in comparison to the non-clinical use. The non-clinical use is the story to be told here.

Chapter 2

INTRODUCTION

2.1 ANABOLIC-ANDROGENIC STEROIDS

2.1.1 Definitions

The term anabolic-androgenic steroid (AAS) is defined to include the male sex hormone testosterone and other endogenous androgenic hormones, as well as chemical synthetic derivatives of testosterone [224]. The definition of the term AAS is derived from substances that have the ability in common to cause both anabolic (promotion of protein synthesis) and androgenic (development and maintenance of secondary male sexual characteristics) effects [216]. At present, there are no AAS available with purely anabolic effects [217]. In this thesis, the term AAS, with exception of Paper I, will be used instead of the public name "anabolic steroids".

The term *misuse* is used in favour of the commonly used term *abuse* for two reasons. Firstly, *misuse* emphasises on the non-medical use of an illegal substance, in distinction from the non-medical intake of a legal drug, substance *use*. Secondly, *misuse* addresses that this non-prescribed intake may yet not have reached the degree of intake that fulfils the criteria for abuse, neither according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM IV) nor with respect to the degree of compulsive intake related to drug-related stimuli (see section 2.2.4) [9,92].

The International Narcotics Control Board (INCB), Vienna, lists two categories of drugs, narcotic drugs and psychotropic substances. At present, control is exercised over more than 116 narcotic drugs under the 1961 Convention. They include mainly natural products such as opium and its derivatives, morphine, codeine and heroin, but also synthetic narcotics such as methadone and pethidine, as well as cannabis and cocaine. Some 105 psychotropic substances are controlled by the 1971 Convention. Most of them are contained in pharmaceutical products acting on the central nervous system. Broadly speaking, these are the hallucinogens, the stimulants, the depressants and some analgesics. In this thesis the term psychotropic substances includes opioids and refers to cocaine, amphetamine, methamphetamine, Lysergic acid diethylamide (LSD), Methylenedioxydiethylamine (MDMA), cannabis, psilocybin and opiates.

2.1.2 Biochemical Synthesis, Structures, and Metabolism

Structural modifications of testosterone

The testosterone molecule has been chemically modified in order to enhance the anabolic or androgenic effects, bypass its high first-pass metabolism, delay the release of the hormone into the circulation, and/or to retard catabolism [176]. Structural modifications performed are summarized into three major categories; 1) alkylation at the 17- α -position, 2) esterification of the 17- β -hydroxyl group, 3) modifications of the ring structure of the steroid [349]. Synthetic AAS usually contain a combination of structural changes of the ring structure and either 17- α -alkylation or 17- β -hydroxy esterification.

The advantage of 17- α -alkylations, commonly undertaken with a methyl- or ethyl- group, is that the products are catabolised slower by the liver and hence are orally active. Among the 17- α -alkylated AAS are methyltestosterone, fluoxymesterone, oxymesterone, methandrostenolone, oxymetholone, stanozolol, oxandrolone, ethylestrenol, norethandrolone, and danazol, of which all but the first one also have been subjected to ring structure alterations. Esterification with carboxylic acids at the 17- β -site of the steroids makes parental administration possible. Esterification reduces the polarity of the molecule, makes it more soluble in lipid vehicles and therefore slows down the rate of absorption from the site of injection. Esters must be hydrolysed prior to biological activation. Testosterone propionate, testosterone cypionate, and testosterone enanthate which are 17- β -esterified AAS are only modified at the 17- β -site, while methenolone enanthate, and nandrolone have been subjected to ring modifications in addition to the esterification. Modifications of the ring structure have been performed to allow both oral and parenteral administration. Substitution at the 1, 2, 9, and 11 carbons are common, either in order to slow the rate of inactivation or to increase the potency. The 19-nor-androgens such as nandrolone are more potent than testosterone because the removal of the 19-methyl group provides a more planar structure, which more easily binds to the steroid site [204].

Biosynthesis and metabolism

The pathways of biosynthesis and metabolism of testosterone are illustrated in Figure 1. In man, testosterone is synthesised from cholesterol. The Leydig cells of the testes and cells of the adrenal cortex synthesise the majority of testosterone in males, while cells of the corpus luteum and the adrenal cortex are responsible for the synthesis of this steroid in females. In addition to the adrenocortical and gonadal steroid synthesis, steroid hormones (neurosteroids) could also be produced in the brain [236].

The metabolism of testosterone can serve as a prototype for all AAS. Enzymes that convert testosterone to its distinct metabolites are also active towards other AAS, when similar chemical groups and configurations are present. Changes of the perhydrocyclopentano-phenantrene ring are grouped into two kinds of metabolic pathways, phase-I and phase-II [290]. Phase-I metabolism generally convert

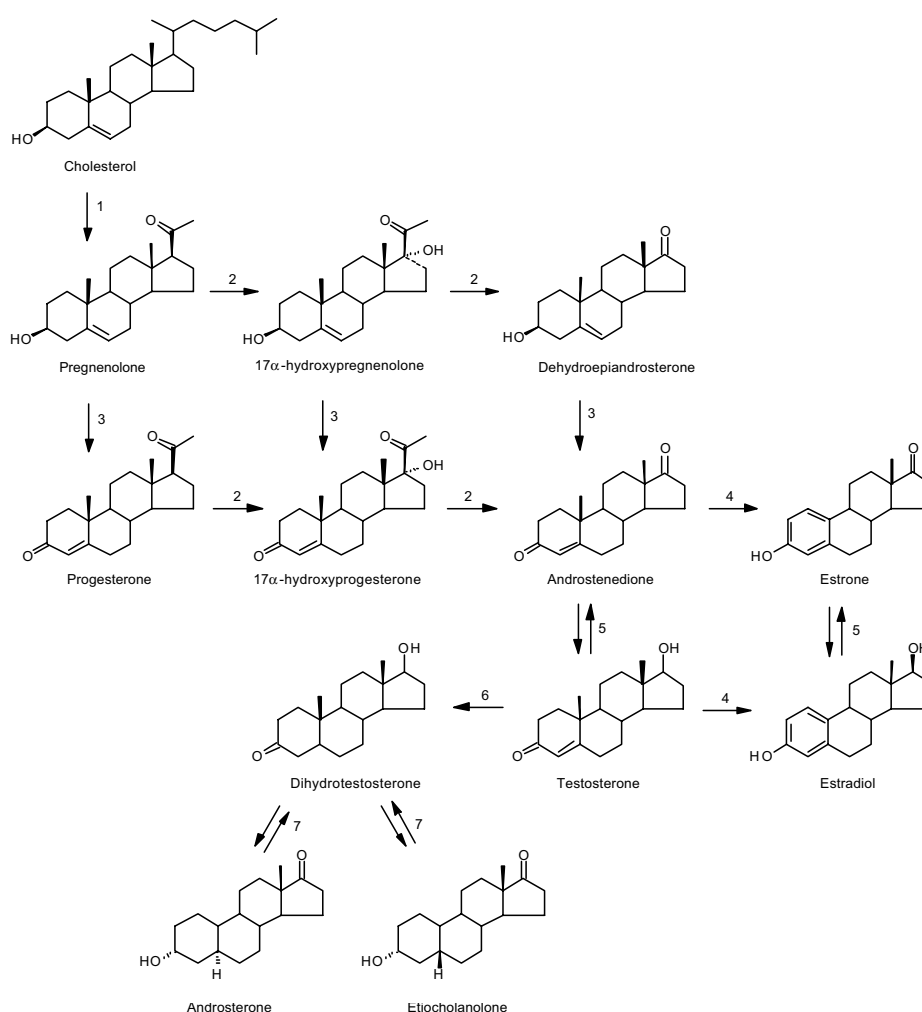


FIG. 1. The pathway for biosynthesis and metabolism of testosterone.

1) P450_{sc} Cholesterol side-chain cleavage enzyme, 2) P450 17 α -hydroxylase, 3) 3 β -hydroxy steroid dehydrogenase, 4) Aromatase, 5) 17-hydroxysteroid dehydrogenase, 6) 5 α -reductase (5 β -reductase), 7) 3 α -hydroxylase

the steroid by enzymatically catalysed reactions into more polar compounds through oxidation, reduction or hydroxylation. Phase-II metabolism represents conjugation reactions, which are characterised by glucuronidation or sulfatation of the steroid or its metabolite. The major metabolites of testosterone are the active compounds dihydrotestosterone and estradiol and the inactive androsterone and etiocholanolone. Dihydrotestosterone is much more androgenic than testosterone. One of the most potent AAS, is nandrolone (19-nortestosterone), which mainly is converted to norandrosterone and noretiocholanolone Figure 2. Also the enzyme

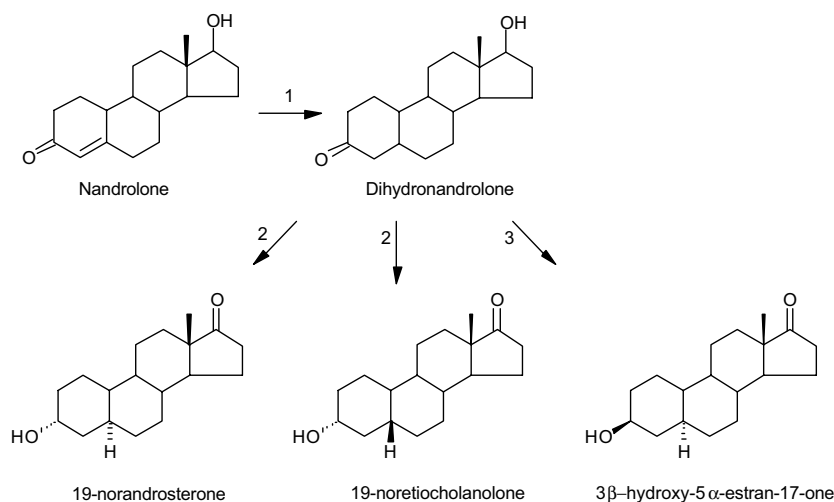


FIG. 2. The pathway for biosynthesis and metabolism of testosterone.
 1) 5 α -reductase (5 β -reductase), 2) 3 α -hydroxylase, 3) 3 β -hydroxylase

5 α -reductase converts nandrolone (19-nortestosterone) to dihydrondrolone (19-nordihydrotestosterone), which is less androgenic than nandrolone [244]. Nandrolone can also be partially aromatised to estrogens, even though aromatisation of 19-nor-steroids is less common than aromatisation of testosterone [26,299,305].

2.1.3 Physiological and Pharmacological Perspectives

Mechanisms of action

Steroid hormones mediate their effects either *via* slow intracellular genomic actions in the nucleus or through rapid non-genomic actions at the plasma membrane. The classical action of steroids is the genomic action, which lasts 30-60 minutes. The androgen receptor is, along with the estrogen, corticoid (mineral corticoid, glucocorticoid), and thyroid hormone, a member of the nuclear receptor superfamily [223]. The unoccupied androgen-, estrogen-, and progestin-receptor proteins are located in the nucleus of the cell, while the majority of the glucocorticoid receptors are located in the cytoplasm [133,140]. Steroid hormones bind to the hormone-binding domain of the steroid receptors. The nuclear steroid-receptor complexes are dimerised into the formation of homodimers. Active homodimers bind *via* the DNA-binding domain of the receptor to certain genes, Hormone Response Elements (HRE), which for androgens are called Androgen Response Elements (ARE), and gene transcription is stimulated [24,223]. In addition to ARE certain gene sequences of androgen response enhancers have been identified to be specific for the androgen-receptor complex [67]. Both testosterone and dihydrotestoster-

one bind to the androgen receptor, but dihydrotestosterone binds with a higher affinity and activates the gene expression more efficiently [90,343]. The affinity of nandrolone to the androgen receptor is weaker than for dihydrotestosterone, but higher than for testosterone [280]. The nature of the anabolic action of AAS on muscle is unclear. It could be caused by effects linked to differences in the affinity to the androgen receptor but it may also be mediated through mechanisms that are independent of the androgen receptor [359]. Rapid non-genomic actions of steroids could either occur by non-receptor mediated actions at the membrane or be exerted through membrane-bound receptor sites [63]. Rapid non-receptor mediated actions can be due to alterations in membrane fluidity [339]. Neurosteroids and A-ring reduced steroids, in general induce rapid effects by acting through ligand-gated ion channels, i.e. neurosteroid sites at the GABA-A and the NMDA receptor [70,71,81,235,243,315]. Recent studies indicate that AAS are also able to modulate the GABA-A receptor on the plasma membrane [39,70,71,120-122,168,225]. Apart from the neurosteroid sites, rapid effects are also suggested to occur through membrane bound steroid receptors and non-steroidal receptors [63].

Physiology and therapeutic use

Testosterone and other AAS possess both androgenic and anabolic effects. In males, the androgenic properties consist of the development of primary sexual characteristics and is crucial for pubertal changes. The anabolic effects are usually considered to derive from mechanisms promoting the protein synthesis, muscle growth, erythropoiesis, stimulation, and inhibition of skeletal growth in the young [244]. Testosterone is metabolised to dihydrotestosterone by 5 α -reductase and to estradiol by aromatase. The effects of testosterone are either mediated by testosterone itself or by its metabolites dihydrotestosterone and estradiol [349]. The physiological relevance of testosterone is mainly development of internal genitalia, increased skeletal muscle mass and strength, and erythropoiesis. Dihydrotestosterone is relevant for the development of external genitalia, and hair follicles and estradiol is important for epiphyseal maturation and prevents osteoporosis [38,307,350].

In Sweden, testosterone and nandrolone decanoate are the only registered pharmaceuticals. To date, the clinical treatment of AAS is restricted to hypogonadism, impotence, catabolic and wasting states, angioneurotic edema, osteoporosis, blood dyscrasias, palliative breast carcinoma and male contraceptives [115].

2.1.4 Misuse in Society

Extent of misuse

The misuse of AAS was long confined to body building and professional sports, but is nowadays a problem that involves a broader population, including adolescents and young adults. In 1995, at the time of the initiation of the epidemiological part of the present thesis there were only a limited number of epidemiological stud-

Table 1. Anabolic-androgenic steroid prevalence estimates among adolescents and young adults

Reference	Country	Tool	N	Gender	Age	Prevalence Lifetime misuse %
<i>National Studies</i>						
Lindström, 1990	Helsingborg & Malmö, Sweden	BSQ	138	M	20-28	38.4
CAN, 1995	8 cities, 1 community, Sweden	SQ		M	16-19	1-5
CAN, 2002	Sweden	SQ	5,349	M	15-16 (9 th grade)	1.0
CAN, 2001	Sweden	CQ	36,085	M	18	0.8
CAN, 2001	Sweden	CQ	39,842	M	All	1.0
ESPAD, 1999	30 Countries in Europe	SQ	100,000	M-F	15-16	1.0
				M	15-16	2.0
				F	15-16	1.0
Nilsson, 1995	Falkenberg, Sweden	SQ	688	M	14-19	5.8
				F	14-19	1.0
Nilsson, 2001	Falkenberg, Sweden	SQ	2,785	M	16-17	3.0
				F	16-17	0.0
<i>Other European Countries</i>						
Scarpino, 1990	Italy	AI	1,015	M-F		16FR : 26O
				TI	216	M-F
Williamson, 1993	United Kingdom	SQ	687	M		4.4
Korkia & Stimson, 1997	United Kingdom	GQ	1,310	M	17-56 (29 [Ⓜ])	9.1
				F	18-35 (27 [Ⓜ])	2.3
Wichstrøm, 2000	Norway	SQ	8,508	M-F	15-22	0.8
				M		1.2
				F		0.6
<i>Africa</i>						
Schwellnus, 1992	South Africa	SQ	1,361	M-F		0.6
				M		1.2
Lambert, 1998	South Africa	SQ	2,547	M-F	16-18	
				M		2.8
				F		0.7

AI: Interview among athletics, BGQ: Gym Questionnaire among body builders, CAN; The Swedish Council for Information on Alcohol and other Drugs, CQ: Conscript Questionnaire, ESPAD; European school survey project on alcohol and other drugs, F: Females, FR; Frequent use, GQ: Gym Questionnaire, M: Males, N: Number of subjects, O; Occasional use, SQ: School Questionnaire, TI: Interview among technicians.

Ⓜ Mean

ies performed on AAS. In Table 1, prevalence estimates of lifetime misuse of AAS among adolescents and young adults are presented from a set of recent national and international studies.

Lifetime misuse of AAS is reported to vary between 1-5% among male adolescents in Sweden [141,142,210,247,248]. The committee of the European school survey project on alcohol and other drugs (ESPAD), including 30 European Countries, reports a lifetime AAS misuse to be 2% among adolescent males [154]. In Norway, 1.2% of the males and 0.6% of the females, aged 15-22 years, reported lifetime misuse of AAS [340]. In U.K. lifetime misuse among young males visiting

Table 1. Continued

Reference	Country	Tool	N	Gender	Age	Prevalence Lifetime misuse %
<i>America</i>						
Centre for Drug free Sports, 1993	Canada			M		4.1 □
Melia, 1996	Canada	SQ	16,119	M-F	11-18	1.5 □
Windsor, 1989	Texas, USA	SQ	1,010	M-F		2.8
				M	17 ☉☉	5.0
				F	17 ☉☉	1.4
Buckley, 1988	USA	SQ	3,403	M	12 th grade	6.6
Komorski, 1992	Arkansas, USA	SQ	672	M	11 th grade	7.6
				806	F	1.5
Radakovich, 1993	Minneapolis, USA	SQ	1,624	M-F	7th grade	
				M		4.7
				F		3.2
Yesalis, 1993	USA	NHS	32,595	M-F	12-34	0.5
				M-F	12-17	0.6
				M-F	18-25	1.3
				M-F	26-34	0.6
				M	12-34	0.9
				F	12-34	0.1
DuRant, 1995	50 states & Colombia, USA	SQ	6,253	M	15-19	4.08
				6000	F	1.20
Tanner, 1995	Denver, USA	SQ	3,438	M	8-17	4.0
				3,492	F	1.3
Scott, 1996	Nebraska, USA	SQ	2,136	M	13-19	4.5
				2,522	F	0.8
Stilger & Yesalis, 1999	Indiana, USA	FSQ	873	M	High School	6.3

AI: Interview among athletics, BGQ: Gym Questionnaire among body builders, CAN; The Swedish Council for Information on Alcohol and other Drugs, CQ: Conscript Questionnaire, ESPAD; European school survey project on alcohol and other drugs, F: Females, FR; Frequent use, FSQ: School Questionnaire among football players, GQ: Gym Questionnaire, M: Males, N: Number of subjects, NHS: National House Hold Survey on Drug Abuse, O; Occasional use, SQ: School Questionnaire, TI: Interview among technicians. ☉ Mean; ☉☉ Median; □ Past 12 months

gymnasiums was 9.1% [189] and among adolescent males 4.4% [344]. Outside of Europe lifetime misuse of AAS is reported to vary. In Australia the prevalence of misuse was 1.2-3.2% among males and 0.2-2.0% among females [25,146]. In different South African studies lifetime misuse was estimated to be 1.3% in 1992 and 2.8% in 1998 among male adolescents [194,300]. Canadian male adolescents report a misuse between 2.5 and 7.0% [234]. In US, prevalence estimates are between 4-12% among adolescent males and 0.5-2% among females [53,104,159,182,272,301,314,322,351,365].

Doses and schedules of misuse

AAS are misused in complex regimens in terms of doses, time-courses, and/or intake of multiple steroids. The three basic regimens are cycling, pyramiding and stacking [262]. AAS are commonly misused in 2-3 cycles per year and each cycle usually lasts 6-12 weeks. Patterns of cycling also include the intake of different steroids within the cycle, either administered in phases or combined at the same time, i.e. stacking [261]. Administered doses vary from therapeutic doses by endurance and sprint athletes, up to doses more than 100 times the therapeutic levels in weight lifters and body builders [261,278]. Pyramiding is characterised by a step-wise increase followed by a gradual decrease in doses over the period of the cycle. Steroids simultaneously administered may include oral and injectable forms. The aim of applying different regimens is to avoid and minimise the risk of side-effects, receptor densitisation, and the development of tolerance.

Legal aspects and the black market

Regarding legal aspects of AAS handling, rules connected to doping in the sports world have to be separated from laws in society. The Medical Commission was created in 1961 in order to deal with the increasing problem of doping in sports. The International Olympic Committee (IOC) annually publishes a "*List of prohibited substances and methods*", which serves as a guide for various national and international sports federations. In 1975, AAS were for the first time listed by IOC. The different international sports federations are responsible for penalty regarding doping for their specific sport. Otherwise, athletes in Sweden can be banned for up to two years from participation in competitions, sport events, and from the involvement in assignments related to sports (Stadgar för Sveriges Riksidrottsförbund, 1999). Beginning in 2003, the World Anti-Doping Agency (WADA) will be responsible for the "*List of prohibited substances and methods*" as well as the establishment of new international rules in sports regarding doping.

In Sweden, three main laws, apart from rules in sports, are applicable on AAS handling. I: Law of prohibition of certain doping agents (*Lag (1991:1969) om förbud mot vissa dopningsmedel, Lag (1999:44) om ändring i lagen (1991:1969) om förbud mot vissa dopningsmedel*), II: Law of pharmaceuticals (*Läkemedelslag (1992:859)*), III: Law of penalty for smuggling (*Lag (2000:1245) om ändring i lagen (1991:1969) om förbud mot vissa dopningsmedel*). Since 1 April, 1999 the maximum penalty for aggravated crime is four years of imprisonment.

The AAS and other doping agents available on the black market in Sweden are mainly produced by legal pharmaceutical companies all over the world, but the majority are distributed from Thailand, the Mediterranean countries and Eastern Europe [275]. The Swedish Police and Customs Agent estimate that confiscation has increased during the past decade. The most frequent occurring doping agents on the black market are the AAS of which nandrolone decanoate, stanozolol, boldenon undecanoate, and various derivatives of testosterone are the most commonly AAS found (Gunnar Hermansson, National Criminal Investigation Service, "Rikskriminalpolisen", personal communication).

2.2 HEDONIA TO ADDICTION MAKES USERS DEPENDENT

Rewards often share the ability to induce subjective experiences of hedonia. Pleasurable feelings and novelty may arise from food, sex, gambling, sports, scientific discoveries, and intake of tobacco, alcohol, psychotropic- and other substances. In animal models rewards are estimated as something that elicit approach behaviour. The major keys that underlie hedonia and novelty as well as the drives for the onset of certain behaviours such as drug use, and further the transition from use to dependence, have long been subjected to separate theories in neurobiology, psychology and sociology. This chapter will present some models of the non-medical onset of substance use (2.2.1), give a quick insight in mechanisms of pleasurable feelings, reward, reward-related learning, and incentives (2.2.2), define the terms use, abuse, addiction and dependence (2.2.4), that so far have been derived from research on tobacco, alcohol and psychotropic substances.

2.2.1 Models of Substance Use Onset

Large-scale environmental risk factor theory

The large-scale environmental risk factor theory is formulated to describe the onset of substance use and misuse from a psychological and social context [348]. This model has basis in Jessor & Jessor's *Problem behaviour theory* [162]. Because misuse of certain substances is illegal for adolescents, paradigms of deviant behaviour are needed in order to achieve comprehensive models of substance misuse and abuse. An adolescent who may be predisposed to try some deviant behaviour has several potential barriers to overcome. The problem-behaviour theory views the involvement in deviant behaviour as a result from a combination of variables, arrayed from so-called distal to proximal [162]. This theory has been extensively applied in research on adolescent substance misuse, such as heavy drinking and marijuana misuse, as well as behavioural traits such as risky sexual behaviour [103]. *Distal factors* represent, according to the large-scale environmental risk factor theory [348], diffuse influences that characterise a large part of the population, occur early in the causal chain, and may lead to substance misuse. These factors are general attributes of the interpersonal environment and include sociodemographic variables such as socio-economic status, geographic variables, family structure, ethnicity, as well as other factors related to early childhood, media advertising, and the availability of certain substances. *Somewhat-distal factors* could be referred to beliefs and competencies of particular families and individuals including parental support, academic competence, self-control, beliefs and expectancies. *Somewhat-proximal factors* are viewed to predispose the final steps in the pathway of onset and are represented by negative life events, perceived norms, feelings of anger and helplessness, and deviance-prone attitudes when a subject normalise a deviant behaviour to be accepted as a positive value. *Proximal factors* are generally characterised to be the final steps in the chain of substance-use-onset and take place immediately before taking a substance, either temporally or causally. Proximal factors are peer influences, per-

ceived similarity to prototype- the social image, and the motivational intentions of use [348].

Stress-coping model

The stress-coping model was originally formulated by Lazarus & Folkman [198]. The vulnerability to life stress is regarded as a general risk factor, predisposing for various kinds of problems. Coping is defined as a series of responses, initiated and repeated as necessary to handle the remaining, continued, or transformed nature of the stressor [308]. The perception of a given stressor does not possess exactly the same reality across people. Individual differences prime people to react differently, to take different routes, as they encounter the inevitable stressors in their life journeys. Each person brings his or her set of characteristics to form a vehicle for dealing with whatever stressors may be encountered and this has given rise to the formulation of Snyder's *Coping machines* [308]. Coping processes are proposed to operate by retarding the development of problems in combination with buffering protective factors such as certain types of competence variables and personality related variables [348]. The stress-coping model has been extended to include also substance misuse. In this context, life stress is posited as a risk factor for substance misuse and the intake of substances is ascribed coping functions itself [348]. In this model low social, behavioural and academic competencies, accompanied by the absence of parental support, are shown to be directly related to misuse [348].

Sensation seeking

Sensation seeking is defined as a human trait that is characterised by "varied novel, and complex sensations and experience and the willingness to take physical and social risks for the sake of such experience" [373]. Zuckerman's first sensation seeking hypothesis [372], was developed in the light of Hebb's suggestion that an optimal level of arousal would regulate behaviour [150]. According to this original theory, the propensity to seek novel and intense sensations and experiences represented an attempt to reach and maintain an optimal level of cortical arousal. Nowadays sensation seeking is viewed as a general sensitivity to reinforcement with an alteration of chatecholamines and their monoamino oxidase (MAO) levels. Low MAO activity has been associated with high sensation seeking, impulsivity, aggressivity, criminality, sexuality, substance use and abuse [253,289,374].

2.2.2 Hedonia, Reward-related Learning, Incentive states

The predominant key, providing the power of the actual perception of pleasurable feelings related to natural and drug stimuli, is today believed to be the medial forebrain bundle with its neurochemical components of ascending and descending pathways [92,93]. The predominant theory that underlies the actual perception of hedonia and reward-related associative learning has long been ascribed mechanisms of the mesocorticolimbic dopamine system (section 2.3.1) [91,92,183,184,310].

Hedonia, in terms of natural and drug rewards is associated with stimulation of dopamine transmission in the nucleus accumbens shell [94,259]. The existence of a reward centre in the brain has emerged both from studies indicating that animals respond for electrical self-stimulation via electrodes implanted in distinct brain regions [250], and self-administer drugs abused by humans [183,353-355]. Rewards are positive stimuli with a primary motivational value as they predict events useful for survival without requiring an associative learning process [92]. *Reward-related-associative learning* takes place when certain stimuli, predictive for the drug intake [297,298], give rise to motivational conditioning and when such stimuli themselves have the capacity to induce rewarding properties [91,92]. Associative learning on the other hand is the mechanism by which neutral stimuli acquire motivational value [295]. During conditioning associative memories between the subjective perception of the positive/aversive effect and environmental stimuli are formed. Thus, environmental stimuli such as the Pavlovian bell, which repeatedly have been associated to predict the effects of a primary reinforcer, become conditioned and accordingly gain the ability to act as secondary reinforcers. Motivational stimuli may act as primary reinforcers if such stimuli are genetically predetermined [92]. Nevertheless recent theories, such as the incentive salience of rewards, suggest dopamine to be implicated in the incentive states (craving) rather than hedonic perception (liking). Herewith, dopaminergic-neurons are only suggested to mediate the assessment of the associative link between external stimuli and internal rewarding or aversive states [27,310].

2.2.3 Impulsivity, Disinhibition and Aggression

The psychological term of self-control [62], also of importance in substance use onset, is closely connected to impulsivity and disinhibitory behaviour. Reduced serotonergic and elevated plasma testosterone levels have both been associated with disinhibition and aggressiveness [46,68,101]. Serotonin function is inversely correlated with impulsivity to a higher extent than with aggression [101], while testosterone has been primarily implicated in aggressive rather than impulsive behaviour [14]. The intermediate and ventromedial hypothalamic areas are stated to represent the attack area of the rat [304]. The amygdala, prefrontal cortex (PFC), mediodorsal thalamic nucleus, ventral tegmental area (VTA), and periaqueductal gray (PAG) are associated with this attack area and thus of importance in aggressive behaviour. Other regions, e.g. the hippocampus and the nucleus accumbens, that are relevant for the regulation of emotional status and connected with the regions that belong to the aggression systems, have also the potential ability to modulate aggression [304].

2.2.4 Use, Abuse, Dependence, and Addiction

The transition from substance use, *via* abuse, to dependence and addiction is outlined in a step-wise process of drug taking behaviour [8,231]. In this thesis *substance misuse* refers to the non-prescribed use of an illegal substance that equals the term *substance use* as defined as the non-prescribed intake of a legal drug. Such intake implies occasional drug intake that may or may not be accompanied by clinically significant impairment or distress on a given occasion. These are both characterised by controlled drug use when the subject comes into contact with a drug of addictive liability and responds to the drug and to drug related stimuli in a controlled manner, close to normal motivated responding. These are not listed as medical disorders in either of the two commonly used diagnostic manuals, the DSM IV or the International Classification of Diseases (ICD-10), stated by the World Health Organisation (WHO). Repeated drug exposure may induce a failure to self-regulate drug intake and cause subjects to enter the stage of drug abuse, which later on also may induce dependence.

The states, *substance abuse* and *substance dependence* are defined in separate paragraphs in the DSM IV by the American Psychiatric Association (APA). Even though substance abuse is a less severe disorder of compulsive drug intake, than is substance dependence both conditions have been characterised as maladaptive patterns of substance use leading to clinically significant impairment or distress according to DSM IV. In general these are characterised as regular, sporadic, or intensive use of higher doses of drugs leading to social, legal, or interpersonal problems. In DSM IV, substance abuse is manifested if one or more out of four listed criteria are fulfilled within a 12-month period (for precise definition see DSM IV). *Substance dependence* is manifested if three or more out of seven criteria have occurred within a 12-month period, but is in general characterised by the condition of substance abuse to which signs of tolerance and withdrawal are added (2.2.3).

Tolerance and *withdrawal* are the result of processes that are recruited in order to maintain homeostasis. Tolerance is defined as the reduction in response after repeated administration of a fixed drug dose, or the need to increase the drug dose in order to achieve a pharmacological effect of the same magnitude as the initial response. Repeated drug administration, on the other hand, can also cause physiological adaptations that result in withdrawal symptoms. The psychic withdrawal symptoms are characterised by anxiety, dysphoria, irritability, aggression, and depression and may also be related to the altered brain neurotransmission during abstinence.

Scientifically, four major components of reinforcements are outlined to be the most important motivating factors for the development, maintenance, and persistence of drug addiction [342]. These are positive reinforcement, negative reinforcement, conditioned positive reinforcement, and conditioned negative reinforcement. *Positive reinforcement* is characterised when the drug is used in order to acquire the rewarding effects, and future drug intake occurs due to the reinforcing properties of the drug itself. *Conditioned positive reinforcement* is identical to positive reinforcement but mediated through drug-associated conditioned stimuli. *Negative reinforcement* arises when the drug is repeatedly consumed in order to avoid an

undesirable state. In some individuals positive and negative reinforcement, with or without conditioning, may simultaneously occur.

In the initiation of compulsive drug use positive reinforcing effects are critical for establishing self-administration behaviour, and have been postulated to be the key for drug dependence [352]. Substance abuse has been outlined as a state when the subject is able to control drug intake in the absence of drug-related stimuli, but elicits compulsive drug seeking in the presence of such stimuli [94]. Drug addiction could be equated with substance dependence as defined by the APA. However, some scientists argue that the term *addiction* should be used instead of *dependence*. This would signify that the term addiction is associated with drug-seeking behaviour outside of clinics, while dependence also could arise from medical purposes. Negative reinforcement has a predominant role in the maintenance of drug use after the development of dependence and is suggested to contribute to both continued drug use and relapse after cessation [191]. This phenomenon is in agreement with the *opponent process theory*, which identifies the counteradaptive responses, aversive states, and the associated reduction in mesolimbic dopamine neurotransmission after repeated drug exposure, as key elements in the development of addiction [309]. Addiction has also, in agreement with the opponent process theory, been presented as a cycle of spiralling dysregulation of brain reward systems that progressively increases, resulting in compulsive substance use and loss of control over drug taking [186,191,313]. Sensitisation has here been described as the increased response to a drug that follows its repeated intermittent presentation, whereas counteradaptation with the following negative reinforcement, are suggested to arise after chronic binge intoxication. Negative affective states may possess motivating properties in maintaining self-administration and drug-seeking behaviour by changing the set point of positive reinforcers [186,313]. In these paradigms, motivational factors of drug addiction have been formulated, e.g. dysphoria, withdrawal induced anhedonia and motivational dependence [186].

In such addictive states, the homeostatic regulation of reward has reached outside the physiological capacity into allostasis [313]. *Homeostasis* refers to a self-regulating process that maintains stability within the physiological range and holds all the body parameters of the organism's internal milieu within limits that allow an organism to survive [313]. *Allostasis* is the principle of maintenance of stability outside the normal homeostatic range, when an organism must vary all the parameters of its physiological system to match them appropriately to chronic demands. Under these circumstances, stability is achieved through change, and the organism resets the system parameters at a new set point [313]. *The allostatic state* is the chronic deviation from the homeostatic level. *The allostatic load* refers to the cost from the chronic deviation to the brain and body, subjected to repeated drug exposure, in terms of pathological states and accumulated damage [229].

2.3 THE MONOMAMINERGIC ENVIRONMENT

2.3.1 The Brain Dopamine Systems

Anatomy

In 1950s, Bertler, Rosengren, and Carlsson discovered that reserpine caused catecholamine depletion at the nerve terminal in vertebrate brain and tissues [29,31,61]. Dopamine was quite soon suggested to act as an independent neurotransmitter in the CNS (Central nervous system) and monoaminergic blood-brain barrier mechanisms were suggested [30,32-34,58,60]. The introduction of the histochemical method of formaldehyde fluorescence by Falck and colleagues [112], made it possible for Dahlström and Fuxe to outline 12 different groups of catecholamine positive neurons in the brain stem, referred to A1-A12 [83].

In the CNS, three dopamine containing systems are identified; the mesocorticolimbic, nigrostriatal and tuberoinfundibular pathways illustrated in Figure 3.

The mesocorticolimbic dopamine system can be subdivided into the mesolimbic, mesocortical and the mesoamygdaloid projections [179] that all originate in the VTA (A10) [40,83,333]. The limbic terminals are located in the AcbSh and the AcbC, the amygdala, the olfactory tubercle and the septal area [11,40,333]. The cortical regions constitute prefrontal, cingulate and the entorhinal cortex [11,40,333]. The nigrostriatal pathway has its nucleus (A9) in the SN pars compacta and projects to the dorsal striatum consisting of the caudate nucleus and the putamen [10]. The tuberoinfundibular pathway originates in the arcuate nucleus and terminates in the pituitary [82,124].

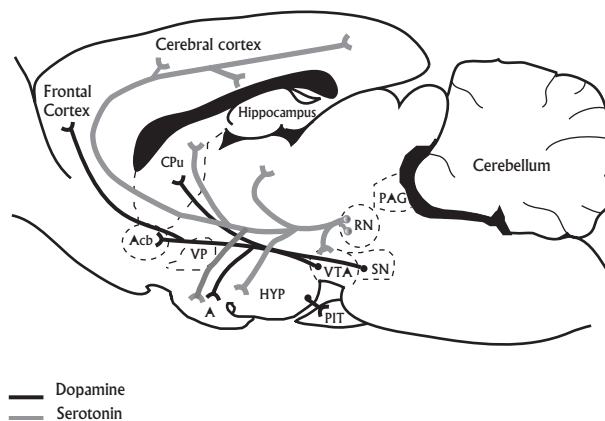


FIG. 3. Dopaminergic and serotonergic pathways in rat brain. A: Amygdala, Acb: Nucleus accumbens, CPu: Caudate putamen, HYP: Hypothalamus, PAG: Periaqueductal grey, PIT: Pituitary, RN: Raphe nucleus, SN: Substantia nigra, VP: Ventral pallidum

Dopamine biosynthesis and metabolism

Dopamine is synthesised from L-tyrosine in the cytoplasm of the nerve terminal [74]. The first rate-limiting step is exerted by tyrosine hydroxylase converting L-tyrosine to L-dihydroxyphenylalanine (L-DOPA). L-DOPA is an intermediate molecule that is rapidly decarboxylated by L-aromatic amino acid decarboxylase (AADC) to dopamine. Synthesised dopamine is packaged in storage vesicles by means of vesicular-monoamine transporter proteins (VMATs). Dopamine is released into the synapse via calcium dependent exocytosis. The major part of released dopamine is transported back into the terminal by a specific dopamine transporter (DAT) [129]. Dopamine is metabolised to dihydroxyphenylacetic acid (DOPAC) by the enzyme monoamine oxidase (MAO), mainly MAO-A, in presynaptic dopamine neurons and in extraneuronal glial cells. Dopamine can alternatively undergo degradation to 3-methoxytyramine (3-MT) and homovanillic acid (HVA) by MAO and catechol-o-methyltransferase (COMT). DOPAC is the predominant metabolite in rat brain, while HVA is the major metabolite in the human brain [74].

Dopamine Receptors

The physiological and pharmacological actions of dopamine involve various kinds of neurons and are mediated *via* activation of dopamine receptor. CNS dopamine receptors are encoded by five distinct genes that express five receptor subtypes (dopamine D₁-D₅). [54,128,130,251,327,367]. The D₂ subtype gene produces two major splice variant mRNAs (D_{2 long} and D_{2 short}) [54,130]. The protein products of the five dopamine-receptor genes are subdivided into two classes based upon sequence, homology, ligand binding and second messenger coupling. The classes are the D₁-like (D₁ and D_{1b (rat)}/D_{5 (human)}) and D₂-like (D₂, D₃, and D₄) receptor families [249]. D₁-like receptor subtypes stimulate adenylate cyclase and thus enhance the formation of the second messenger cyclic adenosine 3', 5'-monophosphate (cAMP) via interaction of the G protein G_s [175], whereas the D₂-like receptors inhibit the activation of adenylate cyclase via the G protein G_{i/o} with a subsequent decrease in cAMP [252]. Both receptor families are located postsynaptically, but the D₂- and the D₃- subtypes also act presynaptically as autoreceptors [41,59,203,282].

Dopamine receptors are widely distributed in the brain even though the density of the receptors vary [240]. Dopamine D₁-receptor mRNA has been found in the CPu, Acb, and the olfactory tubercle. In addition, dopamine D₁-receptors have mainly been detected in the limbic system, hypothalamus, and thalamus. The dopamine D₂-receptor and its mRNA are mainly found in the CPu, the olfactory tubercle, and in the Acb, where the receptor is postsynaptically coexpressed with preproenkephalin. Dopamine D₂ receptor mRNA is expressed in dopaminergic neurons in the hypothalamus, VTA, and SN pars compacta. Dopamine D₂-receptor mRNA is among other regions also present in the prefrontal, cingulate, and temporal cortex, in the septal region, amygdala, and granule cells of the hippocampal formation. In the CPu, the dopamine D₁-receptor is located on striatonigral GABAergic neurons coexpressing preprotachykinin

and prodynorphin and the dopamine D₂-receptor on striatopallidal GABAergic neurons coexpressing preproenkephalin [126]. In the AcbSh, the dopamine D₁-receptor is co-expressed with preprotachykinin on GABAergic projections to the VTA, while similar projections from both the shell and the core are terminating in the ventral pallidum, VP [215]. The dopamine D₂-receptor is coexpressed with preproenkephalin on GABA neurons projecting from the AcbC to the VP [215], as well as on neurons that to some extent may project from the AcbSh to the VTA [170].

Functional properties of Brain Dopamine

Dopamine is the major catecholamine in the brain. The mesocorticolimbic dopamine pathway is implicated in the regulation of cognition and emotion. The foremost attribute of this system is its role in the brain reward systems (2.2.2-2.2.3), but it is also well characterised as a therapeutic target for antipsychotic drugs in people with psychotic disorders such as schizophrenia [80,87]. The core of the Acb is apart from the shell [151], predominantly connected to the locomotor functions of the nigrostriatal pathway. Degeneration of nigrostriatal dopaminergic neurons is observed in patients with Parkinson's disease, with severely dysfunctional voluntary motor control. The tuberoinfundibular dopamine system controls prolactin release from the anterior pituitary [241].

2.3.2 The Brain Serotonin Systems

Anatomy

In 1953, the presence of serotonin, also known as 5-hydroxytryptamine (5HT) in mammalian tissue was initially described by Twarog and Page [332], and in 1955 Brodie and co-workers demonstrated that 5HT possesses a functional role in the brain as a neurotransmitter [47]. In 1964, nine discrete groups of 5HT containing neurons named to B1-B9, were described by Dahlström and Fuxe [83]. The 5HT neurons originate in the medial (B8; MRN) and the dorsal raphe nuclei (B6 and B7; DRN). The projections originating in the MRN innervate the VTA and the hippocampus, whereas the projections from the DRN terminate in the striatum, prefrontal cortex (PFC), and the SN. The Acb is innervated both from the MRN and the DRN [312], Figure 3.

Serotonin biosynthesis and metabolism

Biosynthesis of 5HT is derived from the essential amino acid L-tryptophan. The rate-limiting step is characterised by the hydroxylation of L-tryptophane to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase (TPH) requiring the cofactors oxygen and tetrahydrobiopterin. 5-hydroxytryptophan is further converted to 5HT by AADC. Newly synthesised 5HT is actively transported from the cytosol into vesicles, stored in nerve terminal, and released into the synapse

by calcium-dependent exocytosis in response to an action potential. Extracellular 5HT is partly metabolised by MAO-A present in non-serotonergic neurons, such as in dopaminergic and glial cells. The remaining extracellular 5HT is rapidly transported back into the 5HT neuron by the presynaptic 5HT transporter. In the cytoplasm, 5HT can either be directly transported into the vesicles or be degraded by MAO-B and the aldehyde dehydrogenase yielding 5-hydroxyindole acetic acid (5-HIAA), the main metabolite of 5HT. The MAO-B present in serotonergic neurons has a lower affinity for 5HT than does MAO-A, and degradation by MAO-B occurs only when 5HT concentrations are in the millimolar range [74].

Serotonin Receptors

To date, at least fourteen distinct 5HT-receptor subtypes have been cloned, of which thirteen belong to the G-protein-coupled receptors [23,371]. The 5HT₁ family is negatively linked to adenylate cyclase via G_i and G_o complexes. This family consists of 5HT_{1A}, 5HT_{1B} (human 5HT_{1Dα}), 5HT_{1D} (human 5HT_{1Dβ}), 5HT_{1E}, and 5HT_{1F}-receptors. The 5HT_{1A} receptors are found in the MRN and the DRN where they act as somatodendritic autoreceptors regulating firing activity. These receptors are also distributed in the frontal and cingulate cortex, the hippocampus, the septum and the amygdala. The 5HT_{1B} receptors are located both pre- and postsynaptically and predominantly occur in the VTA, the SN, the globus pallidus (GP), the dorsal subiculum (DS), and the superior colliculi (SC). The presynaptic 5HT_{1B} receptors may either act as auto- or heteroreceptors [285]. The 5HT_{1D} receptors are present in the DRN, the basal ganglia, the SN, and the GP [23]. The 5HT_{1E} receptors are identified in the brain but display low affinity for serotonergic compounds and the 5HT_{1F} receptors are sparsely distributed. The 5HT_{2A}, 5HT_{2B}, and the 5HT_{2C} receptors represent the 5HT₂ receptor family. These receptors act through G_q proteins and stimulate phosphoinositol hydrolysis. A high density of the 5HT_{2A} subtype is present in the striatum, the Acb and the cortical areas, where the 5HT_{2A} subtype mainly is localised postsynaptically on non-serotonergic neurons. The 5HT_{2C}-receptors are found in the cortex, hippocampus, olfactory tubercles and choroid plexus. The 5HT₃-receptor is a ligand-gated non-selective Na⁺/K⁺ ion channel, which depolarises the target neuron via cation influx when activated [360]. The 5HT₃-receptors are found in the frontal cortex, the entorhinal cortex, the area postrema and in limbic regions, such as the hippocampus, the amygdala, and the Acb. These receptors are heteroreceptors modulating the activity of other neurotransmitters such as dopamine and noradrenaline. The 5HT₄, 5HT₆, and 5HT₇-receptors stimulate adenylate cyclase and increase cAMP *via* interaction with the G_s complex. The signal transduction mechanism of the 5HT₅-receptor is not yet known. The 5HT₄-receptor is located in the hippocampus and the colliculi, the 5HT₆-receptor subtype mRNA in the striatum, Acb, and cerebral cortex. The 5HT₇-receptor subtype is present in the amygdala, the thalamic, and in hypothalamic regions [23].

Functional properties of Brain Serotonin System

The brain 5HT is implicated in a wide range of psychological processes including motor functions, sleep, sexual behaviour, anxiety, mood, neuroendocrine functions,

inhibitory control, impulsivity, aggression, and intake of food and addictive drugs [23,161]. Dysfunction of the 5HT system has accordingly been associated with several psychiatric disorders such as affective illness, schizophrenia, anxiety, drug abuse and obsessive-compulsive disorder. Subjects suffering from impulsive behaviour and impulse control disorders often display signs of reduced 5HT neurotransmission (low 5-HIAA levels and low platelet MAO) [2-4,101,211,212]. Treatment with selective serotonin reuptake inhibitors (SSRI's) is effective in order to ameliorate some of these disorders [109].

2.4 PERSPECTIVES OF AAS MISUSE IN SOCIETY

2.4.1 Doping, Sports and Body Image

Misuse of AAS is commonly perceived to be the domain of competitive elite athletes in terms of doping and highly associated with body building [156,210]. AAS are most frequent in sports related to size and muscular strength such as weight lifting, football, wrestling, and track and field events [53,106,314]. In the US, body building appears to be gaining in popularity and is estimated to comprise 5 million people [132].

Body image is, in contrast to an unbiased objective view of the outer attractiveness, referred to an "internal" image or representation that we have of our own physical appearance [326]. The modern society's praises of a muscular male body ideal, both through media and toys, have led to an increase in body image concerns among men [202,267,268]. Consequently, body dissatisfaction and eating disorders are no longer restricted to females. The male body ideal focuses on mesomorphic (muscular) characteristics, rather than endomorphic (fat) and ectomorphic (thin) aspects [311], while the female ideal is characterised by the desire to be thin and loose fat [69,139,228]. A condition called muscle dysmorphia has been identified, in which muscular men perceive themselves as thin and underdeveloped [260]. Along the path to a more muscular physique it is not surprising that eating disorders and states as "reverse anorexia", the male response to the predominant female condition anorexia nervosa [264] has developed among body builders [132].

2.4.2 Violence

The relationship between AAS and acts of violence [65,269,324], of which significant others (mainly girlfriends and wives) quite often are victims [65], have become a part of reality in society during recent years. Three subgroups of AAS perpetrators have been outlined namely the "roid rage," "terminator" and the "stürmschnapps" group [324]. The violent act of subjects belonging to the "roid rage" group [262,263,324,362] has been preceded by a slight provocation and is characterised by being intense, repetitive and long lasting [324]. Perpetrators in the "terminator" group, all committed executioner-like homicides in a non-impulsive manner, seemingly with time to reconsider, after having been subjected to provocation from

victims [324]. A schnapps given to the soldiers of the Austrian-Hungarian army before storming has given rise to the name of the third subgroup of AAS perpetrators, the "stürmschnapps" group, which consists of subjects taking AAS in order to induce aggression prior to a planned criminal event [324]. Patterns of AAS misuse seems to be strictly gender specific in favour of males. At the time of the initiation of this thesis, some studies conducted in the US indicated that misuse of AAS had extended amongst adolescents [53,365]. Since American trends are often adopted in Sweden, these changes in patterns are quite alarming. Knowledge about the profile of AAS misuse was mainly restricted to aspects of doping, body building, desires of improved appearance, multiple drug use, and violent acts [53,107,365], while the importance of factors regarding sociodemography, school, and personality related aspects were not well understood. Thus, it was, and still is, urgent to test the hypothesis if the problem behaviour theory and models of the initiation of substance misuse can also be extended to include patterns of AAS misuse.

2.5 PSYCHIATRIC AND BIOLOGICAL PERSPECTIVES OF AAS

There is a wide spectrum of effects on the physique and mental health documented to be associated with intake of AAS [176,216,217,263], which is not surprising considering the various dose regimens that are applied. The literature presents mainly case reports or retrospective studies, of which subjects are encountered in misuse and abuse. To date, there are too few controlled prospective studies performed in order to draw any firm conclusion about how the sort and magnitude of the physical and psychiatric effects are related to dimensions of time and dose. Thus, such comparisons should be regarded with caution.

2.5.1 Physical side effects

AAS-induced effects on the external physique are, besides muscle mass and strength [35], acne, striae, baldness, and gynecomastia [6,110,261,302,316]. Suppression of the endogenous production of luteinising hormone (LH) and follicle-stimulating hormone (FSH) is reported with reduced testosterone secretion, testes atrophy, a deficient spermatogenesis, and impotence to follow [36,123,219,329,330]. In men, elevated estradiol levels close to those of females are reported as a consequence of the aromatase metabolism, which cause gynecomastia [123]. Cardiovascular diseases such as altered serum lipoprotein levels (high levels of low density lipoprotein (LDL) and low levels of high density lipoprotein(HDL)), disturbances in blood coagulation, affected triglycerides, hypertension, liquid retention, myocardial infarction, enlarged heart muscle, and sudden death related to arrhythmia or stroke are further documented [98-100,117,157,177,178]. The 17α -alkylated steroids are highly associated with liver dysfunctions including jaundice, hepatic malignancy, hepatic carcinoma especially peliosis hepatis [20,57,79]. Disturbances in the thyroid function are also reported [7]. Certain side effects exclusively related to females are

deepening of the voice, hirsutism, clitoromegaly, galactorrhoea, and an irregular menstrual cycle, and amenorrhoea [43,88,135].

2.5.2 Psychiatric and neurobiological alterations

One of the first controlled prospective studies investigating the neuropsychiatric effects of AAS is worth discussion [317]. This study is a two-week double blind, placebo controlled crossover trial of methyltestosterone. The sample investigated consisted of 20 male volunteers, who were free of medical and psychiatric illness, athletic training, medication, and had no prior history of AAS abuse. All men were subjected to four treatment conditions of 3 days each; placebo baseline, 40mg/day, 240 mg/day, followed by placebo to induce withdrawal. The high dose of methyltestosterone induced significant alterations in positive mood states such as euphoria, energy, sexual arousal, in negative mood states in terms of irritability, mood swings, violent feelings, and hostility, and in cognitive impairment including distractibility, forgetfulness, and confusion. One subject became manic and another hypomanic. In addition, other controlled studies have found AAS to evoke major mood disorders [263], mania, hypomania, and aggression [265], and violent behaviour [148,190]. Furthermore, to share personality characteristics such as antisocial behaviour with alcoholics [362], and to induce high activity scores and increase the serotonergic metabolite 5-HIAA in CSF [86].

Some researchers regard certain effects to be confined to the early state of misuse, while other effects rather are viewed to appear after prolonged misuse [77]. Pleasurable states that may arise in the early career of misuse are enhanced energy, motivation, enthusiasm, self-esteem, self-confidence, libido, and euphoria [86,119,261,317]. Other reported AAS-induced conditions such as insomnia, diminished fatigue, and the ability to train despite of pain may initially be desirable [119,261,317], but are of course ultimately problematic. Side-effects that may appear at the beginning of the misuse are irritability, anger, confusion, anxiety, agitation, hostility, mania, hypomania, psychosis, and paranoia [48,55,76,85,102,262,263,317]. After prolonged abuse of AAS adverse effects may arise, such as impulsivity, disinhibition, suspiciousness, and aggression. These side effects may underlie a series of violent acts against significant others (commonly girl friends), attempts of suicide, criminal acts, child abuse, suicide, and attempts of murder [1,49,65,72,73,75,125,262,265,266,269,270,345]. After discontinuing the AAS misuse, withdrawal effects such as depression, agoraphobia, insomnia, nightmares, mood swings, anxiety, apathy, loss of appetite, and concentration problems usually arise [21,51,78,261,270].

Several studies indicate that AAS may induce dependence [48,75,149,263,366]. The potential implication of AAS in dependence has been investigated with respect to the DSM III or IV criteria of substance dependence in humans. In a recent study by Copeland and co-workers 78% of 100 current AAS misusers presented at least one symptom of abuse or dependence, 25% exhibited AAS abuse using DSM IV criteria, and 23% met the criteria of AAS dependence [76]. These findings are

consistent with previous studies by Brower and co-workers in which a major part of the AAS users qualified for dependence according to the DSM III criteria [50,51]. However, because there are no reliable animal models of AAS self-administration due to the long duration of onset and maintenance and AAS-induced effects on locomotion are not clear-cut [239,284], the issue whether AAS could be classified as a drug capable of promoting the development of dependence substance is still a matter of controversy.

Some AAS-induced signs of increased sensitivity towards external stimuli and aggressive states have also been observed in behavioural studies of rats [165,209,230]. Neurobiochemical mechanisms that underlie the psychiatric conditions observed among AAS abusers are not yet well understood. Recent studies of the male rodent brain indicate that AAS affects substrates of the HPA-axis [293,294], FOS activity [167], dopaminergic [324], opioid [15,164,166,213], tachykinin [143], glutamate [199,200], and serotonergic systems [209,323]. These are all systems that are implicated in the regulation of reward, impulsivity and aggression. Most of the studies conducted, have focused on the implication of various systems in terms of estimated activity or mRNA content of peptides, monoamines, or steroids and their corresponding precursors and metabolites. In 1998-1999, at the time of the initiation of the neurobiological part of this thesis, there was particularly a lack of evidence for the role of different receptors in AAS-induced effects.

Chapter 3

OBJECTIVES OF THE THESIS

The objective of this thesis is to test the hypothesis that models of AAS misuse share similarities with misuse of psychotropic substances both from epidemiological and neurobiological points of view.

The specific aims of the projects were:

1. To investigate the extent of misuse of AAS and other doping agents among adolescents through a survey conducted in a large city in Sweden.
2. To evaluate models of both doping agents and AAS misuse among adolescent males, reflecting the importance of factors regarding sociodemography, school, sport, personality, violence and the intake of other substances for the AAS misuse.
3. To test the hypothesis that models of psychotropic substance misuse can be extended to include also AAS misuse.
4. To test the hypothesis that AAS, shown to share similarities with psychotropic substances in the epidemiological portion of this thesis and earlier psychiatric studies, may affect the protein expression and gene regulation of dopamine receptors and other dopaminergic substrates in regions of the male rat brain regulating rewarding properties.
5. To test the hypothesis that AAS, shown to be implicated in impulsivity and violence, may affect the density of serotonin receptors in regions of the male rat brain regulating cognition, disinhibition, emotional status, and aggression.

Chapter 4

STUDY SUBJECTS AND METHODS

4.1 EPIDEMIOLOGICAL METHODS

4.1.1 Study Population (Paper I, II, and III)

In 1995, data were collected from all students in the first (16-17 years of age) and third (18-19 years of age) senior high school years in Uppsala, Sweden. Of the students who participated in the survey, 1,592 were in their first year and 1,150 were in their third year. The non-participation rate was 19.2%, 99% of which consisted of students absent from class and 1% of students who did not answer the survey.

4.1.2 Study Design

The studies presented in Paper I, II, and III were cross-sectional. Students were administered a multiple-choice questionnaire, which they answered anonymously while supervised by a teacher. The questions used in this survey were mainly standard questions from school surveys conducted by the Swedish Council for Information on Alcohol and other Drugs (CAN) and the National Institute of Public Health. Some new questions were developed e.g the questions about doping and AAS.

4.1.3 Outcome Variables

Doping agent misuse (Paper I, II)

The questionnaire asked students about their misuse of doping agents. Lifetime misuse refers to use at least once during a respondent's lifetime. The commonly understood term, 'anabolic steroids,' was listed in the survey instead of 'anabolic-androgenic steroids,' and testosterone was listed as a separate kind of doping agent in order to avoid misunderstandings among respondents. The extent of lifetime misuse of doping agents was assessed by the question 'Have you ever used any of the following doping agents without a doctor's prescription?'. The most common doping products were listed as choices, for each for each category—testosterone, anabolic steroids, and peptide hormones (growth hormone or human chorionic gonadotrophin). In each category of substance, choices also included 'another product' and 'a product whose name I have forgotten.' For each selected choice the respondent could answer, 'yes, as a tablet,' 'yes, by injection' or 'no.' Additionally, respondents were asked whether they used 'doping agents other than testosterone, anabolic steroids, or peptide hormones.'

Anabolic-androgenic steroid use (Paper I, II, III)

The extent of the testosterone and the anabolic-androgenic steroid misuse, respectively, were presented separately in Paper I, in order to distress the number of subjects who had rated ‘anabolic steroids,’ and testosterone, separately in the questionnaire. In the data analyses evaluating the importance of different background factors (Paper II and Paper III) for the misuse of AAS, testosterone was included in the scientific definition of AAS.

The most common doping products listed as choices were for testosterone (Sustanon®, Testoviron-Depot® and Undestor®) and for anabolic steroids (Metahapoctehonol®, Parabolan®, Primobolan®, Winstrol®, Anasteron®, Anadur® and Decadurabolin®).

4.1.4 Factors of Importance Analysed in the Survey

Sociodemographic and school variables

Gender, age, school grade, urbanisation, parental influences, and ethnicity were taken into consideration. The degree of urbanisation was estimated from the questions, ‘Where have you been living during the main part of your life?’, ‘Where do you live right now?’, ‘How long have you been living where you live now?’ The first question was followed by listed alternatives of different cities and sizes, the second by alternatives with regard to Uppsala and its immediate surroundings, and the third by various year spans. The students were asked whom they shared their household with to provide an indirect estimate of the influence from parents. Ethnicity was estimated in terms of immigrant status and was based on the questions, ‘Were you born in Sweden or abroad?’, ‘How long have you been living in Sweden?’ *High* immigrant status was given to senior high school students who had moved to Sweden at the age of 9 years or older, whereas adolescents who were native born or had moved to Sweden before age 9 years were given a *low* immigrant status. The questions regarding school achievement and truancy were based on self-perceptions and framed as follows. ‘What do you think about your school achievement in comparison to that of other youths in your age?’ ‘Do you play truant?’

Sports activities

A set of questions was assessed in order to obtain knowledge about respondents’ sports activities ‘Are you active in sports in your spare time?’ and ‘If so, do you practice any of these sports activities?’ Among other sports, strength training was listed.

Personality related factors

The student’s self-esteem and perceived peer relations were investigated in a set of 17 statements (Table 1). Each statement had responses on a five-point scale ranging from ‘do not agree at all’ to ‘completely agree’.

Motives for use

The doping agent misusers were also questioned about their main reasons for the use. The respondents could rate either one or several alternatives from the choices; to get a more attractive body/larger muscles, to enhance sports performance, because it was fun to try, to become intoxicated, to become braver, because friends do so, and another reason.

Use and misuse of other substances

The variable tobacco use included both smoking and snuff. The occurrence of this use was assessed by the questions ‘Do you smoke?’ and ‘Do you take snuff?’ Different answers were listed according to frequency of use. Tobacco users were defined as those who responded that they were presently smoking or taking snuff.

The questionnaire asked for the frequency of heavy alcohol consumption as consuming at least half a bottle of spirits, one bottle of wine, four bottles of beer (5.2% volume), or six bottles of beer (4.2% volume) on the same occasion. The survey also asked about lifetime use of prescription and non-prescribed tranquillisers or sedatives. One question asked for lifetime misuse of psychotropic substances including; cannabis (hashish, marijuana, cannabis oil), opiates (heroin, morphine, methadone, opium), cocaine (cocaine, crack), amphetamine, methamphetamine (ICE), LSD, MDMA (ecstasy), and psilocybin.

Violence

The question about violence was framed as ‘Have you been exposed to violence during the past term in Uppsala?’

4.1.5 Data analyses

All statistical analyses were carried out using SAS statistics program [180,286].

Descriptive analysis

Cross-tabulations were initially carried out to evaluate factors, categorical and continuous, potentially associated with the dependent variables, doping agents (Paper II), AAS (Paper II, Paper III), and psychotropic substances (Paper II, Paper III). In order to identify significant differences between groups of subjects Chi-square statistics or Fisher’s exact tests were applied for comparison of proportions and Mann-Whitney *U*-tests to compare medians for quantitative ordinal-scale variables. Correlation analyses between quantitative ordinal-scale variables were performed with Spearman’s rank correlations.

Logistic regression analysis

Logistic regression analyses were carried out to estimate the association between different suggested determinants and the dependent variables, doping agents (Paper II), AAS (Paper II, Paper III), and psychotropic substances (Paper II, Paper III). Factors potentially associated with investigated dependent variables were tested in bivariate logistic regression analyses. Bivariate logistic regression analysis

was only applied to study the specific associations in misuse between the AAS and individual psychotropic substances (Paper II).

To identify different groups of doping agent misusers, background factors were analysed for different delineated subgroups. The doping agent misusers were subdivided according to their reasons for misuse or by their activity in sports in their spare time. The question concerning reasons for misuse was dichotomised. One group consisted of those who had reported that the reason for taking doping agents had been because it was fun to try, to become intoxicated, to become more 'brave', because friends do so, or that they had used doping agents for another reason than was stated in the question. In the other group those who had answered that they had misused doping agents to get a more attractive body/larger muscles or to enhance sports performance were included. We examined if there were differences between the groups with respect to background factors.

Different multiple logistic regression models were created to determine the combination of variables that could best explain misuse, incorporating the significant individual factors from the bivariate analyses. Multiple logistic regression models were used to hold constant suspected confounding variables and to evaluate whether the observed associations were independent of one another. As regression models were constructed, variables were omitted when they were highly interrelated with each other or showed no independent association with dependent variables investigated.

Factor analysis

Factor analysis was employed to create measures of the background factors self-esteem and perceived peer relations (Paper III). A two-factor orthogonal varimax solution of the 17 items showed that two dimensions could be reliably measured. The first factor—including 10 items with loadings > 0.40 —clearly mirrored self-esteem. The internal consistency of the 10 items, as expressed in Cronbach's alpha coefficient, was 0.83. Six items describing perceived peer relations had factor loadings > 0.40 in the second factor. Coefficient alpha amounted to 0.76. The item—'I am a curious person'—did not load in either factor and was not included in the scales. Individual scale values were constructed by averaging the individual ratings in each scale.

4.2 NEUROBIOCHEMICAL TECHNIQUES

4.2.1 Animals, Treatments and Ethics

Animals

Adult male Sprague-Dawley rats served as subjects and were supplied by Alab, Sol-lentuna, Sweden (Paper IV, V, VI, VII). The animals were housed four per cage at a temperature of $22 \pm 1^\circ\text{C}$, at a humidity of $55 \pm 5\%$, in air-conditioned rooms with a controlled light-dark cycle (12 hour light: 12 hour dark cycle). The rodents

had free access to water and food (R36 food pellets, Labfor, Lactimin, Vadstena, Sweden). During an adaptation period of one week animals were allowed to adapt to the department facilities and be handled by the experimenter. The rats were randomised into their respective treatment groups presented in Paper IV, V, VI, VII. All experiments of this thesis were approved by the local ethical committee of the Swedish National Board for Laboratory Animals.

Drug treatment

The AAS treated rats were given nandrolone decanoate (Deca-Durabol®, Organon, Oss, Netherlands) and the control rats were given the vehicle arachidis oleum (Apoteket AB, Sweden). All rats were subjected to chronic treatment with daily intra muscular injections for 14 days. In Paper IV, V, and experiment I of Paper VI the nandrolone dose was 15 mg/kg/day. In Paper VII and experiment II of Paper VI, four different doses of nandrolone decanoate were used (1, 5, 15, and 45 mg/kg/day). All injections were conducted during the light phase.

4.2.2 Biochemical Methods

Overview

- Paper IV The total specific binding to dopamine D₁-like and D₂-like receptors were estimated *in vitro* by autoradiography.
- Paper V The total specific binding to the dopamine transporter, the dopamine D₁-like and D₂-like receptors were estimated *in vivo* using Positron Emission Tomography, PET.
- Paper VI The mRNA content of the dopamine D₁-receptor and the dopamine D₂-receptor subtypes were investigated by Northern Blot solely in experiment I, while mRNA levels of tyrosine hydroxylase, dopadecarboxylase, the dopamine D₁-receptor and the dopamine D₂-receptor subtypes were all assessed by *in situ* hybridisation in experiment II.
- Paper VII The total specific binding to the serotonin 5HT_{1B} receptor and 5HT₂-like receptors were estimated *in vitro* by autoradiography.

Tissue preparations

Animals from the experiments in Paper IV, VI, and VII, were sacrificed by decapitation on day 15, approximately 24 hours after the last nandrolone or oil injection. The brains selected for *in vitro* autoradiography (Paper IV, VII) and *in situ* hybridisation (Paper VI), were rapidly removed and frozen in 2-methyl butane at -20°C to -30°C and stored at -80°C until further used. The brains were cut in a cryostat at -19°C into coronal sections of 12 µm (Paper IV) or 10 µm (Paper VI and VII), respectively. In Paper IV, sections were collected at bregma +1.6, -2.8, and -5.2 mm, in experiment II of Paper VI at bregma +1.6 and -5.2, in Paper VII at bregma + 4.7,

+1.6, -1.3, -2.8, -5.2 mm, and [255]. The sections were thaw-mounted on gelatine-coated slides, dried with a fan for 60 minutes and stored at -80°C.

Radiochemistry (Paper IV, V, VII)

The radioligands used for autoradiography were in Paper IV [¹²⁵I]-(+)-SCH 23982 (D₁-like receptor antagonist) and [¹²⁵I]-NCQ-298 (D₂-like receptor antagonist) and in Paper VII [¹²⁵I]-(\pm)-iodocyanopindolol (5HT_{1B} receptor antagonist) and [¹²⁵I]-(\pm)-DOI (5HT₂ receptor agonist). All radioligands had a specific activity of 2,200 Ci/mmol and were obtained from NEN Life Science Products (Boston, USA). The tracers used for the *in vivo* PET analysis, in Paper V, were [¹¹C]-(+)-SCH23390 (D₁-like receptor antagonist), [¹¹C]-Raclopride (D₂-like receptor antagonist), and [¹¹C]-FE - β -CIT (cocaine analogue acting as dopamine transporter ligand) [113,145]. The ¹¹C used for the syntheses was generated by a 17 MeV cyclotron (Scandtronix, Uppsala, Sweden) and converted to methyl-[¹¹C]-iodide for the methylation reactions of respective precursor to generate the radioligands. The radiochemical purity of each batch was greater than 95 % and the specific radioactivity in the range 1 – 10 GBq/micromole.

Autoradiography (Paper IV, VII)

The autoradiographic procedure to label the dopaminergic D₁-like and the D₂-like receptors is presented in Paper IV, and the experimental settings to analyse the serotonergic 5HT_{1B} and 5HT₂ receptors in Paper VII. Briefly, the slide-mounted sections were brought to room temperature with a fan for 60 minutes. Sections were subjected for preincubation at room temperature for 10-30 minutes in a buffer carefully chosen for each kind of receptor investigated. Incubation was performed with hot radioligand for 90-120 minutes (time length depended on radioligand used), either in the presence (non-specific binding) or absence (total binding) of a cold ligand selective for the receptor of interest. The incubation buffer was supplemented with certain antagonists to adjust for unselective binding of the radioligand. Sections were rinsed 2 x 5 or 2 x 10 minutes in their corresponding cold binding buffers, quickly dipped in distilled water at 4°C, dried under cold air, placed in X-ray cassettes and co-exposed with plastic standards (Autoradiographic [¹²⁵I] Micro-scales (2.2-160 nCi/mg), Amersham, Stockholm) to autoradiographic film (Amersham Hyperfilm) at 4°C for 1-7 days.

The films were manually developed (Kodak D19,Unifix). The autoradiograms were digitized using a dia-scanner (DuoScan T1200, Agfa), and the optical densities were converted to fmol/mg wet weight based on the coexposed standards using NIH-Image software (NIH Image 1.62, NIMH, Bethesda, MD) and Scion-Image software (Scion Image NIMH, Bethesda, MD). Brain regions were identified with a rat brain atlas [255].

PET-scan (Paper V)

All PET scans were performed on day 15 of each treatment period, using a dedicated animal PET camera (Hamamatsu SHR-7700). Three overlapping treatment periods were arranged for each of the three radioligands. On each day of PET imaging, one

set of 6 animals (3 controls; 3 AAS treated) were simultaneously scanned. Chloral hydrate (400 mg/kg, i.p., Sigma) was administered as a bolus dose 45 minutes prior to the scanning. Thirty minutes after the bolus injection i.v. continuous anaesthesia (chloral hydrate, 100 mg/kg/hr) was given. In order to administer anaesthesia as well as radiolabelled tracer, a cannula was implanted in the tail vein of the rats. The PET camera used has a 3.5 mm resolution and was operated in 2D-mode with simultaneous acquisition of 31 tomographic slices, each with a thickness of 4 mm. Each PET session consisted of a blank scan (60 minutes), transmission scan (30 minutes), and a total dynamic scan including 16 frames (5 frames 1 minute; 5 frames 3 minutes; 4 frames 5 minutes; 2 frames 10 minutes). The emission acquisition was initiated at the moment of injection of the tracer, typically at 8 MBq/rat as an i.v. bolus. The images were reconstructed with attenuation and scatter corrections. In a summation image, including the last 4 time frames, the brain regions of interest (ROIs), striatum and cerebellum, were identified using the rat brain atlas, and time activity curves in ROIs were obtained. The tracer's specific binding in the striatum was analysed using the Lammertsma reference method [195]. The time activity data of cerebellum was used as reference region. With these tracers used, specific binding should be absent in cerebellum and this region would only show the distribution of free and non-specifically bound tracer in the tissue. The analyses were performed with a non-linear fit of the kinetic model to the uptake data using an in-house generated Math-Lab. The binding potential (BP) was used as an index of specific binding.

Northern Blot (Paper VI)

Northern blot analysis was conducted in order to analyse the mRNA expression of the dopamine D₁- and the dopamine D₂-receptor subtypes. The cDNA that served as probes for the dopamine D₁- and the dopamine D₂-receptor subtypes, were synthesized by reverse transcriptase polymerase chain reaction, RT-PCR. Total RNA, used in the RT-PCR and the northern blot analysis, was isolated using the single step acid guanidinium thiocyanate-phenol-chloroform method [66]. In the RT-PCR, 5 µg of total RNA from the rat striatum was reversed transcribed using random primers (pd(N)₆, Amersham Pharmacia Biothech, Sweden) and RNase-H-reversed transcriptase (SuperscriptTM II, Life Technology AB, Sweden) according to manufactures manual. The obtained cDNA was amplified with Taq-polymerase (AmpliTaq® DNA polymerase, Stoffel fragment, Perkin-Elmer Wallace, Sweden) and oligonucleotides of the respective receptor subtypes included in the PCR reaction. The cDNA probe used for the dopamine D₁-receptor was the 225 bp fragment spanning from bp 704-928 reported by Zhou and co-workers [367]. The dopamine D₂-receptor cDNA probe used could be recognised as the 503 bp fragment spanning from bp 738-1240 in the sequence published by Bunzow et al. (1988) [54]. The D₂-receptor cDNA obtained was selected to capture both the D₂-receptor mRNA isoforms, D2_L and D2_S. The PCR products were ligated into a pCR3.1 vector and transformed into competent cells using a cloning kit (Eukariotic TA cloning kit, Invitrogen, the Netherlands) according to the protocol

supplied with kit. The obtained DNA product was then verified by sequence analysis (T7Sequencing kit, Amersham Pharmacia Biotech, Sweden). Northern blot procedure has been described elsewhere. The hybridisation signals from the radiolabelled dopamine D₁- and dopamine D₂-receptors were normalized to that of GAPDH (glyceraldehyde 3-phosphate dehydrogenase) mRNA, detected with a *Pst*I fragment of a human GAPDH cDNA. Signals were semi-quantified by a digital image analysis system using NIH Image software (NIH Image 1.62, NIMH, Bethesda, MD).

***In situ* hybridisation (Paper VI)**

Synthetic oligonucleotide probes complementary to rat TH and rat AADC were synthesised by Scandinavian Gene Synthesis, Sweden. Oligonucleotide probes complementary to rat D₁- and D₂-receptors were purchased from Metabion GmbH, Germany. The TH probe sequence was 5'-TGCGTGGGCCAGGGT-GTGCAGCTCATCCTGGACCCCTCCAAGGAGCG-3', complementary to nucleotides 769-816 published by Grima et al. (1985) [134]. The AADC probe sequence was 5'-AGCATCAATGTGCAGCCATACACCC-TCCTGGTTGCA-GATGGGACCCAC-3', complementary to nucleotides 769-816 in the sequence published by Tanaka et al. (1989) [321]. The dopamine D₁-receptor probe was 5'-AATCGATGCAGAATGGCTGGGTCTCCTCAGAGCCACAGAAG-GGCACCA-3', complementary to nucleotides 880-928 reported by Zhou et al. (1990) [367]. The sequence of the dopamine D₂-receptor probe was 5'-GCAA-GATCTTCATGAAGGCCTTGC-GGAACTCGATGTTGAAGGTGGTGT-3' complementary to nucleotides 1193-1241 according to Bunzow et al. (1988) [54]. The tissue slides were incubated overnight at 42°C in a specific hybridisation solution, and then rinsed and dehydrated (Paper VII). Labelled sections and plastic standards (Autoradiographic [¹⁴C] Microscales (30-880 nCi/g, Amersham, Stockholm) were placed in X-ray cassettes and exposed to autoradiographic film (Hyperfilm-βmax, Amersham, Stockholm). After four weeks the films were developed manually (Kodak D19, Unifix). The autoradiograms were digitized and the optical density calibrated to pmol/mg wet weight based on the co-exposed standards using NIH Image software (NIH Image 1.62, NIMH, Bethesda, MD), and the hybridisation signal was quantified.

Statistics

The data from all experiments were statistically verified using the SAS Statview computer software 501. In parametric statistics, Student's T-test (unpaired or paired data) or Analysis of Variance (ANOVA) was applied for factorial estimates where appropriate. The ANOVA was followed by *post hoc* comparisons between independent groups by means of the Fischer's Protected Least Significant Difference (PLSD) test. In analyses, a probability value (*P*) equal to or less than 0.05 was considered statistically significant.

Chapter 5

FINDINGS

5.1 EPIDEMIOLOGICAL FINDINGS

5.1.1 Extent of Misuse

Various doping agents (Paper I)

Lifetime misuse of doping agents was 3.4% of the senior high school males in the first year and 1.8% of the male students in the third year in Uppsala, Sweden. Among senior high school females 0.5% in the first year and 0.2% in the third year had misused doping agents at least once. Taken together, a total of 2.7% of the adolescent males (37 subjects) and 0.4% of the females (5 subjects), reported lifetime misuse of doping agents. Among male doping agent misusers, 51.4% had taken Metahapotehohonol with its active substance metandienone. In Paper I, misuse of testosterone was analysed as listed in the questionnaire, i.e. separately from the misuse of AAS. Among senior high-school males, lifetime misuse of testosterone was 1.7% in the first year and 0.7% in the third year, which gives a total of 1.3%. Among the females, 0.3% reported lifetime misuse of testosterone. A total of 1.3% of the males and 0.2% of the females had misused peptide hormones, of which growth hormone was the most common. Of the senior high school males, 0.6% in the first year and 0.4% in the third year, a total of 0.5% had misused growth hormone.

Anabolic-androgenic steroids (Paper II, III)

Scientifically testosterone is included in the term AAS. In data analysis of Paper II and III, the term AAS also included testosterone. Lifetime misuse of AAS was among males 2.7% in the first year and 1.3% in the third year, which gives a total of 2.1% or 29 subjects. Among females a total of 0.2% (3 subjects) reported that they had misused AAS at least once. Of the adolescent males reporting lifetime misuse of AAS, 27.6% had tested these substances but had not taken a full cycle, another 27.6% had taken some cycles, 6.9% had misused several cycles over a period of a year or two, and 10.3% had misused several cycles for a period longer than two years, 27.6% of the misusers did not answer this question.

The question regarding the first initiation of the AAS misuse was answered as follows: Of the male respondents reporting lifetime misuse in the first year, 18.2% had initiated their misuse during the last year, 36.4% in the 8th or 9th grade of the comprehensive school, and 18.2% in the 7th grade or earlier. Of the male respondents reporting lifetime misuse in the third year, 57% rated that they had initiated their misuse during the third senior high school year, 14.3% had initiated their misuse during the last year.

Psychotropic substances

Lifetime misuse of psychotropic substances was 15.3% among adolescent males and 8.9% among adolescent females. The most frequent misused psychotropic substance was cannabis, which was estimated to be 14.8% among males and 8.3% among females. Of adolescent males 0.8% had misused amphetamine, 0.5% cocaine, 0.8% LSD, and 0.7% MDMA (ecstasy).

5.1.2 Attitudes, Availability and Knowledge

Doping agents (Paper I)

The proportion of subjects having had the opportunity to try doping agents highly exceeded the actual number of misusers. This was estimated to 15.9% of the adolescent males and 4.6% of the females. Concerning access, 30.2% of the boys in the first year and 25.1% of those in the third year thought that they would be able to gain access to doping agents. Among the girls the corresponding proportions were 13.9% in the first year and 10.4% in the third year. 14% of the boys and 4.8% of the girls definitely knew a person who could give or sell doping agents.

Achieved information about alcohol, doping agents and psychotropic substances

Respondents, who rated that they received too much information about doping agents and psychotropic substances in school or that teaching about these substances had been very good, were subjected to the highest misuse of doping agents and psychotropic substances, respectively. Further, the same respondents also rated the highest proportion of misusers. On the other hand, of the adolescent males who had misused anabolic-androgenic steroids 31.0% reported that they received far too little information about doping agents, 17.2% somewhat too little, 17.2% that the amount of information was sufficient, 10.3% that the amount of information was a bit too much, and 24.1% that they had received far too much teaching about doping.

5.1.3 Perceived Effects of the AAS Misuse

The male adolescents who had misused AAS at least once, rated the perceived effects as follows; 20.7% reported an intense and 34.5% a slight increase in muscle mass, 10.3% reported an intense and 24.2% a slight increase in self-esteem, 24.1% rated a slight increase in liquid retention. Furthermore, 13.8% responded with intense and 20.7% with some aggression, 17.2% experienced a slight euphoria, 3.5% reported difficult cardio-vascular complications, 14.0% some cardio-vascular complications, 3.5% had some bitch tits, and 3.5% had difficult and 17.3% had some problems with acne.

5.1.4 Descriptive Findings of Doping Agents and AAS

Motives for misuse (Paper I)

Data analysis was only performed on males, because the number of misusing females were too few to be adequately included. The main motive for misusing doping agents was to achieve a more attractive body/larger muscles, which was rated highest by 48.7% of the male doping agent misusers. Furthermore, 18.9% of the male adolescents with a lifetime doping agent misuse rated the motive to enhance sports performance, 13.5% because it was fun to try, 8.1% to become intoxicated, 5.4% to become braver and another 5.4% because of friends.

Descriptive bivariate analysis (Paper II, III)

The importance of sociodemographic factors, school variables, sports factors, personal aspects, use of other substances, and violence was investigated for each of the outcome variables, lifetime misuse of doping agents and AAS, respectively. Findings of the descriptive analysis are presented in Table 2 and Table 3. Misuse of doping agents and AAS were quite more common among senior high school males in the first year in comparison to the third. Senior high school males who had been living in cities with more than 300 000 inhabitants during their main part of their life were subjected to a higher proportion of doping agent misuse, which however, was not significant for AAS misuse. Adolescent males who were living alone also had misused doping agents to a higher extent than those who still lived with their parents. None of these background factors were significant for the misuse of AAS.

Adolescents, who had arrived in Sweden when they were 9 years or older, i.e. with a high immigrant status, were associated with higher estimates of both the doping agent and AAS misuse. Truancy several times a week was the only school factor that seemed to be significantly related to the doping agent and AAS intake. There was no significant association between sports and misuse of doping agents and AAS. Nevertheless, among those who were active in sports there was a clear association between high frequency of training and reported misuse. Further, strength training was significantly associated with both the doping agent and the AAS lifetime misuse. Good perception of peer relations and high self-esteem, were the two personality related aspects that were protective against doping agent and AAS misuse.

Use and misuse of other substances such as tobacco, heavy alcohol consumption several times a week, prescribed as well as un-prescribed tranquillisers and sedatives, as well as psychotropic substances were highly associated with doping agent and/or AAS misuse in bivariate analysis. The psychotropic substances with the highest proportion of AAS misusers were LSD, ecstasy, amphetamine, opiates, and cannabis. Adolescent males who had been exposed to violence more than once during the past term in Uppsala reported the highest estimates of misuse.

Multivariate models (Paper II, III)

Findings of the multiple logistic regression analysis relating different background factors to the misuse of doping agents are shown in Table 4. The variables strength training, use of tobacco, heavy alcohol consumption, truancy and living alone seemed to be independently associated with the life time misuse of doping agents. The variable heavy alcohol consumption appeared to be non-significant when the factor psychotropic substance misuse was included in the model.

The final multivariate model of AAS misuse showed that having a high immigrant status, average/low self esteem, average/bad school achievement, truancy, strength training, heavy alcohol consumption, and use of prescription tranquillisers and sedatives were independently significant contributors to the AAS misuse after controlling for age, presented in Table 5.

Table 2. The number of senior high school males and percentage of proportions reporting lifetime misuse of doping agents and AAS with regard to sociodemographic factors.

	Numbers	Proportion of doping agent misuse (%)	Proportion of AAS misuse (%)
Total	1353	2.7	2.1
Sociodemographic factors			
<i>School grade</i>			
First (16-17)	807	3.4	2.7
Third (18-19)	546	1.8	1.3
<i>P-value</i>		ns	ns
<i>Living during the main part of their life</i>			
Stockholm/ Göteborg / Malmö (>300 000)	37	8.1	2.7
Bigger city (>90 000)	647	3.1	2.3
City (>10 000)	97	3.1	3.1
Village (>2000)	309	2.9	2.6
Country (<2000)	251	0.8	0.8
Missing value	12	0.0	0.0
<i>P-value</i>		*	ns
<i>Living alone</i>			
Yes	43	9.3	7.0
No	1235	2.5	1.9
Missing value	75	2.7	2.7
<i>P-value</i>		*	0.07
<i>Immigrant status</i>			
High	66	7.6	6.1
Low	1202	2.4	1.8
Missing value	85	3.5	3.5
<i>P-value</i>		*	*

Significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns; not significant.

Table 2. The number of senior high school males and percentage of proportions reporting lifetime misuse of doping agents and AAS with regard to school factors, personal aspects and violence.

	Numbers	Proportion of doping agent misuse (%)	Proportion of AAS misuse (%)
School factors			
<i>Satisfaction with school</i>			
Rather low/Very low	75	4.0	4.0
In between	246	4.1	3.3
Very high/Rather high	1023	2.4	1.8
Missing value	9	0.0	0.0
<i>P</i> -value		ns	0.06
<i>Perceived school achievement</i>			
Bad/much below average/ below average/average	731	3.2	2.9
Better than average/ much better than average/excellent	606	2.3	1.3
Missing values	16	0.0	0.0
<i>P</i> -value		ns	ns
<i>Truancy</i>			
Several times in the week	40	15.0	12.5
Once a week	109	7.3	7.3
2-3 times per month	226	2.2	1.3
Once a month	215	3.3	3.3
Some time per term	500	1.2	1.0
No, never	252	2.0	0.4
Missing value	11	0.0	0.0
<i>P</i> -value		***	***
Personal factors			
<i>Perceived peer relations</i>			
Low/Average	497	3.8	3.2
High	856	2.1	1.5
Missing values	0	0.0	0.0
<i>P</i> -value		0.06	*
<i>Self-esteem</i>			
Low/Average	404	4.2	4.0
High	949	2.1	1.4
Missing values	0	0.0	0.0
<i>P</i> -value		*	**
Violence			
<i>Exposed to violence last term in Uppsala</i>			
No	1139	1.8	0.2
Yes, once	131	5.3	2.2
Yes, more than once	59	11.9	8.3
Missing value	24	8.3	0.0
<i>P</i> -value		***	***

Significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns; not significant.

Table 3. The number of senior high school males and percentage of proportions reporting lifetime misuse of doping agents and AAS, with regard to use and misuse of other substances.

	Numbers	Proportion of doping agent misuse (%)	Proportion of AAS misuse (%)
<i>Use of other substances</i>			
<i>Use of tobacco</i>			
Yes	525	4.8	4.2
No	823	1.5	0.9
Missing value	5	0.0	0.0
P-value		***	***
<i>Heavy alcohol consumption</i>			
A couple of times/week	44	18.2	15.9
Some time in the week	201	2.0	2.0
Some time in the month	527	2.3	1.7
Some times a year/More seldom/ Never happens/Do not drink alcohol	540	1.7	1.1
Missing value	41	9.8	7.3
P-value		**	***
<i>Use of prescription tranquillisers or sedatives</i>			
Yes	52	15.4	15.4
No	1,119	1.9	1.3
Missing values	182	4.4	3.3
P-value		***	***
<i>Use of tranquillisers or sedatives without doctor's prescription</i>			
Yes	74	6.8	6.8
No	1,081	2.1	1.6
Missing values	198	4.6	3.5
P-value		*	**
<i>Having had the opportunity to try psychotropic substances</i>			
Yes	612	4.6	4.3
No	729	1.0	0.3
Missing value	12	16.7	8.3
P-value		***	***
<i>Having had the inclination to try psychotropic substances</i>			
Yes	356	5.1	4.2
No	974	1.8	1.2
Missing value	23	8.7	8.7
P-value		**	***
<i>Lifetime use of psychotropic substances</i>			
Yes	207	8.7	7.7
No	1121	1.4	0.9
Missing value	25	12.0	12.0
P-value		***	**

Significance levels: ***p < 0.001, **p < 0.01, *p < 0.05, ns; not significant.

In comparison, a model presenting the importance of similar factors for the misuse of psychotropic substances was evaluated. In this model of which age was controlled for (OR = 1.18 and CI = 0.82-1.70), it turned out that perceived high peer relations (average/low peer relations OR = 0.63 and CI = 0.41-0.97), average/low self-esteem (OR=1.54 and CI=1.01-2.33), truancy (OR=2.64 and CI=1.67-4.15), use of prescription tranquillisers or sedatives (OR=3.00 and CI=1.51-5.98), tobacco use (OR=4.01 and CI=2.74-5.86) and heavy alcohol consumption (OR=5.76 and CI=2.71-12.23) were independently and significantly associated with the psychotropic substance misuse.

Table 4. Multiple logistic regression analyses for males in the first and third senior high school years, relating background factors to lifetime misuse of doping agents (Paper II)

	Odds ratio	95% CI		P-value
		Low	High	
<i>Age</i>				
Third senior high school year	0.44	0.18	1.07	0.0691
First senior high school year ☹				
<i>Strength training</i>				
Yes	9.47	3.80	23.63	0.0001
No ☹				
<i>Use of tobacco</i>				
Yes	2.74	1.20	6.30	0.0173
No ☹				
<i>Heavy alcohol consumption</i>				
A couple of times per week	4.32	1.44	13.02	0.0093
Once a week—Never ☹				
<i>Truancy</i>				
At least once a week	4.05	1.77	9.27	0.0009
2-3 times per month—Never ☹				
<i>Living alone</i>				
Yes	4.69	1.25	17.63	0.0223
No ☹				

☹ Reference group

Subgroup of doping agent and AAS misusers

All the doping agent misusers were subdivided into an estimated group and a reference group, with respect to the reported main motive for their misuse. The estimated subgroup consisted of those who rated it was fun to try, to become intoxicated, to become more brave, because friends do so as their main motives for misuse. The reference group included those who reported misuse both because they wanted to enhance sports performance and improve appearance. Bivariate

Table 5. Multiple logistic regression analyses for first- and third-year senior high school males relating socio-demography, personality, school, sports-, and use of other substances to lifetime misuse of AAS (Paper III).

	Odds ratio	95% CI		P-value
		Low	High	
<i>Age</i>				
Third senior high school year	0.58	0.18	1.89	0.3644
First senior high school year ☹				
<i>Immigrant status</i>				
High	9.11	2.35	35.31	0.0014
Low ☹				
<i>Self-esteem</i>				
Average/ Low	4.39	1.51	12.80	0.0067
High ☹				
<i>Perceived school achievement</i>				
Average-Bad	3.77	1.07	13.25	0.0383
Better than average-Excellent ☹				
<i>Truancy</i>				
At least once a week	3.54	1.21	10.38	0.0211
2-3 times per month-Never ☹				
<i>Strength training</i>				
Yes	16.34	4.33	61.72	0.0001
No ☹				
<i>Heavy alcohol consumption</i>				
Yes, a couple of times per week	9.13	2.19	38.09	0.0024
Once a week-Never ☹				
<i>Use of prescription tranquillisers or sedatives</i>				
Yes	18.41	5.17	65.51	0.0001
No ☹				

☹ Reference group

logistic regression analyses, even though not significant possibly due to few individuals, revealed that the estimated group of users in comparison with the reference group to a higher extent was implicated in use of tobacco (OR=2.8), heavy alcohol consumption (OR=2.1), and misuse of psychotropic substances (OR=2.0). Further, they lived to a greater extent alone (OR=3.6) and played truant (OR=4.4).

5.2 NEUROBIOLOGICAL EFFECTS OF CHRONIC NANDROLONE ADMINISTRATION ON MONOAMINGERGIC RECEPTORS

5.2.1 Body Weights

Two weeks of nandrolone treatment at a dose of 15 mg/kg/day caused a significant reduction in body weight gain in all studies (Paper IV, V, VI, VII). The body weights after chronic nandrolone treatment at 1, 5, 15, and 45 mg/kg/day are presented in Figure 4. Body weight gain was reduced at the three highest doses $F_{4,35}=13.36$; $P=0.0001$.

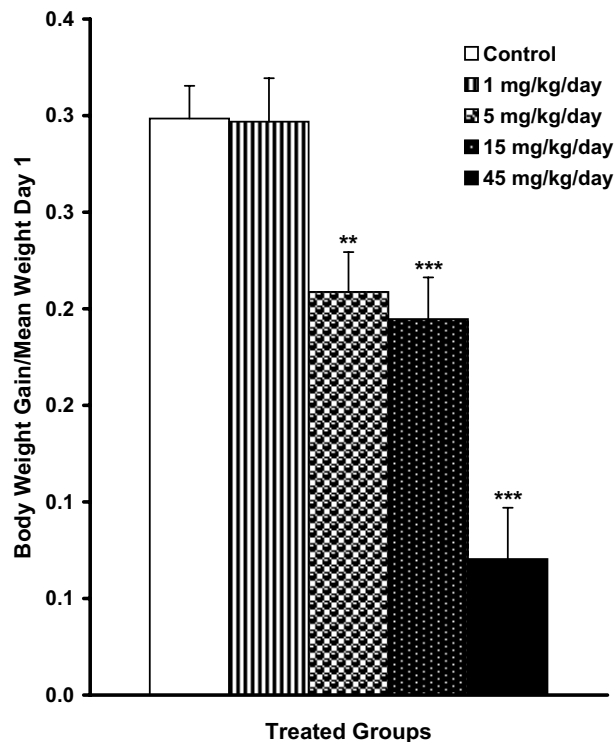


FIG. 4. Body-weight gain during the treatment period. Columns and error bars represent mean \pm SEM for division of paired weight differences by the mean weight of all rats included on day 1. Statistical analysis presented is based on absolute weight differences between day 1 and day 14. Significant levels for Fischer's PLSD are stated in the diagram: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

5.2.2 Dopamine

Regions of interest

Representative autoradiograms from control rats, illustrating total binding of [¹²⁵I]-(+)-SCH 23982 (D₁-like receptors) and [¹²⁵I]-NCQ-298 (D₂-like receptors) in the investigated regions, assessed by *in vitro* autoradiography, are presented in Figure 5 (Paper IV).

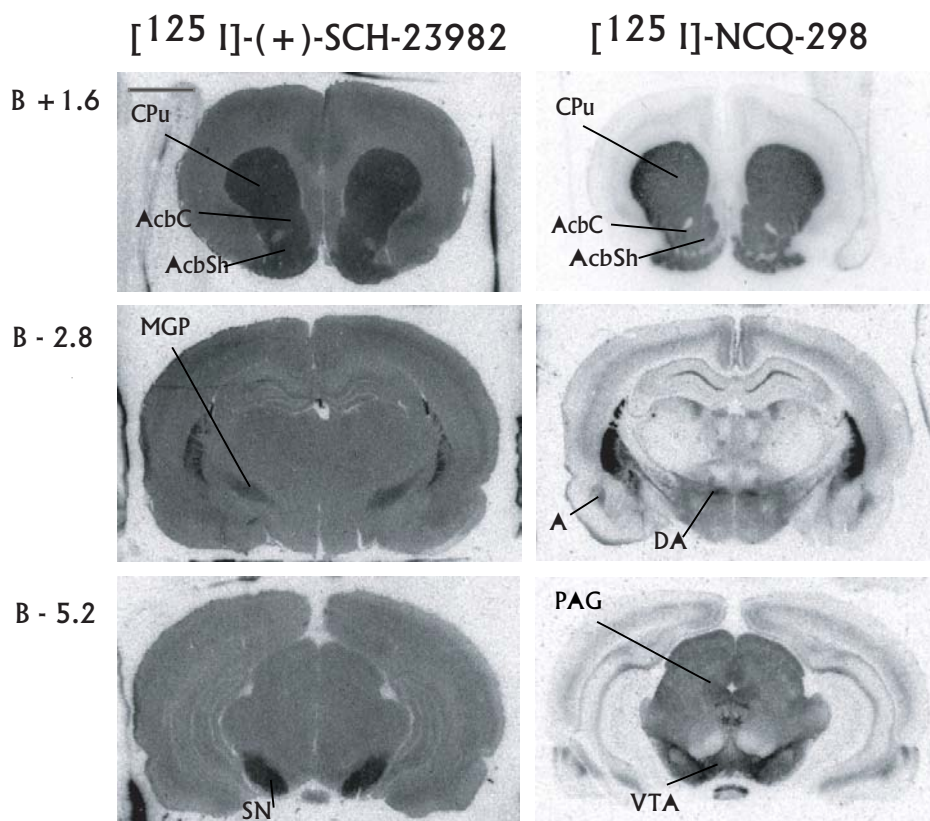


FIG. 5. Representative coronal autoradiograms showing total binding of the D₁-like receptor ligand [¹²⁵I]-(+)-SCH-23982 and the D₂-like receptor ligand [¹²⁵I]-NCQ-298 at bregma +1.6, -2.8, and -5.2 to -5.6. Scale bar 3mm. The brain regions studied were the CPU; Caudate putamen, AcbC; Nucleus accumbens core, AcbSh; Nucleus accumbens shell, MGP; Medial globus pallidus, DA; Dorsal hypothalamic area, PAG; Periaqueductal gray, SN; Substantia nigra and VTA; Ventral tegmental area.

Representative computer derived PET images are shown for the radioactivity uptake of [^{11}C]-(+)-SCH23390 and [^{11}C]-FE- β -CIT estimating the density of D_1 -like receptors and the dopamine transporter *in vivo* are shown in Figure 6 (Paper V). Digitised autoradiograms presenting the *in situ* hybridisation signal are illustrated in Paper VI. The mRNA content of the dopamine D_1 -receptor subtype was estimated in the CPu, AcbSH, and the AcbC and of the dopamine D_2 -receptor subtype in the CPu, AcbSH, AcbC, VTA, and the SN. The mRNA content of TH and AADC was investigated in the VTA and the SN.

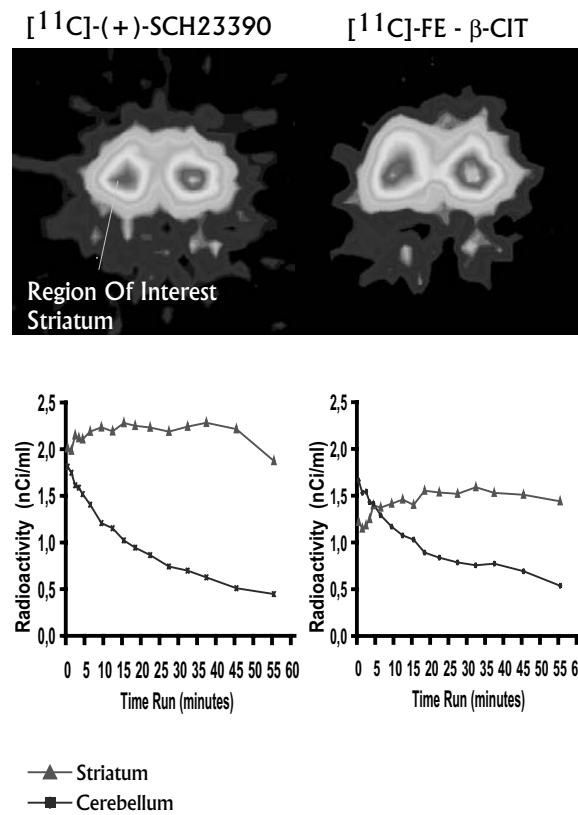


FIG. 6. Representative coronal computer derived PET images illustrating the striatum at bregma +1.6 in the male rat brain. [^{11}C]-raclopride labeling the dopamine D_2 -like receptors (left); [^{11}C]-(+)-SCH23390 labeling the dopamine D_1 -like receptors (middle); [^{11}C]-FE- β -CIT labeling the dopamine transporter (right). b) Time activity curves. Radioactivity uptake (nCi/ml) in the striatum of control rats. The diagrams for the different tracers follow the same arrangement as above.

Chronic treatment with nandrolone affects the dopaminergic system

The effects of chronic treatment with nandrolone decanoate, on the dopamine system are presented in Figure 7. Two weeks of nandrolone treatment at a dose of 15 mg/kg/day resulted in a significant up-regulation of the binding potential for the dopamine transporter by *in vivo* analysis using PET, while the effect on [¹¹C]-(+)-SCH23390 labelling dopamine D₁-like receptors did not reach significance (Paper V). The subjects included in the [¹¹C]-Raclopride analysis were not statistically verified because of the risk for type II error (n=5).

Figure 8 presents the findings from the *in vitro* autoradiography (Paper IV) and the *in situ* hybridisation (Paper VI). Using autoradiography chronic treatment with nandrolone decanoate down-regulated the total binding of [¹²⁵I]-(+)-SCH 23982 to the dopamine D₁-like receptors significantly in the CPU, AcbSH, and AcbC, whereas the [¹²⁵I]-NCQ-298 binding to the dopamine D₂-like receptors were up-regulated in the CPU, AcbC, and the VTA. In the AcbSH the [¹²⁵I]-NCQ-298 binding was down-regulated. The results of the *in situ* hybridisation indicated on a significant down-

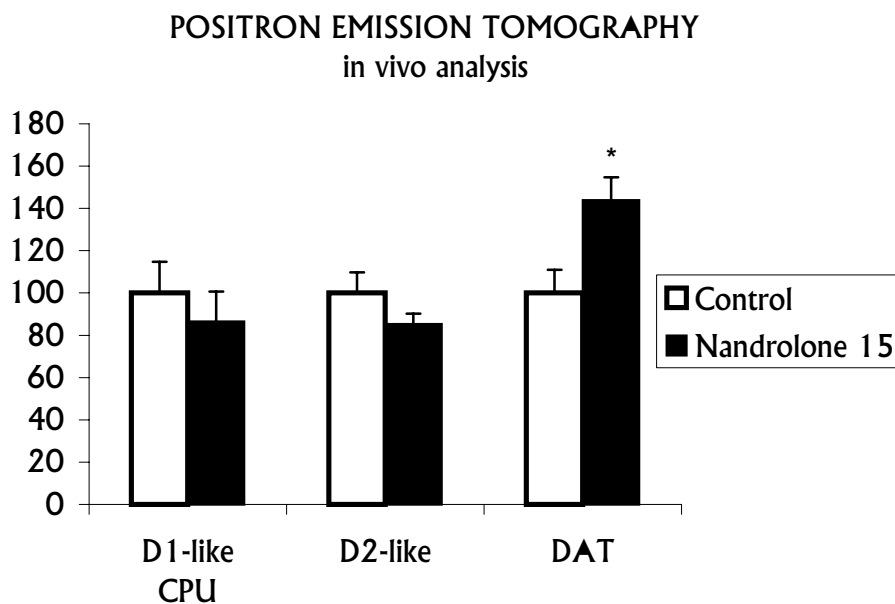
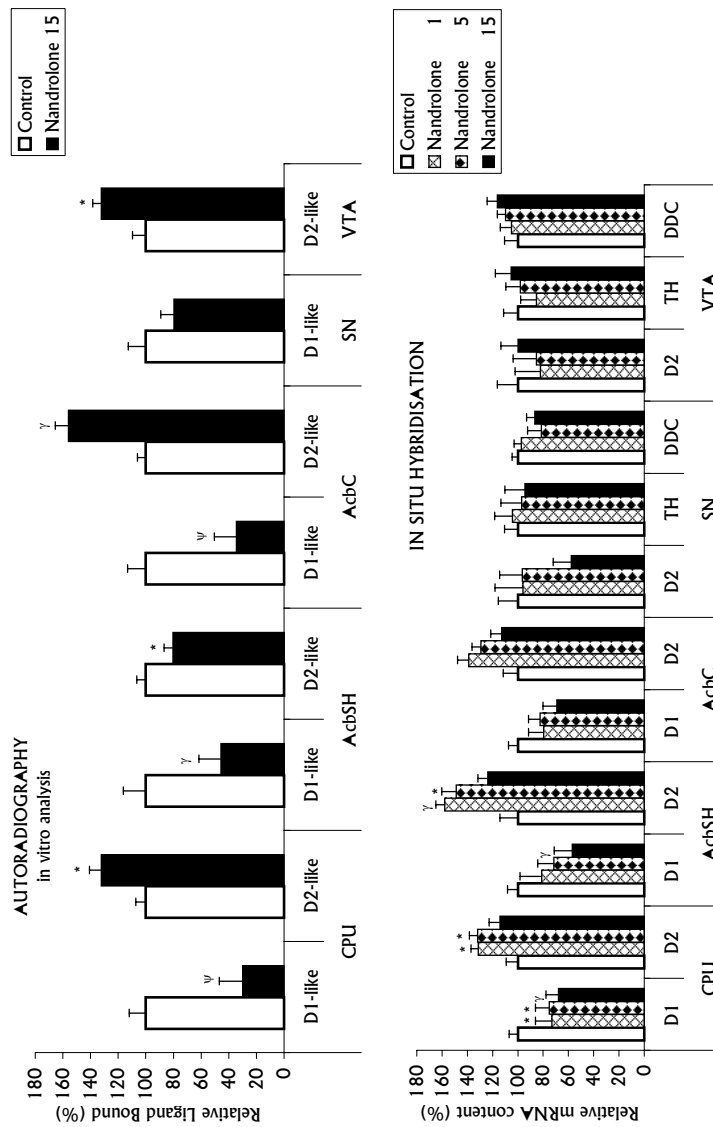


FIG. 7. Male Sprague-Dawley rats after two weeks of treatment with daily i.m. injections of nandrolone decanoate. Columns and error bars represent mean ± SEM. Positron Emission Tomography of [¹¹C]- raclopride, [¹¹C]-(+)-SCH23390 and [¹¹C]-FE-β-CIT in the striatum of rats after nandrolone treatment at a dose of 15 mg/kg/day. The data are normalised to mean of control levels (originally quantified in binding potential). Sample size: [¹¹C]- raclopride (controls; n=5, nandrolone; n=5; data were excluded from statistical verification), [¹¹C]-(+)-SCH23390 (controls; n=8, nandrolone; n=11), [¹¹C]-FE-β-CIT (controls; n=7, nandrolone; n=7). Significance levels, according to Student's T-test is denoted by *P < 0.05.

FIG. 8. Male Sprague-Dawley rats after two weeks of treatment with daily i.m. injections of nandrolone decanoate. Columns and error bars represent mean \pm SEM.

In vitro autoradiography. Total specific binding of dopamine D₁-like receptor ligand [¹²⁵I]-(+)-SCH-23982 and dopamine D₂-like receptor ligand [¹²⁵I]-NCQ-298 estimated in the CPU, AcbC, AcbSh and VTA of male Sprague-Dawley rats at a dose of 15 mg/kg/day. The data are normalized to mean of control levels (originally quantified in fmol/(mg wet weight)). Significance levels, according to Student's T-test are denoted by ***P < 0.001, **P < 0.01 and *P < 0.05.

In situ hybridisation. The regulation of the content of mRNAs for TH, AADC, the dopamine D₁ receptor- and the dopamine D₂ receptor subunit, after nandrolone treatment at three different doses. The data are normalized to mean of control levels. Significance levels, according to Fischer's PLSD are denoted by ***P < 0.001, ** P < 0.01 and *p < 0.05.



regulation of the mRNA content regulating the dopamine D₁-receptor subtype at all three doses in the CPu ($F_{3,23}=3.187, p<0.05$), and at the highest dose in the AcbSH ($F_{3,23}=3.439, p<0.05$). Even though the ANOVA ($F_{3,23}=2.568, p=0.079$) was not significant, Fischer's post hoc comparisons revealed that the D₁-receptor subtype mRNA was significantly down-regulated at the highest dose in the AcbC. The mRNA content of the D₂-receptor subtype was significantly up-regulated at the two lowest doses in the CPu ($F_{3,24}=3.025, p<0.05$) and the AcbSH ($F_{3,24}=3.764, p<0.05$). The alteration of the mRNA content for the D₂-receptor in the AcbC, met no significance in the ANOVA, but indicated on an up-regulation in the AcbC at the lowest dose in group comparisons of Fischer's (PLSD).

In northern blot, the mRNA content was only affected for the D₂-receptor subtype. A significant up-regulation was shown in the VTA and a down regulation in the SN (Paper VI).

5.2.3 Serotonin

Regions of interest

Computer derived coronal brain sections from male rats illustrating total binding of [¹²⁵I]-(\pm)-iodocyanopindolol and [¹²⁵I]-(\pm)-DOI, labelling the 5HT_{1B} receptor and the 5HT₂-receptors, respectively, in investigated brain regions are presented in Figure 9 (Paper VII).

Chronic treatment with nandrolone affects the serotonergic system

Chronic treatment with nandrolone decanoate induced significant alterations of the [¹²⁵I]-(\pm)-iodocyanopindolol and the [¹²⁵I]-(\pm)-DOI binding in several regions of the male rat brain, Figure 10 (Paper VII). All doses caused a significant down regulation of the 5HT_{1B} receptor density in the hippocampus CA1 and in the MGP and a significant up-regulation of the 5HT₂ receptor density in the AcbSH. Alterations in receptor density were also observed in the LGP, VMH, amygdala and in the intermediate layers of various cortical regions as presented in the figure.

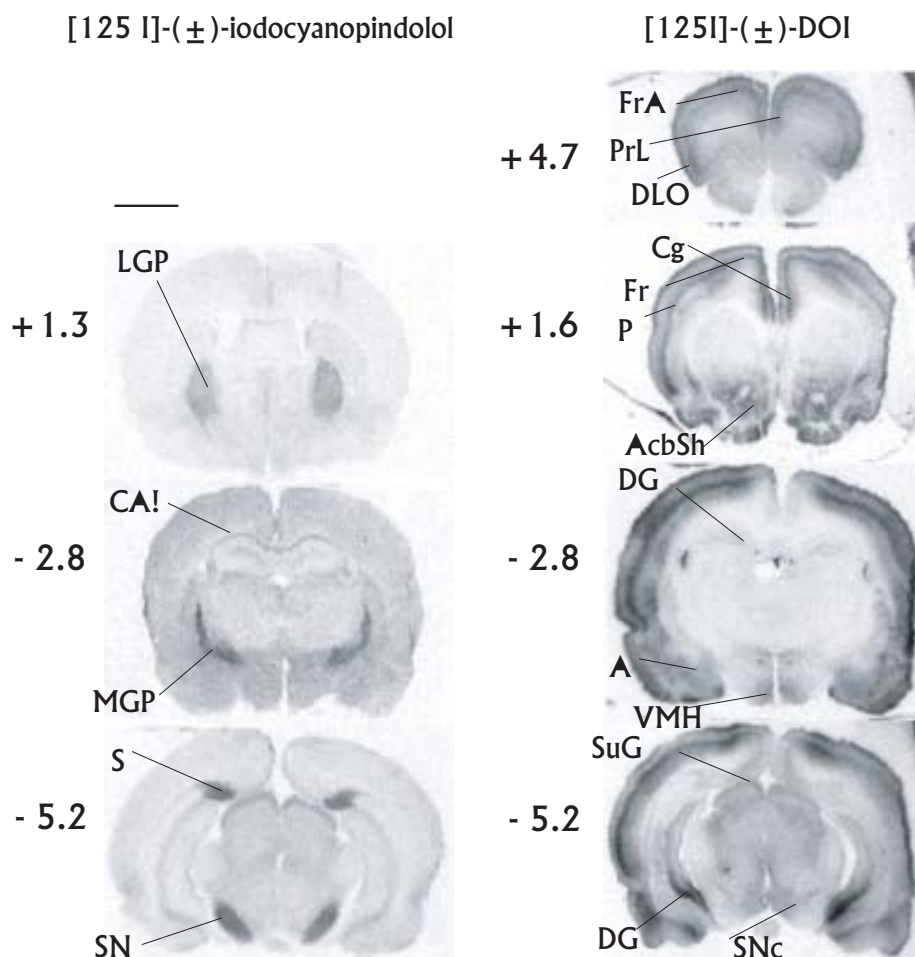


FIG. 9. Representative autoradiograms from control rats illustrating total binding of [¹²⁵I]-(-±)-iodocyanopindolol (12 μM) labelling the 5HT_{1B} receptors in the presence of isoprenaline (30 mM) and total binding of [¹²⁵I]-(-±)- DOI (200 pM) labelling the 5HT₂ receptors. Abbreviations at bregma + 4.7 mm: FrA; Frontal Association Cortex (intermediate layers), PrL; Prelimbic cortex, DLO; Dorso lateral orbital cortex, at +1.6: Cg; Cortex cingulate, Fr; Fronal, P; Parietal, AcbSh; Nucleus accumbens shell, Abbreviations at bregma -1.3 mm.; LGP; Lateral globus pallidus, at -2.8; MGP; Medial globus pallidus, CA1; Hippocampus CA1, VMH; Ventromedial hypothalamus, A; Amygdala, DG; Dentate gyrus, at -5.2; S; Hippocampus dorsal subiculum, SN; Substantia nigra, SNc; Substantia nigra compacta, at -5.8; SuG; Superficial graylayer of the superior colliculus. Scale bar 3 mm.

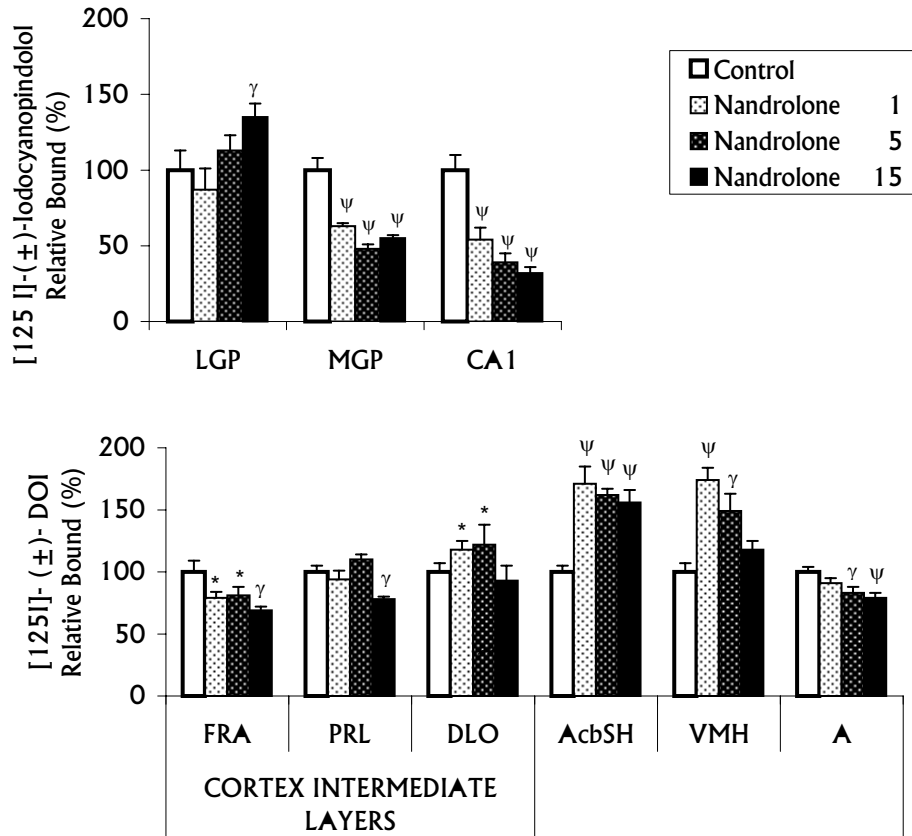


FIG. 10. Two weeks of treatment with daily i.m. injections of nandrolone decanoate at three different doses. Total specific binding of $[^{125}\text{I}](\pm)\text{-iodocyanopindolol}$ ($5\text{HT}_{1\text{B}}$ receptors) and $[^{125}\text{I}](\pm)\text{-DOI}$ (5HT_2 receptors). Significant levels for Fischer's PLSD: $\Psi P < 0.001$, $\Upsilon P < 0.01$, $*P < 0.05$.

Chapter 6

DISCUSSION

6.1 Epidemiology of AAS Misuse (Paper I, II, III)

This thesis provides evidence, through an epidemiological survey conducted in a big city in Sweden, that misuse of doping agents, specifically AAS, are not only attributed to elite athletes and body builders, but have also extended to include adolescents. Adolescents misuse these agents in order to improve appearance, enhance sports performance, become intoxicated, braver, because it is fun to try and because friends do so (Paper I). In agreement with recent national and international studies, misuse of AAS in Uppsala seems to be gender specific in favour of males [53,105,146,246,322,340,365].

Lifetime misuse of doping agents (2.7%) and AAS (2.1%) among adolescent males in Uppsala, are in agreement with other studies conducted among adolescents in Sweden and many other countries. In the US, AAS misuse is reported to vary between 4-12% (see section 2.1.6). Prevalence data derived from adolescent males could not be extended to estimate the AAS misuse at other ages. Since most studies conducted in Sweden have been restricted to target adolescents it is impossible to hold any accurate information on the lifetime and repeated AAS misuse at other ages, and thus also the total number of current misusers and addicts.

6.1.1 Models of Doping, AAS and Psychotropic Agents

In the model of doping, the variables strength training, use of tobacco, heavy alcohol consumption, truancy and living alone were independently associated with the lifetime misuse (Paper II).

The present thesis highlights a model including the factors having a high immigrant status, perceived average/bad school achievement, truancy, strength training, average/low self-esteem, heavy alcohol consumption and use of prescription tranquillisers/sedatives to be independently associated with the male AAS misuse (Paper III).

In this thesis, a model of psychotropic substance misuse was also evaluated in order to compare factors of importance for AAS with those of importance for psychotropic substances. The model of psychotropic substance misuse showed that perceived high peer relations, average/low self-esteem, truancy, use of prescription tranquillisers or sedatives, tobacco use, and heavy alcohol consumption were independently and significantly related to misuse (Paper III).

6.1.2 Methodological Considerations

Prevalence estimates

Most studies on the extent of AAS misuse have mainly been performed as cross-sectional questionnaires in school and conscription environments. The advantage of using self-reported questionnaires is the ability to sample a quite large population in a non-threatening, non-invasive and rather inexpensive manner. In the present Uppsala study, the importance of confidential handling was emphasised. Thus, the risk of false-negative responses given by adolescents who did not admit AAS misuse for fear that they would be discovered, is low. False-positive responses may arise when adolescents mistake medically approved substances for AAS misuse. Since the present questionnaire highlighted that unprescribed use should be rated and the most common AAS were listed in the questionnaire by group and name, the probability for misunderstandings was limited. In methodological study, the Swedish Council for Information on Alcohol and other Drugs (CAN) has in cooperation with six other countries suggested the validity of school questionnaires to be high [155]. The non-participation rate of the present survey was 19%, which seems to be consistent with the regular truancy level at senior high school. In another methodological study of school surveys, CAN has confirmed that the impact caused by the proportion of drop outs in terms of regular truancy for the final prevalence estimates of drug misuse is low [12].

Cross-sectional data and causality

The present survey is cross-sectional. The direction of the causation in terms of temporality between variables studied could therefore not be scientifically derived from data in this thesis, neither in-between independent variables, nor between independent and dependent variables. Thus, the observed associations are open to alternative interpretations. Some factors associated with the misuse of doping agents, AAS or psychotropic substances may serve as predictors and others as consequences of such misuse. The potential position and the role of the variables investigated will be discussed in the following sections with respect to the large-scale environmental risk-factor theory [348], the stress-coping model [347], and other national and international studies conducted in the field.

6.1.2 The Role of Sociodemography, Sports and other Substances

Sociodemography

Initiation of AAS use is frequently observed in pre-teens, but most commonly occurs in early adolescence. In Uppsala, 18.2% of the males with lifetime AAS misuse in the first senior high school year had tried AAS at the age of 13-14 or younger. In the US, a study by Buckley (1988) demonstrated that one third of AAS misusers has initiated misuse before the age of 15 and another third before 16 years of age. Another study by Yesalis (1993) showed the median age of initiation of AAS misuse to be 15 years, and a study conducted by Tanner (1995) reported that 54% of the AAS users had started to use these agents at 14 years or when they were younger

[53,322,365].

In the literature, prevalence estimates of AAS at different ages mainly comprise adolescents and young adults. Thus, the relationship between AAS misuse and age can only be evaluated among adolescents. Some studies indicate that older adolescents misuse AAS to a higher extent than do younger adolescents [107,182,272,301,363], but documented data in this field is quite contradictory. Other studies, do not present any significant differences between ages and AAS intake among adolescents [106,146,322].

Regarded as a predisposing factor [348], immigrant status, in terms of having been living in Sweden since the age of 9 years, significantly and independently contributed to the AAS intake and accordingly supports previous studies indicating that AAS misuse is overestimated in minorities [53,106,146,272,300]. In contrast, racial differences have not appeared to be of any importance in other studies [237,365]. In the U.S., caucasian students have been reported to have higher usage rates than non-caucasian students [182].

The factor of living in Stockholm, Göteborg and Malmö during the main part of their lives seemed to be a risk factor for the doping agent misuse, but was omitted from the final multivariate model of AAS misuse. In this perspective and in agreement with other studies, the effect of urbanisation is not of great importance for AAS misuse [53,340].

In Sweden, senior high school students usually share household with one or two of their parents. Living alone was independently associated with the doping agent misuse, but did not seem to be of importance for the AAS misuse when factors as immigrant status, self-esteem, perceived school achievement, and use of tranquillisers and sedatives were controlled for. Living alone could either be a risk factor or a consequence of doping agent misuse. Regarded as a predictor, it could reflect lack of parental support, independence, or high socio-economic status, factors that were not investigated. Parental support is protective according to the stress-coping model, because it helps to develop individual competence and ameliorate the effect of stressful circumstances [347]. Other studies have shown that students who only lived with one of their parents and students who were subjected to unsupervised recreation reported higher prevalence estimates of AAS misuse [53,340]. This is in agreement with the documented literature on other substances of abuse, reporting teenagers with dysfunctional parental support to be more likely to become abusers [16,84,108,152]. If the factor living alone is regarded as an outcome of AAS misuse this could be due to aspects like the refusal of the adolescent to stay at home because of drugs. The impact of socio-economic status is not investigated in this thesis. Its relevance in AAS patterns is however also contradictory. In one study low socio-economic status has been shown to influence the misuse of AAS [182], while another indicated a higher AAS prevalence estimate among students with a higher income [146].

School factors

Perceived average bad school-achievement and truancy at least once a week, both appeared to act as independent contributors of the final model of AAS misuse. Low grades in school has been associated with AAS misuse in two studies [106,336]. School achievement is a competence variable proven to be protective for substance misuse [347]. Truancy also was independently associated with psychotropic substance use in its final model of the present thesis. Taken together, several studies points out truancy to be strongly connected to drug intake [19,146]. Consistent with these findings drop outs have been proposed as risk groups for substance misuse [12]. The large-scale environmental onset theory describes school factors as predictors of substance use [348]. However, data in this thesis does not provide information that could be used to conclude whether perceived school achievement and truancy are causal variables or outcomes of investigated misuse.

Regarding intellectual levels as a protective competence variable, it is interesting to note that low MAO subjects with a high intellectual level have been found to express their sensation-seeking behaviour in socially acceptable ways, while low MAO subjects with low intellectual levels were shown to express their need for stimulation by means of alcohol and drugs [357].

Doping, sports, body image, and personality

The main motive for misusing doping agents, e.g. AAS, were to improve muscle mass/strength and the second most common reason was to enhance sports performance (Paper I), which were the major motives rated also in other studies of adolescents [53,106,182,322,340,344]. The impact of strength training was evident in the multiple logistic regression model of AAS intake. Notably, this variable was not of relevance for psychotropic substance misuse, indicating that this aspect of muscle mass and appearance actually is unique for patterns of AAS misuse. Because habits of AAS misuse have appeared to be used as a short cut not only by body builders [42,264], but also by adolescents, an increasing influx of a muscular body ideal through media and toys [202,267] may give rise to more frequent cases of muscle dysmorphia and “reversed anorexia” in Sweden [260,264].

Several studies have found a positive correlation between body satisfaction and self-esteem [17,44,228], and further a poor self-esteem to be related to drug abuse [174,347]. This supports the idea that a good self-esteem is important for positive health behaviour [328]. Since AAS misuse is motivated by the desires to increase muscle mass/strength and enhance sports performance, and is implicated in psychotropic substance misuse, it is interesting to note that the variable high self-esteem seemed to be protective in both the model of AAS and psychotropics, respectively.

Perceived good peer relations were only rated to be of importance for psychotropics, which is supported by the documented literature in this field [16,174,346]. Maybe a potential effect of peer relations among those AAS misusers implicated in psychotropic substance misuse is masked by the subjects with the drive for sports or bigger muscles.

Besides self-esteem and peer-relations there are a set of other items that reflect personality. Adolescents who proceed early and quickly to heavier levels of use of alcohol and illicit drugs tend to score lower on achievement, cognitive structure, and harm avoidance and higher on affiliation, autonomy, exhibition, impulsivity and play [193]. Further, teenage boys with mixed drug abuse have been shown to have significantly lower MAO activity and higher scores on sensation seeking than non-abusers [358]. Interestingly, MAO activity levels [324], as well as high-risk behaviours such as driving too fast, driving after drinking, not wearing a seatbelt, sharing intra venous needles, using injectable drugs, and suicide attempts [107,146,182,238] have been associated with intake of AAS.

Tobacco, alcohol, tranquillisers, psychotropic and other substances

The use of tobacco was included in the multivariate model of doping, but was not a significant variable in determining AAS misuse. Intake of alcohol and tranquillisers were independent contributors in both models.

The association between misuse of AAS and psychotropic substances was statistically significant in bivariate analysis among adolescent males in Uppsala. Interestingly, the variable of high frequency of heavy alcohol consumption was rejected from the multivariate models of both doping agents (Paper II) and AAS (Paper III), when the factor of psychotropic substance misuse was present. This reflects that the same subjects to some extent seem to be implicated in intake of heavy alcohol consumption, psychotropic substances and doping agents, e.g. AAS.

These multivariate models showed that the profile of AAS misuse had a lot of factors in common with the model of psychotropic substance misuse. Further, subgroup analysis with respect to their primary rated motives for misuse, revealed that adolescent males subdivided into the group for which the main motives were to become intoxicated, brave, because it was fun to try and because of peers, were overestimated in behaviours such as lonely living, truancy, use of tobacco, heavy alcohol consumption, and misuse of psychotropic substances, in comparison with the adolescent males rating the motives of improved appearance and sports performance.

Consistent with this thesis, other studies show significant associations between misuse of AAS and other substances, i.e. legal as well as illicit drugs [105,146,246,365]. Although the extent of AAS misuse is limited compared to psychotropic drugs, changes in habits of the AAS trend is quite alarming, since both the number of available AAS and psychotropic substances on the black market as well as the abuse of psychotropic substances have been shown to increase during the last 5 years [154,275].

Additional substances taken by AAS misusers and abusers include nutritional supplements, which are not regarded as harmful per se, but may act as a gateway for AAS misuse [268]. Furthermore, self-treatment with different pharmaceuticals such as diuretics, beta-blockers, and analgesic drugs in order to counteract AAS-induced side-effects is also a part of the AAS profile of misuse [234].

6.2 NEUROBIOLOGICAL PERSPECTIVES

Chronic administration with supra-therapeutic doses of the commonly misused AAS nandrolone decanoate is in the present thesis, by means of *in vivo* analysis using PET, *in vitro* autoradiography and gene transcript analysis using *in situ* hybridisation and Northern blot, shown to affect various correlates of the dopamine system in regions of the male rat brain. These regions have been implicated in mediating motor behaviour, reward, reward-related associative learning and/or incentives [27,92,310]. Chronic nandrolone treatment is also shown to affect serotonergic receptors by *in vitro* autoradiography in brain areas regulating cognitive functions, impulsivity, aggression and other emotional states.

AAS-induced alterations in the monoamine systems and causality

The mechanisms that underlie the nandrolone-induced alterations of the monoaminergic receptors are caused by direct or indirect interactions of nandrolone and/or its metabolites with the monoamine system. The changes in receptor density and/or the gene-transcript expression of the receptors could either be responses to alterations in monoamine activity and/or be caused by completely different mechanisms. Such mechanisms could be represented by direct steroidal interactions either with the gene sequence and transcripts in the cell nucleus or with membrane bound receptors that may not at all affect the dopamine or the serotonin activity.

The AAS nandrolone may exert its effects through androgenic steroid receptors, but considering the supra-therapeutic doses used, cross reactivity that engage other steroidal receptors such as the glucocorticoid, mineral corticoid, estrogens and progestin receptors may not be excluded. Some pharmacological actions of AAS may be caused by interactions with specific steroid sites of the membrane bound GABA-A receptor [39,70,122,168,225]. Changes in the monoamine system could be caused by influences from alterations in closely related neurobiological systems prior to the dopamine alterations. Consequently, observed AAS-induced alterations in the HPA-axis [293,294], FOS activity [167], opioid [15,164,166,213], tachykinin [143], glutamate [199,200], and melanocortin systems as well as the reduced body weight gain [205] may either cause the observed alteration in the monoamine systems or be results of such changes.

6.2.1 AAS and the Dopamine System (paper IV, V, VI)

Reflections about AAS-induced receptor alterations and the dopaminergic homeostasis

Earlier studies demonstrate that testosterone induces conditioned place preference that involves the regulation of the dopamine D₁- and D₂-receptors [296] and that intermittent treatment with AAS affects the dopamine activity in terms of increased catecholamine metabolism [323]. In the present thesis rats were subjected to supra-therapeutic doses of nandrolone reflecting conditions that might be similar to states related to continuous “binge” intoxications. Several studies suggest that

dopaminergic systems undergo significant changes following chronic administration of psychomotor stimulant drugs. Chronic continuous administration of high binge-like doses of addictive drugs may evoke characteristic withdrawal syndromes [186,191]. Interestingly, chronic ethanol administration has been reported to induce both short term and long term increases in dynorphin B levels in the Acb [207], indicating on enhanced counteraction of dopamine activity and potentiated aversive effects. Chronic “binge” administration of cocaine has been shown to reduce the dopamine transmission both in the Acb and the CPu [222,337,338]. In contrast, chronic intermittent administration of cocaine and amphetamine increase extracellular dopamine, linked to behavioural sensitisation and enhanced locomotor activity [171,172,256,257]. Regarding the dopamine activity following chronic treatment of nandrolone decanoate, neither a previous study estimating the dopamine content in tissues [209] nor the present findings estimating the mRNAs for TH and AADC induced any changes in the dopamine activity. However, a supposed unaffected dopamine activity is actually challenged by the interesting findings of the dopamine receptors in the present thesis.

In this thesis, two weeks of supra-therapeutic nandrolone decanoate administration induced significant opposite alterations on the dopamine receptor density for D₁-like and the D₂-like receptors in the CPu and the AcbC. The D₁-like receptor proteins were down-regulated, whereas D₂-like receptor proteins were up-regulated. However, in the AcbSh both the D₁-like and the D₂-like receptor density were reduced. The trend of opposite alterations of the dopamine receptors was observed for the expression of the gene transcript of the D₁- and the D₂-receptor subunits at the two lowest treated doses in the CPu and the AcbSh, indicating that alterations of the receptor proteins are presumably linked to the gene regulation. The mRNA content of the D₂-receptor subunit in the CPu and the AcbSh reflects postsynaptically located receptors, in contrast to the estimated D₂-receptor density of the *in vitro* autoradiography.

Interestingly, in a study by Gerfen and co-workers, lesions of the nigrostriatal pathway originating in the SN compacta and terminating in the CPu, down-regulated the mRNA content of the dopamine D₁-receptor subunit and up-regulated the mRNA content for the dopamine D₂-receptor in the CPu [126]. In the CPu the dopamine D₁-receptor subtype is postsynaptically localised on the striatonigral GABAergic neuron co-expressing preprotachykinin and prodynorphin [127]. The gene transcript of the dopamine D₂-receptor subtype is in the CPu postsynaptically localised in the striatopallidal projection co-expressing preproenkephalin [118,127]. Opposite changes of the receptor subunits on the respective GABAergic feedback projections, referred to opposite G-protein coupling, are suggested to induce synergistic effects upon the SN reticulata [126]. Notably, opposing functional properties of the dopamine D₁- and the D₂-receptor subtypes are also observed with regard to sexual behaviour and are further confirmed in other studies [22,160]. In this perspective, the findings of this thesis indicating down-regulations of the D₁-receptors and up-regulations of the D₂-receptors, with respect to both the gene transcript and the receptor protein, could be hypothesised to reflect synergistic

responses to a reduced dopamine transmission following chronic supra-therapeutic nandrolone administration. Thus, it is tempting to speculate whether continuous “binge” intoxications of AAS may lead to counteradaptive states in the late phase of abuse that reflect alleostatic conditions similar to withdrawal states [186,191,313], whereas signs of the dopamine-receptor involvement in testosterone-induced conditioned-place preference rather would arise at the earlier phase of steroid intake [296]. Thus, such a hypothesis might explain why a milder chronic intermittent dose regimen of AAS may enhance the dopamine activity [323] whereas a heavier does not [209].

Regarding receptor alterations, several studies in exception from one [334] indicate that continuous or chronic “binge” administration of cocaine as well as cocaine-induced withdrawal seems to decrease the D₁-receptor density *in vivo* [331] and *in vitro* [114,181,196], as well as to suppress the expression of the gene transcript of this receptor [196,320]. Interestingly, treatment with dopamine D₁-receptor agonists have successfully been shown to reduce the cocaine-induced craving [147] due to the D₁-receptor mediated euphoric effects from cocaine [279]. Taken together, even though cocaine and AAS might have different mechanisms of actions, these drugs of abuse seem to share similarities regarding changes of the D₁-receptor protein and its gene transcript following chronic “binge” intoxications. The findings on the D₂-receptors are contradictory possibly because this receptor subtype could be both pre- and postsynaptically located. Continuous “binge” administrations of cocaine have been reported to cause increased, decreased, as well as unaltered levels of the D₂-receptors [13,221,335,356]. Interestingly, chronic treatment with the neuroleptic raclopride enhanced the expression of the gene transcript for the D₂-receptor subtype [188]. Regarding DAT, the literature documents both unaffected mRNA levels [220] and an up-regulated density following cocaine-induced withdrawal have been reported [232]. Nevertheless, dopamine transport has been shown to increase after striatal dopamine D₂-receptor activation supporting the consistency of our research [233].

The hypothesis that the changes in dopamine receptor density after chronic supra-therapeutic nandrolone administration may reflect alleostasis is supported by findings indicating that identical dose regimens induce imbalances in the opioid and the tachykinin systems [143,164].

The involvement of steroids other than AAS and direct effects on gene transcripts

Nandrolone and its metabolites possibly also act on other steroidal sites besides on the androgen receptor. AAS-induced anabolic (presumably anti-catabolic) effects have been proposed to be mediated through glucocorticoid receptors [5,359]. In contrast to testosterone [37], nandrolone is aromatised to a much smaller extent [26,299,305], but might still exert some of its effects through estrogen receptors especially at supra-therapeutic doses. Notably, acute doses of progesterone and estradiol, alone or in combination enhance the levels of dopamine and its metabolism in the striatum [242]. Furthermore, 17 β -estradiol has been shown to increase mesolimbic and striatal dopamine D₂-receptors [273]. Testosterone, progesterone and 17 β -estradiol have in different studies been reported to affect the splicing ratio

of the long versus the short form of the dopamine D₂-receptor isoforms [138,192], indicating that this effect of testosterone involves the action of estrogens [137]. AAS and their metabolites could be hypothesised to modulate dopamine receptors either through direct actions on trans-membrane bound receptors, or *via* gene regulation through mechanisms that do not necessarily have to affect dopamine activity. Interestingly, estrogens have been shown to up-regulate the gene transcript of human D₁-receptors in a neuroblastoma cell line under physiological conditions [201]. However, the literature provides no studies on estrogens that equal the doses and conditions of continuous “binge” intoxications.

AAS Affects the Serotonin System (Paper VII)

Acts of violence in society have together with impulsivity, disinhibition and aggressive behaviour been linked to the intake of AAS [148,165,190,209,265,324,325]. The present thesis highlights that chronic treatment with nandrolone affects key regions of the aggression centre such as the amygdala and the hypothalamus [304], not only by alterations in recently suggested FOS activity [167], glutamate- and tachykinin systems [143,199], but also in the serotonin system. The amygdala is involved in the regulation of anxiety, among other emotional states. The hypothalamus is as a part of the stress responsive HPA-axis also a crucial component in feeding mechanisms in conjunction with NPY and substrates of melanocortin system [173,291,341]. In turn, feeding mechanisms of the melanocortin system is suggested to act in linkage with reward [206]. Interestingly, three days of sub-acute treatment with nandrolone has been suggested to induce the activity of the HPA-axis [292]. Chronic treatment with nandrolone has been shown to affect the melanocortin system implicated in the regulation of feeding [205]. Supported by findings that treatment with 5HT₂ receptor antagonists have been shown to relieve stress, anxiety and psychosis [116,281], and further to elevate food intake [197], the nandrolone induced alterations of the 5HT-receptor density in the present thesis may besides aggression also underlie observed AAS-induced behavioural changes such as anxiety in human and reduced body weight gain in rats. The down regulation of the 5HT₂ receptor density at the highest doses in the amygdala might reflect responses to counteract AAS-induced states of anxiety. The enhanced density of the 5HT₂-receptors in the hypothalamus at the two lowest doses tested might be hypothesised to be related to the reduced body weight gain of the rats that might be blunted at the highest dose because of secondary neuroadaptive changes.

In the light of the suggested hypothesis that acute and intermittent dose regimens of drugs of abuse may increase the activity of the dopamine system while a heavier regimen may reduce this [187,191], it is interesting to note that acute “binge” patterns of cocaine enhance the HPA-axis activity, whereas continued administration in the length reduces it [293,369]. Furthermore, in the hypothalamus acute administration of ethanol has been shown to enhance the ACTH and the corticosterone levels, while chronic “binge” patterns indicated no or diminished effects [368]. The same study reported reduced hypothalamic POMC mRNA content after acute ethanol administration, whereas this gene

transcript was not affected by chronic “binge” patterns [368]. Consistent with this ethanol study sub-acute administration of nandrolone decanoate increases HPA-axis activity in terms of enhanced hypothalamic ACTH and corticosterone levels [294]. Results regarding hypothalamic POMC mRNA are more complex. Sub-acute administration of nandrolone decanoate did not alter hypothalamic POMC mRNA levels either 1 or 24 hours following the last nandrolone injection. However, 24 hours following nandrolone POMC mRNA levels were significantly lower than 1 h following the last injection. Chronic nandrolone treatment has been shown to reduce POMC mRNA contents in the arcuate nucleus [205], as well as to induce unaltered effects upon the activity of the HPA-axis [293]. Notably, a decreased HPA-axis activity in terms of low salivary cortisol levels have been linked to aggressive behaviour [227].

The hippocampus is crucial for the regulation of memory and learning [258,274], but is also linked to regions of aggression and other emotional states [318]. Aggressive behaviour is counteracted by stimulation with 5HT_{1B} agonists and enhanced in knock-out mice lacking the 5HT_{1B}-receptor [46,64,288,306,370]. The diminished density of presynaptic 5HT_{1B}-receptors in the hippocampus presented in this thesis is in line with reported AAS caused reductions in 5HT levels in this region as well as AAS-induced dominance and aggressiveness [46,209,218]. In the same brain region, another study is indicative for an enhanced ratio of 5-HIAA/5HT [323], which actually also could refer to low 5HT levels [319].

The forebrain cortex is together with the nucleus accumbens implicated in reward-related learning, aggressive and cognitive behaviour [111,276,277]. In the present thesis, the density of the 5HT₂-receptors was significantly reduced at all doses in the frontal association cortex, while it was enhanced in the AcbSh. The implication of the serotonergic system in rewarding properties will preferably be viewed with respect to the closely connected dopaminergic system.

6.2.3 Dopaminergic and Serotonergic Interactions

Neuroanatomical and functional interactions between serotonin and dopamine have been observed with regard to the modulation of reward-related behaviour and motor behaviour [287]. Serotonergic pathways originating in the MRN and/or the DRN innervate the VTA and the SN [153,245] as well as the striatum and the Acb [18]. Infusion of 5-HT into the VTA [136] or the nucleus accumbens [144,254] has been shown to increase extracellular dopamine levels in the nucleus accumbens. Interestingly, chronic supra-therapeutic administration with nandrolone that corresponds to the highest dose-regimen in the present thesis, reports results in low 5-HT levels in the dorsal striatum and the mesolimbic forebrain, as well as reduced 5-HIAA in the striatum [209]. However, the 5-HT levels seem to be unaffected in the striatum and the frontal cortex, while the ratio 5-HIAA/5-HT was increased in these regions following chronic intermittent treatment with testosterone and oxymetholone, but was not affected by nandrolone [323]. The radioligand used in

the present thesis, [¹²⁵I]- (±)- DOI, is unselective for the different 5-HT₂-receptor subtypes. Thus, from this data it is difficult to ascertain whether the observed receptor alterations represent a change in all the 5HT₂-receptor sub-types or whether the alterations correspond to specific subgroups of 5HT₂-receptors. The 5HT_{2C}-receptor is known to be implicated in a tonic serotonergic inhibitory control of the mesocorticolimbic dopamine pathway, because disinhibition of this receptor by both 5HT_{2C} selective and 5HT_{2B/2C} antagonists is shown to enhance the dopamine release in the rat Acb, the PFC and the striatum [89,95-97,131]. The 5HT_{2A}-receptor, on the other hand is suggested to facilitate control, exerted by endogenous 5-HT, upon dopaminergic release in the nucleus accumbens [89]. Enhanced 5-HT₂-receptor densities in the nucleus accumbens shell could reflect postsynaptic alterations in the 5HT_{2A} subtype to adapt to the reduced 5-HT₂ levels in this region, in order to increase the dopamine activity. However, in the striatum the 5HT_{2A} subtype has not been proposed to possess a similar role [89]. Furthermore, the role of the different receptor subtypes seems to be specifically related to different drugs. For example, amphetamine-induced dopamine release is reduced only by 5HT_{2A}-receptor antagonists but is not affected by 5-HT_{2B/2C} blockers. Conversely, morphine-induced dopamine release is potentiated by 5HT_{2B/2C} antagonists but is unaffected by 5HT_{2A} antagonists [271]. The relevance of the different 5-HT receptor subtypes and their interactions with the dopamine system after AAS administration has to be further investigated. For example, stimulations of the 5HT_{1B} receptors induce mesolimbic neurotransmission through disinhibition of the GABAergic interneuron affecting the dopaminergic nuclei in the VTA [144,169,361]. Further, the 5HT₃-receptor, shown to increase dopamine transmission in the mesocorticolimbic pathway also seems to be important for the mechanisms connected to drugs of abuse [163].

6.2.4 The monoaminergic system and AAS and cross-sensitisation

In the previous section it has been hypothesised that chronic supra-therapeutic doses of AAS may lead to within system adaptations with regard to the dopamine system as has been proposed also for other drugs of abuse [185]. Interestingly, rats subjected to challenge of either alcohol [165,209] or amphetamine (unpublished data) on the last day of AAS injections following chronic administration with AAS have been reported to drink more alcohol and to be more aggressive. Furthermore, a bolus injection of cocaine following chronic AAS administration has been shown to potentiate the decrease of the NMDA NR1 mRNA subunit in both the Acb and the PAG [200] compared to the reduction observed when AAS are given alone [199]. In combination with cocaine administration, nandrolone is shown to increase seizure rate when the steroid is given in high intermittent doses (20mg twice weekly), a phenomenon that was not observed after low daily doses (2mg) of nandrolone [214]. Consequently, within system adaptations to a certain substance may give rise to cross-sensitisation. Several drugs of abuse induce cross-sensitisation, e.g. ethanol administration potentiates cocaine-induced dopamine levels in the Acb [208].

Chapter 7

CONCLUDING REMARKS

In the light of modern society's praises of a muscular male body ideal and victories in sports, it is not surprising that habits of AAS misuse has appeared to be a short cut for adolescent males taking these agents to improve muscle mass and strength and to enhance sports performance, not only abroad but also in a big city in Sweden, Uppsala. This trend is more alarming because in addition to those who misuse AAS for the psychological motives of improved appearance and athletic victories, there is now an emerging group of AAS abusers consisting of adolescents highly associated with intake of psychotropic substances and apparently rather take these agents in order to become intoxicated, braver, and because it is fun to try. The rated motives of misuse could according to the large-scale environmental risk factor theory be regarded as proximal factors that take place immediately prior to taking a drug. The epidemiological study conducted in this thesis has taken into account the importance of factors considering sociodemography, school, sports, personality, and intake of other substances and evaluated a multivariate model for the misuse of AAS. Finally, high immigrant status, perceived average/bad school achievement, truancy, average/low self-esteem, strength training, heavy alcohol consumption and use of prescription tranquillisers seemed to be independently associated with lifetime intake of AAS. This model indicates that having a high self-esteem and high-perceived school-performance (possibly reflecting the intellectual level) were protective competence variables against misuse. The factors included in this model could with respect to the cross-sectional application of the study either be regarded as distal independent factors predisposing the proximal and the dependent variable of AAS lifetime misuse or be consequences of such misuse. It is clear that social and psychological mechanisms contribute to the onset of AAS misuse and maintenance. However, not everybody exposed to a similar environment does initiate drug use, become abusers or addicts. The dimension *vulnerability* is crucial. Psychologists as well as neurobiologists posit the vulnerability to life stress as a general risk factor, predisposing various kinds of problems and coping as the function to handle stressors. In this context, life stress is posited as a risk factor for AAS onset and the misuse of AAS is ascribed as a coping function itself. I would like to regard the vulnerability to AAS onset and/or transition to addiction as a result of the contribution from genetic and external environmental factors estimated in terms of internal neurobiological and/or hormonal states at a certain time point.

Regarding the neurobiological mechanisms related to AAS, it still remains a mystery, whether the drive towards onset of misuse predominantly is caused by psychological motives of desire to enhance sports performance or muscle strength, both of which are related to the tissue building properties of AAS, or whether

the drive of onset is due to the actual novelty to experience acute rewarding psychopharmacological effects as is the case for psychotropic substances. Studies indicating that testosterone induce conditioned place preference [296] and that chronic intermittent treatment with AAS affects the dopamine activity in terms of increased metabolism [323] supports the hypothesis that some people initiate AAS intake because of rewarding properties *per se*.

Repeated drug intake does for addictive drugs give rise to a compulsive pattern of drug intake that characterises drug abuse and addiction. The vulnerability to abuse is for several addictive drugs related to the degree of sensitisation in conjunction with the loss of inhibitory control. The impulsive behaviour observed among addicts could either reflect a predisposing personality trait or be induced by the drug intake. Impulsivity and specific personality disorders, such as borderline personality, have been associated with low 5HT levels, but another subgroup has also been identified among offenders with elevated 5HT levels [101]. Subjects with schizoid and autistic disorders, as well as those with obsessive-compulsive disorders, display enhanced 5HT levels [158,226,303]. In this perspective, it is interesting to note that for subjects of AAS perpetrators in the “roid rage” group the act of violence was impulsive, whereas the “terminator subjects” all shared a compulsory strategy, suggesting enhanced 5HT levels [324]. Behavioural differences preceding the violent act may reflect variations in serotonin function in these subjects.

This thesis highlights that the AAS-induced changes in the serotonin receptor density in regions of the male rat brain regulating anxiety, cognition, and aggression both support the hypothesis that the serotonergic system is implicated in several behavioural changes observed among AAS misusers, and that AAS abuse and addiction could be related to a loss of inhibitory control. The AAS-induced neurochemical alterations of dopamine receptors following chronic continuous supra-therapeutic administration are hypothesised to reflect a condition of a disrupted homeostasis. The *initial* neurobiological homeostasis, which is reflected in the traditional counteracting balance of Yin & Yang, might be imbalance. In order to adapt with Yin & Yang aversive states of addiction possibly have adjusted the homeostatic set point in linkage with severe alleostasis. AAS, irrespective of their potential as acute rewards, are in this perspective suggested to be addictive drugs.

Chapter 8

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Chapter 9

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