Voluntary alcohol drinking: Relation to corticosteroids and alcohol-mediated testosterone elevation

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ACADEMIC DISSERTATION

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ABSTRACT

Alcohol dependence and alcoholism are modulated by environmental factors and genetic predisposition. Rat models have been invaluable in the investigation of several aspects of alcoholism in humans. The rodents exhibit a wide range of genetically determined alcohol-drinking preferences. Selective rat breeding programs with different alcohol preference has produced stable lines of rats that reliably exhibit high and low voluntary alcohol consumption (termed here AA and ANA, respectively).

Alcohol consumption is also strongly dependent on environmental conditions. Stressful events evoke an extensive multisystem and integrative physiological response, where a major component is the activation of the hypothalamic-pituitary-adrenal (HPA) axis.

Testosterone has been implicated as mediator of the rewarding effect of alcohol, and the testosterone level is predictive of future alcohol consumption. The objective of the present thesis is to examine in greater detail the relations between alcohol, testosterone and neuroendocrine stress responses, and alcohol drinking.

In the first study (I), the interrelations between endogenous and alcohol-mediated effects on testosterone and corticosterone levels and voluntary alcohol consumption were studied in a crossbred F2 generation of the original AA and ANA rat lines. The second study (II) was a reinvestigation of the effect of subchronic nandrolone decanoate treatment, which in an earlier study had been shown to increase alcohol consumption, and the relation of the effect on the HPA and hypothalamic-pituitary-gonadal (HPG) axes. The third study (III) examined the possible role of benzyl alcohol, present as a preservative in the nandrolone product used in the earlier study, as a confounding factor that could explain differences between the results of the earlier study and the present one. In the fourth study (IV) the interrelations between the effect of corticosterone on alcohol-mediated testosterone changes and alcohol consumption in AA, ANA, F2 and Wistar rats were examined.

The results of Study I shows connections between testosterone elevation and increased alcohol drinking, as well as between testosterone reduction and decreased alcohol drinking, which is in line with the original data of the AA and ANA lines. Elevated endogenous testosterone levels and higher frequencies of alcohol-induced testosterone increases were found in high consumption groups compared to low consumption groups. In Study II, subchronic nandrolone administration led to a reduction in alcohol-mediated testosterone levels, correlating with reduced voluntary alcohol consumption in the alcohol-preferring AA rat line. On the other hand, Study III showed that benzyl alcohol increases voluntary alcohol intake at least in the low-consumption rats, which may explain earlier discrepancies among studies. The results of Study IV were consistent with the hypothesis that corticosterone is involved in the regulation of alcohol-mediated testosterone changes.

In conclusion, our present results suggests that corticosterone is a balancer, which regulates both the alcohol-mediated testosterone increase followed by reinforcement and increased voluntary alcohol drinking in high-drinking rats, and the alcohol-mediated testosterone reduction followed by disinforcement and reduced alcohol intake in low-drinking rats.

Keywords: corticosteroids, testosterone, voluntary alcohol drinking, rat

TIIVISTELMÄ

Ympäristö ja geneettiset tekijät vaikuttavat alkoholiriippuvuuden ja alkoholismin kehittymiseen. Eläinmallit ovat tärkeitä välineitä ihmisen alkoholismiin liittyvien tekijöiden tutkimuksessa. Jyrsijöillä on perinnöllisiä ominaisuuksia, jotka yhdessä säätelevät mieltymystä alkoholin kulutukseen. Alkoholin kulutukseltaan toisistaan eroavia rottakantoja on jalostettu valikoimalla paljon ja vähän juovia yksilöitä erilleen sukupolvesta toiseen. Näin on saatu kehitettyä alkoholimieltymykseltään vakaat linjat fysiologista tutkimusta varten (tässä tutkimuksessa AA ja ANA rottakannat).

Perintötekijöiden ohella alkoholin kulutus on myös vahvasti riippuvainen ympäristötekijöistä. Stressaavat tapahtumat käynnistävät moniulotteisen fysiologisen vastejärjestelmän kehossa. Hypotalamus-aivolisäke-lisämunuaiskuoriakselin (HPA) aktivoituminen vastaa pääasiallisesti elimistön stressivasteen muodostumisesta.

Tutkimukset ovat osoittaneet yhteyden testosteronin ja alkoholin kulutuksen välillä sekä testosteronin että palkitsemiseen liittyvien hermostollisten vasteiden välillä. Testosteronitaso voi olla myös ihmisen tulevaa alkoholin käyttöä ennakoiva tekijä. Tämän tutkimuksen kohteena oli tarkemmin selvittää alkoholin, testosteronin ja elimistön fysiologisen stressivasteen välisiä suhteita.

Ensimmäisessä tutkimuksessa (I) selvitettiin sisäsyntyisen testosteroni- ja kortikosteronitason, alkoholiannoksen sekä vapaaehtoisen alkoholin kulutuksen aiheuttamien muutosten keskinäisiä yhteyksiä. Tutkimuksessa käytettiin alkoholin kulutukseltaan erilaisiksi jalostettujen rottakantojen F2-sukupolven yksilöitä. Toinen tutkimus (II) tehtiin aikaisempien löydösten perusteella, joissa oli havaittu nandrolon dekanoaatti-käsittelyn lisäävän alkoholin kulutusta. Tutkimuksen tavoitteena oli selvittää, miten tämä vaikutus on yhteydessä HPA- ja HPG-akseleihin. Kolmannessa kokeessa (III) selvitettiin, oliko alkoholin kulutusta lisännyt aikaisemmassa tutkimuksessa säilöntäaineena käytetty bentsyylialkoholi, mikä selittäisi ristiriitaiset tulokset nykyiseen tutkimukseen verrattuna. Neljännen tutkimuksen (IV) tavoitteena oli selvittää tarkemmin kortikosteronin ja alkoholin aiheuttaman testosteronitason muutosten välisiä yhteyksiä AA, ANA, F2 ja Wistar rottakannoilla.

Tutkimuksen I tulosten perusteella paljon ja vähän alkoholia kuluttavat rotat eroavat toisistaan testosteronin perustasojen suhteen kuten alkuperäiset AA ja ANA rottakannat. Paljon juovilla rotilla havaittiin korkeammat testosteronitasot ja enemmän alkoholin aiheuttamia testosteroninousuja kuin vähän alkoholia kuluttavilla rotilla. Tutkimuksen II tulosten perusteella havaittiin, että nandrolonikäsittely lievensi alkoholin aiheuttamaa testosteronitason nousujen määrää AA-rotilla ja vähensi alkoholin kulutusta kontrollieläimiin verrattuna. Tutkimus III osoitti, että bentsyylialkoholilla on alkoholin kulutusta lisäävä vaikutus ensisijaisesti vähän juovilla Wistar-rotilla. Tämä selittää ristiriitaa aikaisemman tutkimuksen ja nykyisen tutkimuksen II välillä. Bentsyylialkoholin vaikutusta stressivasteeseen ja testosteronitasojen nousuun ei kuitenkaan havaittu. Tutkimuksessa IV havaittiin kortikosteronin perustason olevan yhteydessä alkoholin aiheuttamaan testosteronitason muutokseen.

Tutkimuksen tulosten perusteella voidaan esittää, että kortikosteroni on yhteydessä sekä alkoholin aiheuttamaan testosteronitason kohoamiseen, mikä voi lisätä alkoholin palkitsevaa vaikutusta paljon kuluttavalla rottakannalla että testosteronitason alenemiseen, mikä voi vähentää alkoholin palkitsevaa vaikutusta vähän kuluttavalla rottakannalla.

Avainsanat: kortikosteroidit, testosteroni, vapaaehtoinen alkoholin juominen, rotta

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ABBREVIATIONS

AA/ANA	Alko, Alcohol/ Alko, Non-Alcohol rat lines (Finland)
AAS	anabolic androgenic steroid
ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
AUD	alcohol use disorders
BA	benzyl alcohol
BEP	beta-endorphin
CPP	conditioned place preference
CRH (CRF)	corticotropin-releasing hormone (factor)
CSF	cerebrospinal fluid
CV	coefficient of variation
DA	dopamine
DSM	Diagnostic and Statistical Manual of Mental disorders
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
GnRH	gonadotropin-releasing hormone
GR	glucocorticoid receptors
HAD/LAD	high-alcohol-drinking/ low-alcohol-drinking rat strains (US)
HPA	hypothalamic-pituitary-adrenal axis
HPG	hypothalamic-pituitary-gonadal axis
i.p.	intraperitoneally
i.v.	intravenous
ICD	International statistical Classification of Diseases and related health problems
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
MAD	median absolute deviation
NAc	nucleus accumbens
ND	nandrolon decanoate
P/NP	preferring/ non-preferring rat strains (US)
POMC	pro-opiomelanocortin
s.c.	subcutaneously
SEM	Standard Error of Mean
sP/sNP	Sardinian alcohol-preferring/ non-preferring rat strains (Italy)
SUD	substance use disorder
UChA/UChB	low-alcohol-drinking/ high-alcohol-drinking rat strains (Chile)
VTA	ventral tegmental area
WHO	World Health Organization
WHP/WLP	Warsaw high-preferring/ low-preferring rat strains (Poland)

LIST OF ORIGINAL PUBLICATIONS

I. Etelälahti, T.J., Saarikoski, S.T. and Eriksson, C.J.P. Associations of corticosterone and testosterone with alcohol drinking in F2 populations derived from AA and ANA rat lines. Alcohol. 2011 Aug; 45(5):421-6. doi: 10.1016/j.alcohol.2011.04.003.

II. Etelälahti, T.J. and Eriksson, C.J.P. On the association between nandrolone-mediated testosterone reduction during alcohol intoxication and attenuated voluntary alcohol intake in rats. Pharmacol Biochem Behav. 2013 Nov; 112:15-21. doi: 10.1016/j.pbb.2013.09.013.

III. Etelälahti, T. J. and Eriksson, C.J.P. Benzyl alcohol increases voluntary ethanol drinking in rats. Pharmacol Biochem Behav. 2014 May 25; 124C:81-85. doi: 10.1016/j.pbb.2014.05.011.

IV. Eriksson, C.J.P., Etelälahti, T. J. and Apter, S. Corticosteroid modulation of testosterone changes during alcohol intoxication affects voluntary alcohol drinking. Submitted 2016 Nov.

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1. INTRODUCTION

Alcohol dependence and alcoholism are basically modulated by environmental factors and genetic predisposition. The exact mechanisms, why alcohol consumption can become compulsive and addictive, are yet unresolved. Two main questions are: what are the factors, environmental and/or genetic, that are involved in the initiation and maintenance of voluntary alcohol consumption and what kinds of changes in the brain leads to the transition from controlled to compulsive alcohol use.

Animal models have been invaluable in the investigation of several aspects of the more complex human condition. Many pharmacological and behavioral effects produced by alcohol in humans are also present in other mammals (Bell et al., 2006; Grant and Bennett, 2003). The main criterion for the development of a valid animal model of alcoholism is the oral ingestion of a considerable amount of alcohol by the animal under free choice conditions (Bell et al., 2006; Lester and Freed, 1973). Arvola and Forsander (1961) tested various species for their voluntary consumption of alcohol: hedgehog, golden hamster, guinea pig, rabbit, albino rat and mouse. The study showed that golden hamsters drank large amounts of alcohol, and rabbits consumed water and alcohol in about equal proportions, but the other species showed a significant preference for water. Nonhuman primates have also been used as experimental animals to study alcohol self-administration. Oral self-administration has been reported for example in rhesus monkeys, chimpanzees and orangutans (Baker et al., 2014; Pieper et al., 1972; Vivian et al., 1999). There are two main categories of animal models for studying the motivational effects of alcohol: conditioning models, where alcohol exposure is controlled by the experimenter and self-administration models, where alcohol exposure is controlled by the animal (Cunningham et al., 2000).

The rodents exhibit a wide range of (partly) genetically determined alcohol-drinking preferences. Selective rat breeding programs for lines with different alcohol preferences have been carried out, e.g. in Chile (UCh-A/UCh-B) (Mardones and Segovia-Riquelme, 1983), Finland (AA/ANA) (Eriksson, 1968), The United States (P/NP, HAD/LAD) (Murphy et al., 2002), Italy (sP/sNP) (Colombo et al., 1995) and Poland (WHP/WLP) (Dyr and Kostowski, 2000), which have produced stable lines of rats that reliably exhibit high and low voluntary alcohol consumption. These studies demonstrate that genetic factors are important determinants of alcohol predilection and consumption.

On the other hand, alcohol consumption is also strongly dependent on environmental conditions. Stressful events evoke an extensive multisystem and highly integrative physiological response (Goldstein and Kopin, 2007; Kopin, 1995). The activation of the hypothalamic-pituitary-adrenocortical (HPA) axis constitutes a major component of the neuroendocrine stress response (Smith and Vale, 2006). The anxiolytic effects of alcohol are well established in human and animal models. This is the cornerstone of the *tension (stress)-reduction hypothesis* (Brady and Sonne, 1999; Pohorecky, 1991). However, in clinical studies the support has not been universal (Wand et al., 1998) and there are also clinical studies that indicate a dissociation between physiological and subjective effects of alcohol (de Wit et al., 2003; Söderpalm and de Wit, 2002).

2. REVIEW OF THE LITERATURE

2.1. General aspects of alcohol drinking, addiction, consequences and classifications

Alcoholic beverages are consumed around the world as an acceptable part of many activities. Low to moderate use of alcohol may facilitate socialization, as it reduces anxiety and has a disinhibiting effect on social behaviors. There is epidemiological evidence for a beneficial effect of low alcohol consumption without heavy drinking episodes. Drinkers with average intake of <30 g/day and no episodic heavy drinking had the lowest ischemic heart disease risk (relative risk = 0.64, confidence interval 0.53 to 0.71) compared with drinkers with heavy episodic alcohol use and lifetime abstainers (Roerecke and Rehm, 2012). In a recent study of da Luz et al. (2014), where lifestyle variables were concerned, it was observed that long-term red wine drinkers (at least one glass of red wine, 4-5 days/week) had similar coronary plaque burden but higher calcium scores, higher high-density lipoprotein, less of a previous history of diabetes, and lower plasma glucose than abstainers.

Excessive alcohol consumption is one of the most serious substance abuse disorders worldwide. The Global Status Report on Alcohol and Health 2014 by World Health Organization (WHO) highlights the actions needed for reducing harmful use of alcohol. An estimated 4.9% of the world's adult population suffer from alcohol use disorder (7.8 % of men and 1.5% of women). The report points out that a higher percentage of deaths among men than among women are from alcohol related causes, in the year 2012 7.6% of men's deaths and 4% of women's deaths. Men also have a greater rate (7.4 %) of total burden of disease expressed in disability-adjusted life years attributable to alcohol than women (2.3 %). Worldwide about 16 % of drinkers engage in heavy episodic drinking. Europe is the region with the highest consumption of alcohol per capita according to the report. Altogether, heavy alcohol drinking has led to several adverse health consequences, such as alcoholism, liver and cardiovascular diseases, brain damage and a number of cancers, metabolic disturbances, nutritional deficiencies and fetal abnormalities (Edwards et al., 1996).

Addiction treatment trials often use the Diagnostic and Statistical Manual of Mental disorders 5th edition (American Psychiatric Association, 2013) definition of alcohol use disorders (AUD), abuse or dependence, to define study participants. The DSM-V definition of alcohol dependence requires significantly harmful impact caused by at least two out of 11 criteria during the same 12-month period. The severity of an AUD is graded mild, moderate or severe. These dependence symptoms include e.g. tolerance, withdrawal, increased amounts of alcohol consumed over time, ineffective efforts to reduce use, interference with personal or professional life, significant amount of time spent obtaining, using and recovering from alcohol, or continued use of alcohol despite harmful consequences (American Psychiatric Association, 2013).

The Tenth Revision of International Statistical Classification of Diseases and related Health Problems (ICD-10) of WHO include 10 codes that correspond to DSM-5. WHO began to revise the ICD-10 in 2009 and the final ICD-11 is expected by 2018.

ICD-10 defines the dependence syndrome as being a cluster of physiological, behavioral and cognitive phenomena in which the use of a substance or a class of substances takes on a much higher priority for a given individual than other behaviors that once had greater value.

2.2. Alcohol addiction, reinforcement, motivation

Alcoholism is a form of drug addiction. Addiction can be defined by a compulsion to seek and take drug, loss of control in limiting intake and the emergence of a negative emotional state when access to the drug is prevented. Alcoholism impacts multiple motivational mechanisms and can be conceptualized as a disorder that includes a progression from impulsivity (positive reinforcement) to compulsivity (negative reinforcement).

2.2.1. Reinforcement and motivation

The constructs of reinforcement and motivation are crucial parts of all animal models of addiction. The primary pharmacological effect of a drug can be associated with either positive or negative reinforcement. Reinforcement is the process by which a stimulus increases the probability of a response. This can also apply to the definition of reward, and the two words are often used interchangeably. However, reward often connotes some additional emotional value, such as pleasure, whereas reinforcement may be either positive or negative.

Positive reinforcement is defined as the presentation of an event that increases the probability of the response. An example is drug seeking in a nondependent individual. According to Koob and Le Moal (2001) the *positive reinforcing effects* of drugs of abuse have their origins and terminal areas in the mesolimbic dopamine (DA) system. The major components of this drug reward circuit are the ventral tegmental area (VTA), the basal forebrain, the dopaminergic connection between the VTA and the basal forebrain, and opioid peptide neurons within these circuits. Other components are the many neural inputs and outputs that interact with the VTA and the basal forebrain utilizing gamma-aminobutyric acid (GABA), glutamate, and serotonin as neurotransmitters (Koob, 1992).

Negative reinforcement occurs when the removal of an aversive event increases the probability of a response. One example is a person who self-administers a drug to provide relief from the aversive aspects of drug abstinence or withdrawal (Koob, 2013a). The negative reinforcement has been hypothesized to derive from dysregulation of specific neurochemical elements involved in reward and stress within the basal forebrain structures involving the ventral striatum and extended amygdala. Specific neurochemical elements in these structures include decreases in reward neurotransmission, such as decreased DA and GABA function in the ventral striatum. Also it includes recruitment of brain stress systems, such as corticotrophin-releasing factor (CRF), in the extended amygdala (Koob, 2013a). Negative reinforcement is not punishment, although both involve an aversive stimulus. In punishment, the aversive stimulus suppresses behavior, including drug taking (e.g. disulfiram) (Koob, 2013a).

Disinforcement has been proposed by Harzem and Miles (1978), and this term could be used in place of punishment and would refer to reductions in response rate or probability as the result of a response-contingent stimulus. As with reinforcement, disinforcement would be defined in a strictly functional manner and would not depend on the intentions of those delivering the stimulus or whether the stimulus was considered pleasant or unpleasant.

The secondary pharmacological effects of a drug also can have motivating properties. For example, conditioned positive reinforcement involves the pairing of a previously neutral stimulus with the acute positive reinforcing effects of a drug. Conditioned negative reinforcement involves the pairing of a previously neutral stimulus with the aversive stimulus effects of withdrawal or abstinence (Koob, 2014). Sideroff and Bindra (1976) defined motivation as a "rough label for the relatively persisting states that make an animal initiate and maintain actions leading to particular outcomes or goals". And from a neurobehavioral point of view, motivation is "a set of neural processes that promote actions in a relation to a particular class of environmental objects" (Sideroff and Bindra, 1976).

2.2.2. Alcohol addiction

In alcohol addiction alcohol drinking develops to binges of alcohol consumption, which can be daily episodes or longer periods of heavy drinking. This is also characterized by a severe withdrawal syndrome. Craving or intense preoccupation of alcohol obtaining that is linked to a stimuli associated with obtaining of alcohol and also with withdrawal and the aversive motivational state (Koob, 2013b).

Drug addiction has generally been conceptualized as a disorder that involves elements of both impulsivity and compulsivity. Impulsivity has been defined as tendency to act without adequate forethought or the propensity to engage in risky behaviors and a predisposition toward rapid, unplanned reactions to stimuli with diminished regard to the negative consequences of these reactions (Crews and Boettiger, 2009; de Wit, 2009; Evenden, 1999). Research has shown the relevance of impulsive personality to substance use risk (Kaiser et al., 2016; Magid et al., 2007; Verdejo-Garcia et al., 2008) and also studies of individuals identified as at risk for substance dependence based on family history (Tarter et al., 2013).

Compulsivity can be defined as elements of behavior that result in perseveration of responding in the face of adverse consequences or perseveration of incorrect responses in choice situations (Wolffgramm and Heyne, 1995). It also has been defined as actions inappropriate to the situation that persists and have no obvious relationship to the goal (Leeman and Potenza, 2012) or in animals as increasing responding for drug under a progressive ratio, which may be considered a measure of compulsivity as animals continue to respond despite the aversive nature of not being rewarded (Hopf and Lesscher, 2014; Orio et al., 2009).

The cycles of impulsivity and compulsivity yields an addiction cycle comprising three stages: preoccupation/anticipation, binge/intoxication and withdrawal/negative affect. The three stages are interacting with each other and ultimately leading to the pathological state known as addiction (Koob and Le Moal, 1997).

2.3. The role of the hypothalamic-pituitary-adrenal axis

Stress is generally defined as any stimulus that challenges physiological homeostasis, in other words, alters the balance or equilibrium of the normal physiological state of the organism. The term "stress" is nonspecific and should always be qualified. Different forms of stress have different physiological consequences.

Physiological responses to stress are regulated by the interaction of environmental and genetic factors. The stress response consists of at least three components: 1) The glucocorticoid response mediated through the hypothalamic-pituitary-adrenal (HPA) axis, 2) activation of the peptide CRH outside the hypothalamus and 3) activation of the sympathetic nervous system that results in the release of epinephrine (adrenaline) and norepinephrine (noradrenaline).

The glucocorticoid response mediated by the HPA axis involves the hypothalamus, the pituitary gland and the adrenal gland (Fig. 1). When activated by a stressful situation, the hypothalamus releases corticotropin-releasing hormone (CRH), which is then transported to the anterior pituitary gland. CRH interacts with CRH-1 receptors and this stimulates the anterior pituitary to produce pro-opiomelanocortin (POMC), which is then processed further into biologically active peptides including β-endorphin (BEP) and adrenocorticotropic hormone (ACTH). The ACTH is carried via the blood to the adrenal glands, where it induces secretion of glucocorticoids, primarily cortisol in humans and corticosterone in rodents. The activity of the HPA axis is regulated by a sensitive negative-feedback loop. When there are increased levels of plasma corticosteroids, there is an increased occupation of glucocorticoid receptors (GR) and this triggers the negative feedback. Glucocorticoids act on the hypothalamus, hippocampus and the pituitary to decrease CRH and ACTH production, thereby switching off the stress response. Glukocorticoids also modulate extracellular concentrations of DA in the nucleus accumbens, and this effect is state-dependent- the effect is greater when the dopamine system is activated. During chronic stress, the repeated increase in glucocorticoids and DA may result in sensitization of the reward system (Marinelli and Piazza, 2002).

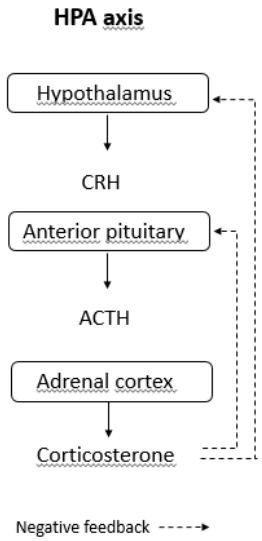

Figure 1. HPA axis

It is suggested that diminished opioid activity, which is either the result of alcoholism or genetically linked to the risk of alcoholism, could induce hypercortisolemia, alter mesolimbic DA production, and lead to abnormal alcohol reinforcement (Gianoulakis, 2001; Rachdaoui and Sarkar, 2013; Wand et al., 1999). Also activation of the mesolimbic dopamine system has long been known to be associated with the acute reinforcing effect of alcohol (McBride and Li, 1998).

2.3.1. Etiology and mechanisms of stress-related alcohol addiction: animal studies

Decades of research has demonstrated that altered HPA axis regulation is associated with problematic alcohol use and dependence and that this dysregulation varies with respect to the stages of progression toward alcohol dependence. Based on preclinical studies it has been suggested that assessing differences in the activity of the HPA axis may help determine whether certain individuals are at high risk for developing alcohol-related disorders and alcoholism (Fahlke and Hansen, 1999).

Animal studies have shown that environmental influences, such as exposure to stress, facilitate the onset of alcohol consumption and that alcohol drinking may be initiated to counteract some effects of stress (Pohorecky, 1981). Stressors can facilitate alcohol consumption by increasing the activity of several neurobiological systems, such as the HPA axis and extra-hypothalamic CRH signaling (Liu and Weiss, 2002). Mesolimbic dopaminergic neurons and GABAergic/serotonergic neurotransmission in the amygdala also integrate stress and alcohol interactions and increase drug self-administration behavior (Clarke et al., 2008; Heilig and Koob, 2007).

Evidence from rat studies has shown that alcohol administration affects the HPA axis activity. An acute exposure to alcohol activates the HPA, leading to a dose-related increase in circulating ACTH and glucocorticoid levels (Lee et al., 2004; Rivest and Rivier, 1994; Rivier et al., 1984). Female rats show a higher glucocorticoid response to acute alcohol than males (Rivier et al. 1984). These results demonstrate the existence of a sex-specific activation of the HPA axis in response to alcohol, implying that sex steroids exert an activational influence on ACTH and corticosterone release in response to ethanol (Rivier, 1993).

Glucocorticoids modulate the activities of the opioidergic, CRH and mesolimbic dopaminergic systems. They have also been shown to interact with the rewarding properties of alcohol abuse, as corticosterone administration increases voluntary alcohol intake in rats and inhibition of corticosterone synthesis reduces alcohol preference in high-preferring male rats (Fahlke et al., 1994; Fahlke and Eriksson, 2000; Fahlke and Hansen, 1999; Rachdaoui and Sarkar, 2013). Glucocorticoids have been shown to possess reinforcing effects: rats will self-administer corticosterone to achieve plasma concentrations of this hormone in the range (1 to 1.5 μM) that prevails during stressful experiences (Piazza et al., 1993). A positive correlation between baseline corticosterone and ethanol preference of Long-Evans rats has also been detected in another experiment concerning isolated rats (Butler et al., 2014).

Opioid peptides in the ventral striatum have been hypothesized to mediate the acute reinforcing effects of alcohol self-administration, largely based on observed effects of opioid antagonists. A relationship between the endogenous opioid system, the HPA axis and reward has been proposed based on preclinical and also on human studies (Oswald and Wand, 2004).

Stress-induced increases in serum glucocorticoid concentrations also inhibit testosterone-biosynthetic enzyme activity, leading to decreased rates of testosterone secretion (Hardy et al., 2005). The effect of stress on reproductive functions are modulated by CRH, pro-opiomelanocortin (POMC)-derived peptides (such as ACTH and BEP) and adrenal corticosteroids. These hormones can influence at all three levels of the HPG-axis: the brain inhibits the gonadotropin-releasing hormone (GnRH) secretion, the pituitary interferes with the GnRH-induced luteinizing (LH) release and the gonads alters the stimulatory effects of gonadotropins on sex steroid secretion (Rivier and Rivest, 1991). Based on studies with alcohol-preferring rat lines, it has been reported that stressful conditions may promote the alcohol-mediated testosterone elevation and consequently the reinforcing effects of alcohol (Apter and Eriksson, 2003; Apter and Eriksson, 2006).

2.3.2. Etiology and mechanisms of stress-related alcohol addiction: human studies

Genetic differences exist in sensitivity and responsiveness to the interaction between stress and alcohol drinking (Clarke et al., 2008; Uhart and Wand, 2009). Many alcohol dependent individuals have a history of affective disorders and altered HPA axis function and there is a possibility that HPA axis dysfunction may contribute to the development of alcohol dependence (Akil et al., 1993; Rose et al., 2010). The ability of alcohol to activate the HPA axis is also dose-dependent (Pohorecky, 1991). Clinical studies have shown a higher incidence of major life events, like divorce or job loss (psychosocial etiology), associated with alcohol dependence (Gorman and Brown, 1992) and greater relapse rates in abstinent alcoholics after severe psychological stress (Brown et al., 1995). Changes in the HPA system are known to occur during and after chronic alcohol intake. Plasma glucocorticoids are raised during alcohol consumption (Adinoff et al., 1998).

According to the study of Zimmermann et al. (2004) HPA axis hyperresponsiveness has been identified in people with a family history of alcoholism. The high-risk group had a significantly higher endocrine response compared to the low-risk group. The study also reports that a moderate alcohol dose decreased cortisol secretion significantly compared to placebo in the high-risk group, but not in the low-risk group (Zimmerman et al 2004) Recently, Mick et al. (2013) reported that a moderate alcohol dose (0.6 g/kg) attenuated the stress response in young adults with a negative family history of alcoholism. Opposite results have been reported by Dai et al. (2002), who found higher baseline and stress-induced ACTH secretion in the low-risk group compared to the high-risk group. These differences might be explained by differences in the quality of stress, pre-selection of participants, the strength of paternal alcoholism, and most importantly, group differences in the baseline levels of ACTH secretion. Differences in ACTH and cortisol responses to social stress in healthy individuals with positive vs. negative family histories for alcoholism support the idea that pre-existing HPA axis reactivity may represent a biological risk factor (Zimmermann et al., 2004). On the other hand, HPA axis dysfunction is also often an important consequence of alcoholism.

Human studies suggest that motivation for drinking can involve both positive and negative factors, both of which can activate the HPA. On one hand, the positive and reinforcing effects of initial alcohol consumption can act as a primer and facilitate further drinking. On the other hand, the anxiolytic effects of alcohol can motivate some individuals to drink to alleviate the negative effects (Wilkie and Stewart, 2005). Epidemiological studies have reported that a variety of stressors are associated with increased alcohol consumption and binge drinking (Perreira and Sloan, 2001; Richman et al., 1996; Thomas et al., 2011).

Several studies have reported a stimulatory effect of alcohol on the HPA axis. In an early study it was shown (Jenkins and Connolly, 1968) that plasma cortisol levels significantly increased in healthy individuals at intravenous (i.v.) alcohol doses of 1 ml/kg (bwt). Thayer et al. (2006) found that healthy men (who self-reported alcohol consumption) had higher levels of excreted cortisol in urine, and that during heavy drinking the inhibitory control of the HPA axis was impaired. Several studies have shown that basal ACTH and corticosterone levels are attenuated in response to chronic exposure to alcohol (Richardson et al., 2008). Disruptions in the CRH system develop over time and are worsened by prolonged exposure to excessive alcohol drinking.

2.4. The role of hypothalamic-pituitary-gonadal axis in alcohol addiction

The HPG axis is constituted by the hypothalamus and pituitary gland in the brain, and the gonads. The hypothalamus and the pituitary gland have regulatory functions, which are mediated by the hormones they produce and secrete. The gonads produce hormones, including estradiol and testosterone, which control sexual characteristics and behaviors.

The hypothalamus produces gonadotropin releasing hormone (GnRH), which is released into blood vessels that connect the hypothalamus and the pituitary gland (Fig. 2). In response to the GnRH signal, the pituitary gland produces two gonadotropin protein hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and these are released into the general circulation and act primarily at the level of the gonads. In men, LH stimulates testosterone production from Leydig cells. Testosterone circulates in the blood and back to the hypothalamus-pituitary unit and regulates the further production and secretion of GnRH and LH (Emanuele and Emanuele, 2001).

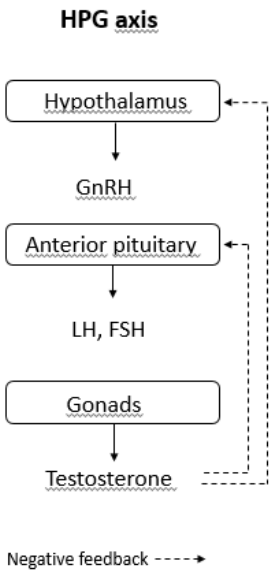

Figure 2. HPG axis

BEP has also a regulatory influence on the complex feedback system of the testosterone homeostasis at all levels of the HPG axis. The opioid BEP is made in the hypothalamus, in the pituitary and in the testes. Hypothalamic BEP restrains the secretion of hypothalamic LHRH and is thus inhibitory to the HPG axis. Hypothalamic BEP increases with both acute and chronic alcohol exposure and increased BEP has been found to result in suppression of hypothalamic LHRH, pituitary LH and testosterone synthesis (Gianoulakis, 1990).

There is evidence for a specific role for BEP during stress with respect to the regulation of LH and the HPG axis, of ACTH and the HPA axis and of prolactin release. Stress has been shown to suppress the release of LH from the pituitary with subsequent suppression of ovarian and testicular function. CRH which is released from the hypothalamus during stress has been shown to cause a similar suppression of the HPG axis. There is evidence that the suppressive effects of stress and CRH on the HPG axis are mediated by BEP. Thus, both CRH and BEP are important in suppressing reproductive function during times of stress.

2.4.1. Role of endogenous testosterone levels in alcohol drinking and addiction

Testosterone levels have been shown to be associated with a variety of behavioral traits in humans. High sensation seeking, impulsiveness, low harm avoidance and also aggressive and antisocial behavior have been associated with high concentrations of testosterone (Kerschbaum et al., 2006). Also, an association has been found between testosterone levels and an antisocial personality, alcoholism and drug use referring to DSM III-criteria (Dabbs and Morris, 1990). It was found that those with the top 10 % testosterone levels were higher on antisocial activities than the remaining 90 %. The relationship between scores on the Eysenck Personality Questionnaire and levels of sex steroids in detoxified male alcoholics and nonalcoholic controls have been studied by King et al. (1995). In addition, elevated testosterone levels have been found in sober random (Hasselblatt et al., 2003; Walter et al., 2007) and antisocial, delinquent or violent (Bergman and Brismar, 1994; Virkkunen et al., 1994) alcoholics compared with controls.

Virkkunen and Linnoila (1993) have associated high cerebrospinal fluid (CSF) testosterone levels with increased aggressiveness of Cloninger type 2 alcoholics. Mean CSF testosterone concentrations were higher among offenders than among healthy volunteers, and the difference was most striking for offenders with antisocial personality disorder. High CSF testosterone has also been associated with aggressiveness or interpersonal violence in a study of alcoholic offenders compared with healthy volunteers (Virkkunen et al., 1994). In addition, Higley et al. (1996) found that CSF testosterone concentrations were positively correlated with competitive aggressiveness.

Neuropsychological literature shows also that individuals with many other forms of substance dependence exhibit cognitive attributes that might be summarized as risk-taking/decision-making deficits, such as increased impulsivity and risk-taking behavior (Verdejo-Garcia et al., 2008). These deficits are also associated with antisocial or psychopathic traits (Mazas et al., 2000; van Honk et al., 2002).

Prior research has demonstrated a link between testosterone and alcohol consumption, and between testosterone and neural responses to rewards. The results of Braams et al. (2016) suggest that neural responses to rewards are correlated with current alcohol consumption, and that testosterone level is predictive of future alcohol consumption.

La Grange et al. (1995) studied the frequency of alcohol use among college students. Among males, high Sensation Seeking Scale V scores, high testosterone levels, and low monoamine oxidase (MAO) activity contributed to the variance in alcohol use. According to the study, there was a positive correlation between alcohol consumption and testosterone for both males and females, however the relationship was stronger among males.

The results of Tarter et al. (2013) show that the adolescence-related activation of the HPG axis can predict the risk of substance use disorder (SUD, which include alcohol drinking) at age 22. This study demonstrates that high transmissible liability index in childhood (age 10-12) predicts cortisol level stability and elevated testosterone concentration in adolescence (age 16), factors which in turn predicts SUD in young adulthood (age 22).

In animal experiments higher basal testosterone levels of alcohol-preferring AA rats compared to low-drinking ANA rats have been reported (Apter and Eriksson, 2003). The researchers postulated that since AA rats have been genetically selected for high drinking preference, this trait might be associated to elevated basal testosterone levels, directly or indirectly. It was also suggested that an additional testosterone surge induced by alcohol could further promote alcohol drinking.

Lakoza and Barkov (1980) reported that testosterone treatment increased voluntary alcohol consumption in castrated male rats. Also the same researchers found that the ethanol-preference was directly dependent on the androgenic properties of the hormone treatment. Ethanol-preference in castrated animal receiving female hormones (progesterone and estradiol) developed dependency slower and less intensively than in rats receiving testosterone. Also, they tested the effect of testosterone compared to its analogues nerobol and 19-nor-D-homotestosterone. The number of rats preferring ethanol to water was highest in the testosterone group and lowest in the group receiving 19-nor-D-homotestosterone, which has the lowest androgenic index. Antiandrogen was found to produce sharp depression in the experimental alcoholism. These studies suggests that high testosterone levels and adrongenic properties of hormones are connected to development of alcoholism.

Conditioned taste aversion studies showed that removal of testes prior to puberty of male rodents induced and maintained an aversion to ethanol (Morales and Spear, 2013). These results support the idea that testicular hormones may play an active role in regulating alcohol intake and preference of adult male rats.

2.4.2. Anabolic androgenic steroid abuse and alcohol addistion

Anabolic androgenic steroids (AAS) are a family of lipophilic hormones derived from cholesterol that includes the natural male hormone testosterone, together with numerous synthetic testosterone derivatives. AAS have both anabolic (protein-synthesizing) and androgenic (masculinizing) actions in the body. These steroids are abused primarily in the context of intense exercise and for the purposes of increasing muscle mass as opposed to drug-induced euphoria. AAS also modulate the HPA axis and may increase the reinforcing value of exercise through changes in stress hormone and endorphin release (Hildebrandt et al., 2014).

There is evidence that AAS are abused, but the potential for positive reinforcing effects and addiction is not fully understood. A two-stage model of AAS dependence has been proposed (Brower, 2002), according to which anabolic effects of AAS on muscle growth account for the initial stage of steroid use. The second step is that users develop dependence on the psychoactive effects after chronic exposure. It has been hypothesized that individuals who progress to AAS dependence are more biologically vulnerable to the dysphoric effect of AAS withdrawal (Kashkin and Kleber, 1989). Verdejo-Garcia et al. (2008) have suggested that there is apparent overlap between AAS dependence and other forms of substance dependence and conduct disorder.

In 1991 testosterone and related AAS were declared controlled substances. However, the relative abuse and dependence liability of AAS have not been fully characterized. Using conditioned place preference and self-administration, animal studies have demonstrated that AAS are reinforcing in a context where athletic performance is irrelevant (Wood, 2008). Also, AAS share brain sites of action and neurotransmitter systems in common with other drugs of abuse (Wood, 2004).

Evidence for a direct role of AAS in promoting alcohol drinking has been obtained in experimental animal studies showing that chronic nandrolone decanoate (ND, an anabolic steroid) administration could increase voluntary alcohol consumption (Johansson et al., 2000).

Human studies have demonstrated that the use of AAS is associated with the abuse of alcohol and other drugs (DuRant et al., 1993; Kindlundh et al., 1999; Lukas, 1996; McCabe et al., 2007). It is not yet clearwhether the use of AAS constitutes a direct risk for excessive alcohol drinking or whether personality and/or other factors independently promote the forms of abuse.

Findings based on retrospective self-reporting (Buckman et al., 2013) suggest that the use of AAS is associated with a higher tendency for alcohol and drug abuse. A larger proportion of performance-enhancing substances users (using e.g. AAS and peptide hormones) reported alcohol use in the past 30 days (83 %) compared with non-users (65.3 %). Also, the users reported significantly greater quantities of alcohol per drinking occasion and they were more likely to report binge drinking as their typical behavior.

The relationship between habitual use of AAS, alcohol and other drugs versus experimental AAS and alcohol use remains less clear. This is because a majority of the published studies to date do not speak about the relationship between habitual AAS use and the use of other drugs, despite evidence suggesting that these two user groups differs from each other (Kanayama et al., 2006; Kanayama et al., 2009).

2.4.3. Reinforcing properties of testosterone and other anabolic androgenic steroids

General reinforcing properties of testosterone and other androgens have been described in several experimental animal studies (Alexander et al., 1994; Arnedo et al., 2000; de Beun et al., 1992; Frye, 2007; Wood, 2004). It has been suggested that testosterone is a prohormone of its more rewarding metabolite 5α-androstanediol (Frye et al., 2001). In addition to the well-established, long-term endocrine modulation of behavior, changes in steroid levels may have more rapid behavioral consequences (Alexander et al., 1994; Harding, 1981; King et al., 1999). Further support for the rewarding effects of testosterone has been obtained in experiments on gonadally intact male hamsters, which self-administer testosterone by either oral, i.v. or intracerebroventicular self-administration (Johnson and Wood, 2001; Wood, 2004).

Conditioned place preference (CPP) studies indicate that the rewarding property of testosterone depends on circulating levels of testosterone markedly above physiological levels. Experiments with doses of 1 and 2 mg/kg have found to induce conditioned place preference and supports also the conclusion that testosterone has rewarding properties in male mice (Arnedo et al., 2002). Also, the effects of testosterone were more pronounced when high doses were used periodically, rather than when the same total amount of testosterone was equally divided among doses (Taylor et al., 1989). Studies examining the neural bases of reward suggest that the rewarding properties of testosterone may be mediated through an interaction with the mesolimbic dopamine system with both D1 and D2 receptors involved (Wood, 2004).

Also opioidergic mechanisms have been implicated (Peters and Wood, 2005). Clark et al. (1996) have suggested that anabolic androgenic steroids (AAS) may influence the sensitivity of the brain reward system. The AAS affect the endogenous opioid peptide system by increasing the BEP immunoreactive fibers in two terminal fields, the bed nucleus of the stria terminalis and the paraventricular hypothalamic nucleus (Menard et al., 1995). Recent findings suggest that AAS treatment produces prolonged changes in rat brain reward circuits associated with drug dependence (Kailanto et al., 2011).

Rodent research has focused on alterations in the opioid system to explain AAS reinforcement (Wood, 2008). AAS administered at high doses in rodents stimulate BEP release in the VTA (Johansson et al., 2000; Johansson et al., 1997), alters the density of kappa, delta and mu opioid receptors in the nucleus accumbens (NAc) and hypothalamic nuclei, and increase the metabolism of opioid peptides (Magnusson et al., 2009). These data support the possibility that AAS exert some mild reinforcing effects via increases in opioid peptides centrally in the brain and possibly through analgesic effects in peripheral tissues.

AASs may elicit a high degree of drug reinforcement through the same neuroendocrine environment that makes exercise reinforcing (Hildebrandt et al., 2011). There are documented overlaps in endocrine effects of AAS administration and intense exercise – among healthy men, acute increases in testosterone are reliably found in response to exercise (Bosco et al., 2000; Gotshalk et al., 1997; Häkkinen and Pakarinen, 1993; Kraemer, 1988; Kraemer et al., 1991; Kraemer et al., 1990; Schwab et al., 1993).

Hildebrandt et al. (2014) explain the strength of AAS reinforcement by an endocrine environment where on-cycle AAS users have elevated BEP and ACTH levels, but lower cortisol levels relative to heavy exercising controls. This profile may be an amplification of the adaptive increase in androgens observed in response to competition, where basal testosterone and specific mood states interact in regulating activation of the HPA axis after social contest (Zilioli and Watson, 2013). It also has been theorized that AAS may enhance the reinforcing value of exercise via BEP release or by capping the HPA response to exercise (Hildebrandt et al., 2014). High doses of AASs also lead to a significant increase in exercise among rodents (Wood, 2002). The same relationship is suggested by Hildebrandt and Greif (2013) as opioids may increase the reinforcing value of exercise and contribute to AAS dependence.

2.4.4. Effect of alcohol on endogenous testosterone levels

In human studies, variable alcohol-mediated effects on testosterone levels have been observed. Moderate to high doses of alcohol (0.8-1.7 g/kg) have lowered testosterone levels in men (Bertello et al., 1983; Rowe et al., 1974; Ylikahri et al., 1974), whereas low doses (0.5 g/kg) have been associated with testosterone elevations (Sarkola and Eriksson, 2003). In women, testosterone elevations have been found independently of the alcohol dose under the use of oral contraceptives and during the ovulation phase of the normal cycle (Eriksson et al., 1994; Sarkola et al., 2000; Sarkola et al., 2001). Thus the effect of alcohol on circulating testosterone in humans is dependent on dose, gender and also situation.

Animal studies show high doses of alcohol (1.5-3.0 g/kg) have generally been reported to lower testosterone levels in male rats (Apter and Eriksson, 2003; Cicero and Badger, 1977; Eriksson et al., 1983; Orpana et al., 1990; Rivier, 1999), whereas no changes and/or even testosterone elevations have been observed with lower alcohol doses (0.3-0.9 g/kg) (Apter and Eriksson, 2003; Cicero and Badger, 1977; Eriksson et al., 1983). However, more recent studies have found testosterone elevations also with high doses of alcohol (1.5-2.0 g/kg) (Alomary et al., 2003; Apter and Eriksson, 2003). In addition, the results by Alomary et al. (2003) demonstrated that alcohol-mediated peripheral testosterone elevations may be directly reflected in the brain.

2.4.5. Alcohol-mediated testosterone elevation and the testosterone hypothesis

Testosterone elevation following alcohol administration has been found to occur more frequently in alcohol-preferring AA rats than in alcohol-avoiding ANA rats (Apter and Eriksson, 2003). Earlier data have displayed lower basal hypothalamic levels of BEP, a feedback inhibitor of testosterone synthesis, in AA rats compared to ANA rats (de Waele et al., 1994; de Waele et al., 1995). Also an increased density of opiate receptors has been observed in the AA line compared to ANA line (de Waele et al., 1994). Given that the AA and ANA lines have been selected for their opposite alcohol preferences, it has been hypothesized that the differences in opiate regulation, and in basal and alcohol-mediated testosterone may be connected to the HPG regulation of voluntary alcohol drinking (Apter and Eriksson 2003).

The data from the study by Apter and Eriksson (2006) also indicate that alcohol-induced reduction of testosterone could be connected to avoidance of alcohol. The reduction of testosterone was detected in the ANA rats, the line genetically selected for non-alcohol preference.

Activation of the HPA axis, stress and depression have commonly been associated with suppressed testosterone production in humans (Hansen et al., 2009; Hardy et al., 2005; Knol, 1991). Based on studies with alcohol-preferring rats, it has been proposed that underlying stress constitutes the condition which promotes the alcohol mediated testosterone elevation and consequently the reinforcing effects of alcohol (Apter and Eriksson 2006).

The testosterone hypothesis

Apter and Eriksson (2003, 2006) have proposed a theory that alcohol-induced testosterone elevations could, through an increasing effect on the feedback regulation of testosterone biosynthesis, elevate the concentrations of hypothalamic BEP and thus promote alcohol drinking. In addition, basal BEP and testosterone levels may both be interconnected with the regulation of alcohol consumption. The higher basal testosterone levels could be related to lower BEP, and this could lead to endogenous hypersensitivity of the opiate receptors through an increased receptor density, which may lead to increased reinforcement by alcohol. Apter and Eriksson 2006 extended the hypothesis by suggesting that stress, at least in AA rats, could be a further promoting factor for the testosterone-mediated reinforcement of alcohol drinking (Fig. 3). This could explain earlier findings of positive associations between isolation-rearing and alcohol mediated reinforcement (Hall et al., 1998a) and increased alcohol consumption (Hall et al., 1998b) in rats.

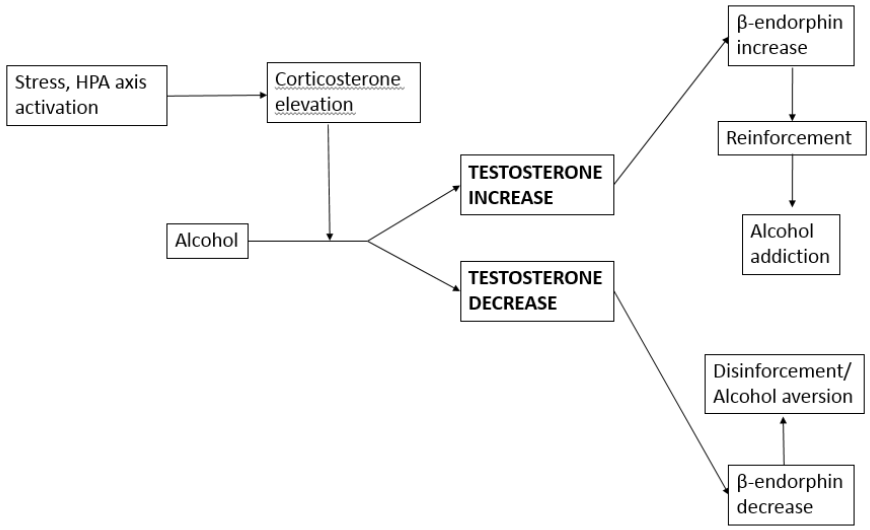

Figure 3. Hypothesis of the role of HPA-axis in the HPG regulation of alcohol drinking

3. AIMS OF THE THESIS

I. To examine the interrelations between endogenous and alcohol-mediated effects on testosterone and corticosterone levels and voluntary alcohol consumption in a crossbred F2 generation of the original AA and ANA lines.

II. To re-examine the previous finding that subchronic Nandrolone Decanoate treatment increases voluntary alcohol drinking in rats (Johansson et al., 2000) and to test how this effect may be related to the HPA and HPG axes.

III. To test the possibility that Benzyl Alcohol treatment may increase voluntary alcohol consumption, which could explain the discrepancy with Johansson et al. (2000), and to test whether this effect could be related to the HPA and HPG axes.

IV. To study the direct role of corticosterone levels in the alcohol-mediated testosterone changes in male AA, ANA and Wistar rats with relevance to the effect on alcohol drinking.

4. MATERIAL AND METHODS

4.1. Animals

In Study I, the male and female alcohol-preferring AA (Alko, Alcohol) and non-preferring ANA (Alko, Non-Alcohol) rats (Alcohol Research Centre, National Public Health Institute, Helsinki, Finland) of generation F89 were crossbred to form a new F1 population, from which 26 breeding pairs were generated to form the final F2 population (n = 283), 157 males and 126 females). In all mating procedures, the use of first-degree relatives was avoided. From the final F2 population, 80 male rats were chosen for the present study and these rats were about 3 months old at the beginning of the experiment.

In Study II, males of two rat line, alcohol-preferring AA rats and low-drinking Wistar rats (Harlan, Horst, The Netherlands) were chosen for the present experiments (n = 40 for each strain). At the beginning of the experiments rats were about 2.5–3.5 months old.

In Study III males of the alcohol-preferring AA and non-preferring Wistar (n = 2 X 20) rats, about 3 months of age, were chosen for the experiments.

Study IV comprises the same rats as in Studies I, II and III, and in addition, in another earlier study of male AA and ANA populations (n = 24 and n = 22 for the AA and ANA, respectively) of generation F80 (Apter and Eriksson, 2006). These rats were about two months old at the beginning of the experiments. Cutoffs were used for the correlation between alcohol consumption and testosterone changes. Thus, high-drinking AA rats below the preference of 50 % (approximately 2.5 – 3 g/kg/day) and low-drinking Wistars above 2.5 g/kg/day were excluded.

The Studies I-IV were approved by the County Administrative Board of Southern Finland and the ethical committee of the National Public Health Institute. All experimental animal procedures were approved by the Institutional Animal Care and Use Committee at the National Public Health Institute and carried out in accordance with the European Communities Council Directive (86/609/EEC).

4.1.1. Facilities and housing conditions

In Study I the animals were individually housed in wire mesh cages (21 X 38 X 19 cm) throughout the experiment. In Studies II and III the rats were housed in plastic cages (Macrolon IV, 56 X 34 X 19 cm), two animals per cage until the stage of voluntary alcohol intake, which was conducted in individual mesh cages (21 X 38 X 19 cm). In Study IV the rats were single housed in either Macrolon cages (21 X 38 X 19 cm; n = 12 for AA and n = 10 for ANA) or group housed in cages (59.5 X 38 X 20 cm; 4 animals per 3 cages).

In all animal studies (I-IV) animal facilities were air-conditioned, with temperature 20-21 ° C, humidity at 47.6 % and a 12 h / 12 h light/dark cycle with lights on at 6 a.m., except for the experiment with reversed light cycle (Experiment 2 of Study II), where lights went on at 6 p.m. The rats had free access to water and standard laboratory pellets (SDS RM1, Witham, Essex, England).

4.2. Drug administrations

4.2.1. Alcohol /Ethanol

During the voluntary alcohol consumption period in Studies I, II and III, the animals had free access to two 100 ml bottles, one with tap water and the other with 10 % (wt/vol) ethanol (Berner Oy, Helsinki, Finland) in tap water. Voluntary alcohol consumption was not tested in Study IV. In Studies I-IV, alcohol doses (0.75 g/kg, Study IV; 1.5 g/kg, Studies I-IV; 2 g/kg, Study 1) were administered intraperitoneally (i.p.). In all Studies I-IV alcohol was administered as a 10 % ethanol (wt/vol diluted in 0.9 % NaCl).

4.2.2. Nandrolone Decanoate

Nandrolone decanoate (ND) (Organon, Oss, the Netherlands) used in Study II was dissolved (50 mg/ml) in sterile oil (*Arachidis oleum*, Yliopiston Apteekki/ University Pharmacy, Finland) and administered by subcutaneous injection (s.c.) (15 mg/kg). It was considered essential to use pure ND, because the commonly used commercial ND product (Deca-Durabolin®, N. V. Organon, Oss, the Netherlands) contains Benzyl Alcohol (10 % v/v) as a preservative, which might cause unwanted effects of its own (Nair 2001).

4.2.3. Benzyl Alcohol

Benzyl alcohol (BA) (Yliopiston apteekki/ University Pharmacy, Finland) used in Study III was diluted (100 mg/ml) in sterile oil (*Arachdis oleum*, Yliopiston apteekki, Finland), which was a dose corresponding to that in the Deca-Durabolin® used by Johansson et al. (2000). The BA solution was administered by s.c. injection (30 mg/kg).

4.3. Blood sampling and analytical methods (Studies I - IV)

Blood samples (200 µl) were taken by puncture from the tip of the tail (Studies I-IV) and immediately diluted with 500 µl saline and centrifuged after coagulation. Serum samples were frozen and kept at -70 °C until the analyses were carried out. Possible consecutive blood samples were taken from the same puncture after removing the coagulated blood plate to minimize the handling stress.

Testosterone concentrations were measured from serum using the testosterone radioimmunoassay kit (Orion Diagnostica, Espoo, Finland). The minimum detectable concentration was 0.1 nmol/L. The intra-assay coefficient of variation (CV) was 9.1 % at a testosterone concentration of 4.8 nmol/L, and the inter-assay CV was 8.3 % at a testosterone concentration of 18.8 nmol/L.

Corticosterone concentrations were determined from serum using an ImmuChem Double Antibody Corticosterone RIA Kit (MP Biomedicals, Orangeburg, NY). The inter-assay CV was 7.2 % and the intra-assay CV was 4.9 % at corticosterone levels of 100-200 ng/mL.

The radioimmunoassay was quantified by a Wallac Wizard 1470 automatic gamma counter (GMI, Inc., Ramsey, MN).

4.4. Experimental designs

4.4.1. F2 (Study I)

All animals were challenged with a priming alcohol dose (Fig. 4). This was followed by a 3-week voluntary alcohol-drinking period with two-bottle choice between tap water and alcohol solution in water. The 40 highest (average for the third week 1.5 ± 0.1 g/kg per day, range: 1.0-3.5 g/kg per day) and 40 lowest (0.6 ± 0.01 g/kg per day, range: 0.34-0.64 g/kg per day) male alcohol drinkers were chosen for Study I. After a washout period of 1 week, about half of the 40 highest (n = 21) and 40 lowest (n = 19) alcohol drinkers were challenged with a second dose (1.5 g/kg) of alcohol. The rest of the animals (matched for same alcohol intake) got an i.p. control injection of saline, same final volume as in the corresponding alcohol test.

The experimental alcohol challenge session started about 7.30 a.m. Blood samples were taken before the alcohol injection in the first sample session (experimental day 1). After the washout period in the second session (day 29) samples were taken before and 1 and 2 hours after alcohol/saline injections.

4.4.2. Nandrolone Decanoate and Benzyl Alcohol (Studies II and III)

For Studies II and III, the rats of each lines (AA and Wistar) were randomly divided into control and treatment groups. In Study II, two experiments with identical designs except for reversed day/night cycle were conducted with both lines, all 8 groups consisting of 10 rats. Study III was conducted with 4 groups consisting of 10 rats. In Study II, the treatment groups received daily subcutaneous injections (s.c.) of ND 15 mg/kg for 14 days, and in Study III, BA 30 mg/kg for 14 days was injected s.c. In both studies the other group of animals were given daily s.c. injections of vehicle oil (*Arachidis oleum*). These first treatment periods were followed by one-week washout periods. After washout, on day 22, all rats were placed into single wire mesh cages for the voluntary drinking period. In Study II drinking period continued 5 weeks (experimental weeks 4-8) (Fig. 4) and in Study III for 3 weeks (experimental weeks 4-6). All injections were given about 8.00 a.m. In Study II, the same animal groups were given a second 14 day nandrolone or oil treatment in experimental days 43-56 for studying the acute effect of ND treatment on voluntary alcohol consumption.

Fluid consumption was recorded daily and the bottles were cleaned and refilled twice a week, whereby their placement was also changed to avoid any effects of place preference.

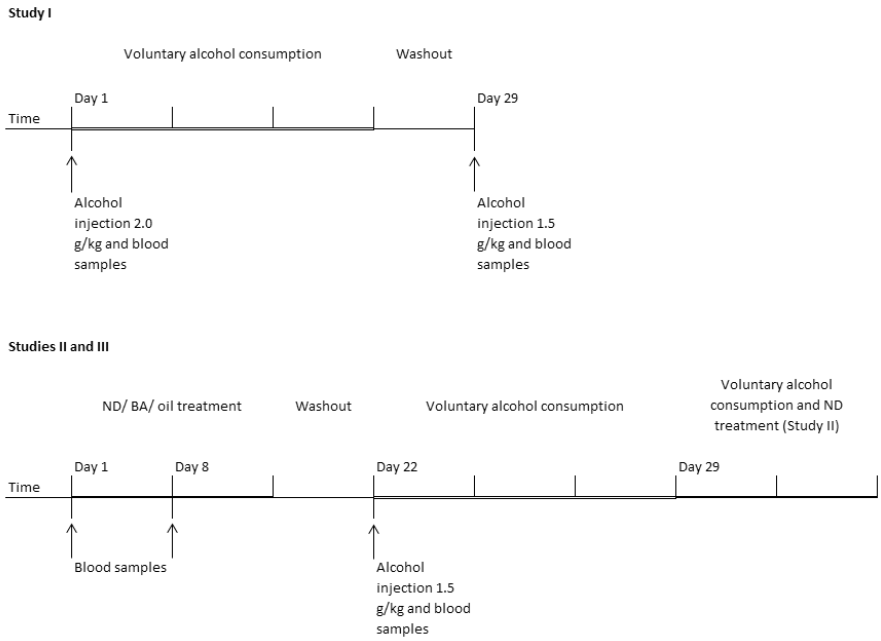

Figure 4. Experimental design in Study I, II and III

4.4.3. The role of corticosterone and alcohol-mediated testosterone changes in the alcohol drinking by AA, ANA and Wistar rats (Study IV)

Study IV comprises 3 sub-studies with the same rats and experimental designs as already described in paragraphs 4.4.1 to 4.4.2 (Studies I, II and III). In addition, the present study (IV) comprises the first substudy with a part of the experimental design of another earlier study of male AA and ANA populations of generation F80 (Apter and Eriksson, 2006). As different from Studies I-III, in Study IV, substudy 1, the AA and ANA rats were tested in both group- and single-cages. Thus, single-caged animals were placed in isolation 1 week prior to the beginning of the experimental period. The treatment conditions involved alcohol administration (at least 1 week between the treatments) with doses 0.75 and 1.5 g/kg involving blood sampling at 0, 1, 2 and 3 hours.

4.5. Statistical analyses

The data were analyzed using SPSS (versions 14.0 (I), 19.0 (III, IV), and 21.0 (II)), SPSS Inc., Chicago, IL.

Nonparametric tests were used when data did not fulfill the requirements of parametric tests, such as normal distribution. Normality was tested with the Kolmogorov-Smirnov and Shapiro-Wilk tests.

Differences and group comparisons of hormone levels in Studies I, II and III were analyzed using the Mann-Whitney *U*-test or the Kruskal-Wallis non-parametric analysis of variance followed by the Mann-Whitney *U*-test for group comparisons. Parametric data of the body weight and food consumption (III) were analyzed with ANOVA for repeated measures and the Student's *t*-test. The individual alcohol effect (the change from starting level) at different time points in Study I was calculated by subtracting the median change of the saline group from the alcohol group at corresponding time points.

Frequency comparisons concerning alcohol mediated testosterone elevations (I, II, III) and reductions between groups were assessed by Pearson's Chi-Square test. Bonferroni corrections were used for assessing combined significance of two separate tests.

Repeated measures ANOVA was used to analyze combined differences in basal hormone levels before both alcohol administrations in Study I. Wilcoxon signed-rank test was used to related samples such as the comparison within groups before and after the alcohol consumption period.

In Study I, the hormone and alcohol consumption data, which did not fulfill the requirements of normal distribution, are presented as median ± interquartile range in the figures. In the text, all data are presented as mean ± SEM (Standard Error of Mean). The figures in Study I and II were made with GraphPad Prism version 4.0 (GraphPad Software, Inc.). The alcohol consumption data in Study III, which did not fulfill the requirements of normal distribution, are presented as median ± median absolute deviation (MAD) in Figures and in the text. The figures in Study III were made with Sigma Plot 9.0.1 (Systat Software, Inc.).

In Study IV, correlation comparisons (both Pearson's r and Spearman's rho) were assessed by Fisher's Z-test.

Significance was assessed at two main confidence levels: 95 % ($\alpha = 1 - 0.95 = 0.05$) and 85 %, suggesting trends, ($\alpha = 1 - 0.85 = 0.15$). All lower levels of confidence are considered non-significant ($p > 0.05$) or not even regarded as trends ($p > 0.15$).

5. SUMMARY OF RESULTS

5.1. F2 (Study I)

5.1.1. Endogenous testosterone levels and effects of alcohol consumption

Basal serum testosterone concentrations before and the alcohol drinking and subsequent 1 week's washout period are depicted in Fig 5. Higher endogenous testosterone levels (8.2 (5.1-13.5) nmol/L) were detected in rats in the high alcohol consumption group compared with low alcohol consumption group (4.1 (2.5-9.1) nmol/L, p = 0.007). This difference was not significant after the voluntary alcohol drinking and washout period (2.2 (0.5-4.0) vs. 1.0 (0.4-2.9) nmol/L, p = 0.427 ns.). The overall significance between the high- and low-consumption groups was p = 0.024 (Mann-Whitney). The basal serum testosterone levels were measured from samples taken at the same time in the morning, the median levels after the voluntary alcohol-drinking period was considerably lower than before the voluntary alcohol drinking (74 % in high drinkers and 75 % in low drinkers).

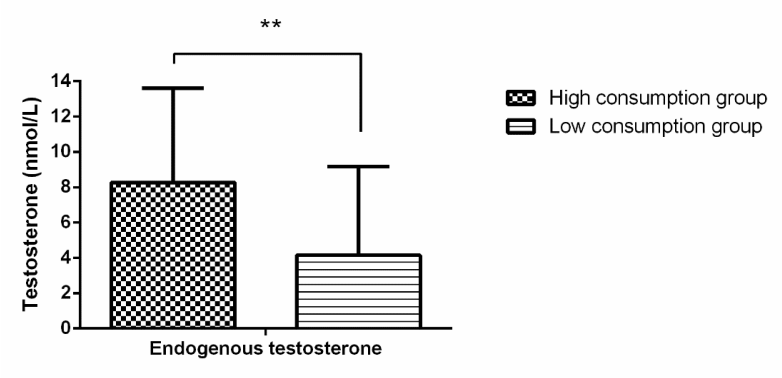

Figure 5. Endogenous testosterone levels at the beginning of the experiments. Data is displayed as median and interquartile deviation. **p < 0.01 for group comparison (Mann-Whitney U-test).

5.1.2. Endogenous corticosterone levels and effects of alcohol consumption

Lower basal corticosterone levels were observed in the high consumption group (86.2 (80.5-119.2) ng/mL) compared to the low drinking group (101.6 (66-138.7) ng/mL, p = 0.137 ns. trend) in the beginning on the experiments. This difference increased after the voluntary drinking and washout period to become statistically significant (130.0 (71.1-190.9) ng/mL) in the high consumption group compared with (184.4 (109.9-264.7) ng/mL, p = 0.029) in the low consumption group ($p < 0.05$). The overall difference between basal corticosterone levels between the two consumption groups was significant, p = 0.021.

5.1.3. Alcohol induced change in testosterone levels

After the voluntary alcohol drinking and washout period the i.p. injection of alcohol had different effects on the testosterone levels of the high and the low consumption groups. The high consumption group showed a significantly higher frequency (38 %) of testosterone elevations compared with the low consumption group (5 %, p = 0.021) after 1 hour after alcohol injection (I, Table 1).

The effect of alcohol (compared with saline) in the low consumption group was a marked lowering of testosterone levels, whereas no significant effect was observed in the high consumption group (Fig 6). No significant group effect in corticosterone responses to alcohol injection were observed in either of the rat groups.

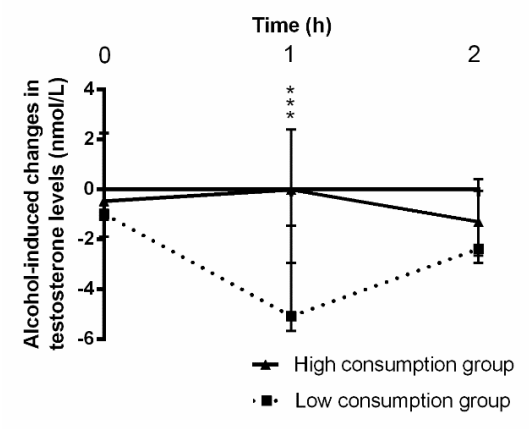

Figure 6. Alcohol induced changes in testosterone levels. Median values of testosterone levels at different time points (subtracted for corresponding median control values) ± interquartile deviation are displayed. ***$p < 0.001$ for group comparison (Mann-Whitney U-test).

5.2. Nandrolone Decanoate (Study II)

Study II includes two separate experiments with chronic ND treatment. The experimental design was identical except for the reversal of the light-dark cycle between the experiments 1 and 2.

5.2.1. The Nandrolone Decanoate effect on voluntary alcohol consumption

Effect of first 2-week ND treatment on alcohol drinking

In both experiments, without ND treatment, significantly higher alcohol consumption was observed in the alcohol-preferring AA compared with the low consumption Wistar rats. The average alcohol consumption in week 6 in Experiment 1 was 4.88 ± 0.72 vs. 1.47 ± 0.29 g/kg/day ($p = 0.005$) and in Experiment 2 3.38 ± 0.72 vs. 0.55 ± 0.16 g/kg/day ($p = 0.007$). In the ND treatment groups the corresponding drinking averages in Experiment 1 was 3.43 ± 0.78 vs. 0.84 ± 0.29 g/kg/day ($p = 0.07$, ns), and in experiment 2 was 1.07 ± 0.55 vs. 0.61 ± 0.14 g/kg/day ($p > 0.05$ ns).

The two-week ND pretreatment significantly decreased the consecutive voluntary alcohol consumption in the AA rats in both experiments (Fig. 7). A similar trend was seen in the Wistar rats in Experiment 1. In Experiment 2, with the reversed light-dark cycle, the ND group medians for the Wistar rats were non-significantly higher compared to controls.

In both rat lines, with or without ND treatment, alcohol consumption was generally higher in Experiment 1 (with treatments and nocturnal phase between 7 a.m. and 7 p.m.) than in Experiment 2 (with the 7 p.m. to 7 a.m. dark phase). Average on week 6: AA no ND, $p = 0.15$ and AA ND $p = 0.07$ (trend), combined significance $p = 0.021$. For Wistar no ND the significance was $p = 0.02$ and in ND group $p > 0.05$, ns.

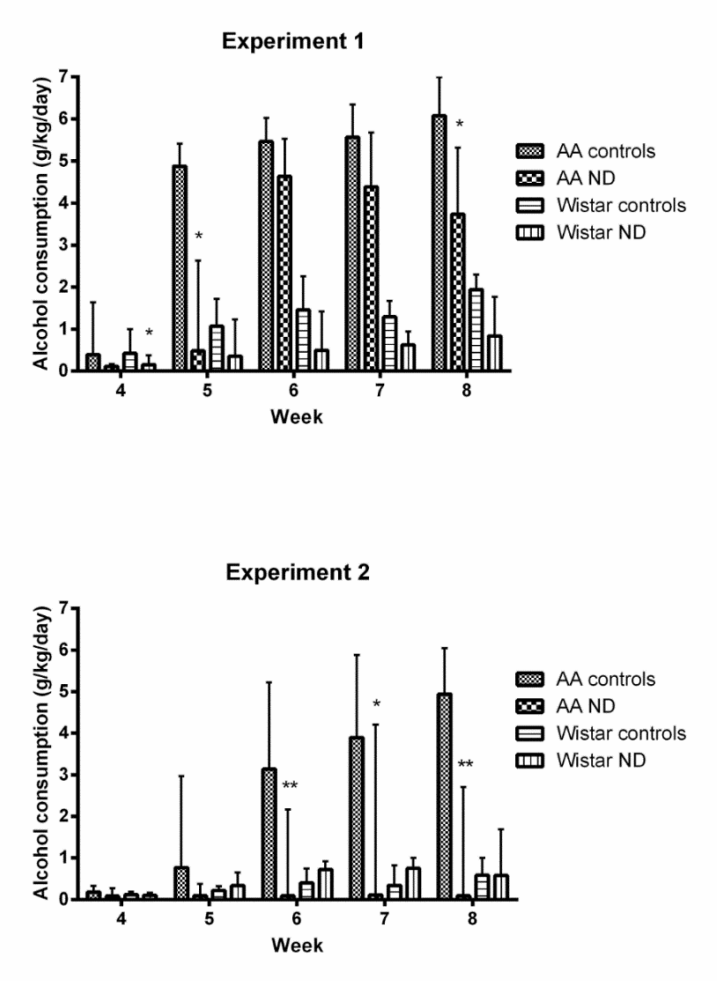

Figure 7. Voluntary alcohol consumption (g/kg/day) five consecutive alcohol drinking weeks. Data are displayed as median ± interquartile deviation. ND groups are compared to corresponding control groups for weekly alcohol consumption. *p < 0.05, **p < 0.01. In Experiment 1 lights were on from 7 AM and off at 7 PM. In Experiment 2 the time schedule was reversed.

Effect of second ND treatment on alcohol drinking

ND treatment during the later phase of alcohol drinking (weeks 7 and 8) further reduced alcohol drinking in the AA rats. Average drinking changes in weeks 7 and 8, were in Experiment 1, +0.94 ± 0.66 vs. -0.06 ± 0.33 g/kg/day, in no ND and ND groups, respectively ($p > 0.05$, ns) and in Experiment 2, no ND +1.27 ± 0.44 vs. ND treated +0.31 ± 0.59 g/kg/day, $p = 0.009$ (combined significance $p = 0.006$) No significant, nor trends, for drinking changes were observed in the low drinking Wistar rats.

Alcohol preference

The ND effects are also clearly seen (Fig. 8) in the alcohol preference data (Experiment 1 and 2, AA and Experiment 1, Wistar) with significantly lower alcohol preference in ND treated animals starting already at the first week of drinking. In Experiment 2 no significant preference differences were recorded in the Wistar rats. ND treatment during the later phase of alcohol drinking (2 last week's average) also tended to further reduce alcohol preference in the AA rats. Alcohol preference change: Experiment 1, no ND +11.28 ± 7.48 % vs. ND -2.95 ± 4.24 %, $p = 0.131$ and Experiment 2, no ND +15.92 ± 6.24 % vs. ND +3.59 ± 6.67 %, $p = 0.121$ (combined significance $p < 0.05$).

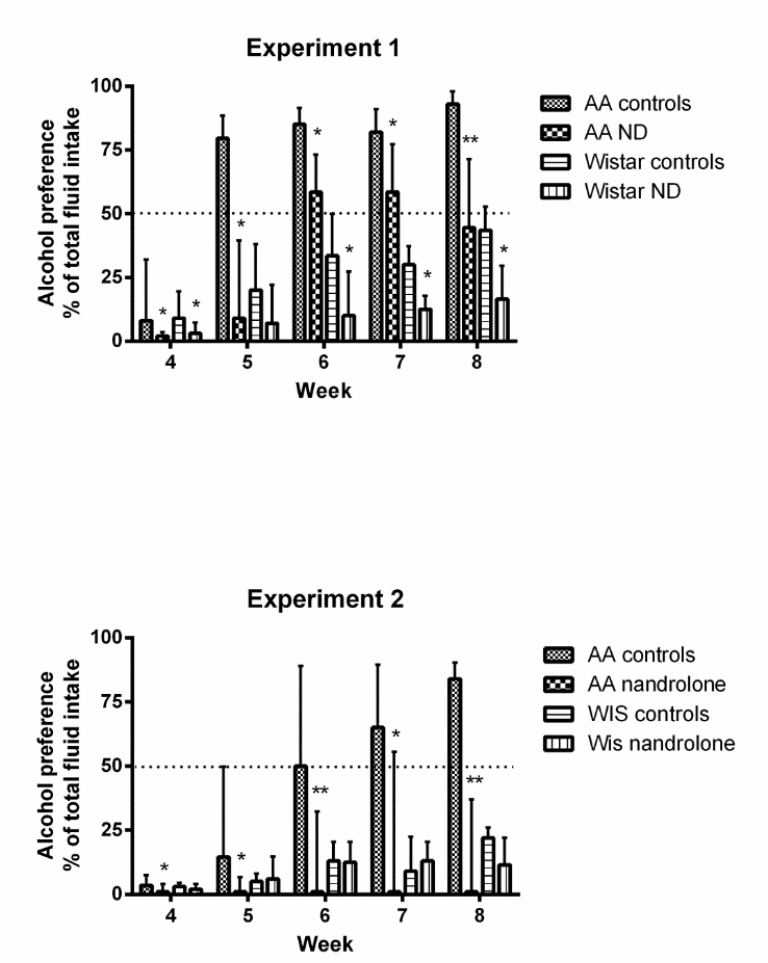

Figure 8. Alcohol preference (% of total fluid intake). For additional details see legend of Figure 4.

5.2.2. Nandrolone Decanoate and endogenous testosterone and corticosterone

Testosterone

Higher testosterone levels were detected in the AA ND group compared to the respective control group ($p = 0.003$) after 1 week's treatment in Experiment 1 (II, Fig. 4). After the washout week there were no significant differences between groups. In Wistar rats the ND treated group had significantly ($p < 0.001$) higher testosterone levels after the treatment and washout period, day 22, compared with the controls (no significant effects after 1 week's treatment).

In Experiment 2 with reversed light-dark cycle, the AA rats displayed only a trend for elevated testosterone in the middle of the ND treatment period ($p = 0.07$). As for the Wistar rats in Experiment 2, the ND group displayed significantly higher testosterone levels in the middle of the treatment period, day 8, ($p = 0.003$) compared to the control group and this effect remained after the wash out period, measured at day 22 ($p = 0.003$) (II, Fig. 4).

Corticosterone

Basal corticosterone levels in ND treated AA rats were significantly lower in both Day 8 ($p < 0.001$) and after Day 22 ($p = 0.005$) when compared to controls in Experiment 1 (II, Fig. 5). Wistar corticosterone levels in Experiment 1 were also lower after the ND treatment in Day 8 ($p = 0.049$) compared with the controls, but after the wash-out period no significant differences remained. In Experiment 2, ND treated AA rats displayed a trend lowered corticosterone levels during the ND treatment ($p = 0.112$ compared with controls). A clearer difference was seen between the groups after the washout period ($p = 0.005$). In Wistars there was no significant difference between groups regarding corticosterone levels in Experiment 2.

5.2.3. Effect of Nandrolone Decanoate on alcohol-mediated testosterone and corticosterone changes

Alcohol-mediated changes in testosterone levels were measured after the ND treatment and washout period. The overall results from combining both rat lines and experiments are depicted in Fig. 9. Results describe the situation after ND treatment but before voluntary alcohol drinking has started. Significant testosterone reductions (ND compared with control groups) were observed both 1 hour ($p = 0.006$) and 2 hours ($p = 0.002$) after alcohol administration.

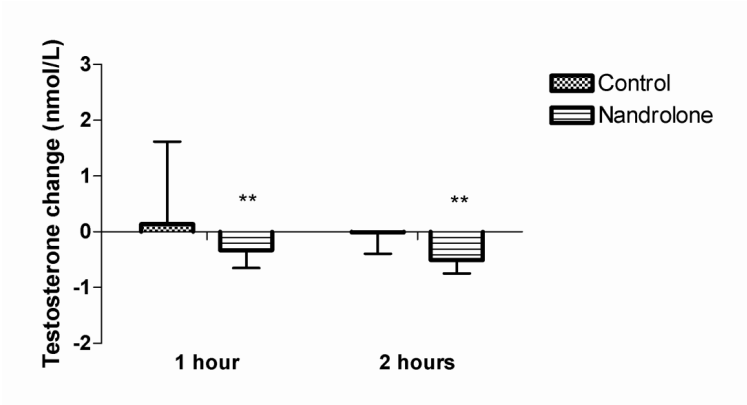

Figure 9. The effect of previous ND treatment on acute alcohol-mediated testosterone changes 1 and 2 hours after alcohol administration. The data depict the combined results of the experiments and rat lines. For additional details, see legend of Figure 7.

In Table 1 the data are presented separately for the AA and Wistar rats, as numbers of individual directional testosterone changes from basal levels to levels at 1 and 2 hours after alcohol administration. One hour after alcohol administration, a distribution difference with a lower frequency of testosterone increases (or no change, one animal) emerged in the ND group compared with the control Wistar rats ($p = 0.037$, experiments combined). In the AA rats this effect was not significant ($p = 0.204$, ns). After 2 hours from alcohol administration the corresponding ND effect was clearer in both AA ($p = 0.018$) and Wistars ($p = 0.034$). No significant ND effects were observed for corticosterone during alcohol intoxication.

Table 1. The effect of ND on the frequency of testosterone changes during alcohol intoxication, changes are calculated as difference to zero value.

Rat line	Treatment	Increase	Decrease or no change	p-value
1 h				
AA	ND	7	13	
AA	Controls	11	9	0.204
Wistar	ND	7	13	
Wistar	Controls	13	6	0.037
2 h				
AA	ND	3	17	
AA	Controls	10	10	0.018
Wistar	ND	4	16	
Wistar	Controls	10	9	0.034

Values express the number of individual directional testosterone changes from baseline to 1 and 2 h after alcohol administration. p-values refer to the differences between ND- treated and control animals (Pearson's Chi-Square).

5.3. Benzyl Alcohol (Study III)

5.3.1. Benzyl Alcohol effect on voluntary alcohol consumption

The results on voluntary alcohol consumption are given for each experimental week separately in Fig. 10. During the first drinking week (week 4) the median voluntary alcohol drinking in the BA group of the AA rats was somewhat higher (difference not statistically significant) compared to the control group ($p = 0.11$). During experimental weeks 5 and 6 the median drinking of AA rats was similar in the BA and the control group ($p = 0.29$ and $p = 0.13$, respectively). Combining the three alcohol drinking weeks together, the total alcohol consumption in the BA treated group was higher compared to the control group both in the AA rats: 4.94 ± 1.31 g/kg vs. 4.17 ± 0.31 g/kg ($p = 0.07$) and the Wistar rats: 1.01 ± 0.26 g/kg vs. 0.38 ± 0.27 g/kg ($p = 0.05$); combined effect of BA treatment in both AA and Wistars was $p < 0.01$.

In Wistar rats, there was no significant difference in alcohol consumption during the first week between BA and control animals. However, group differences were emerging with a trend during week 5 ($p = 0.07$) and more clearly in experimental week 6 ($p = 0.04$) as seen in Fig. 10.

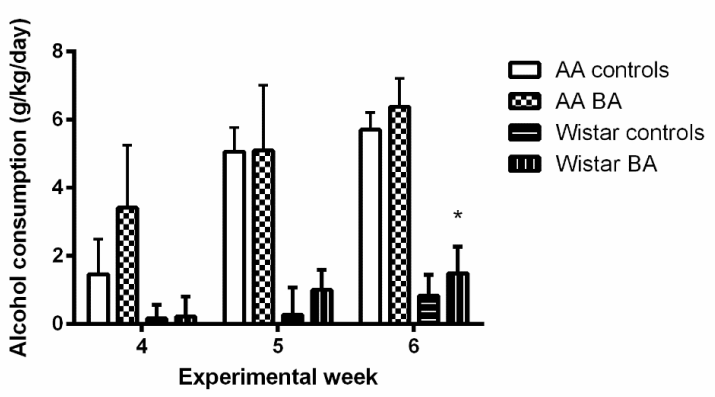

Figure 10. AA and Wistar alcohol consumption during 3 drinking weeks in rats treated with the BA or with vehicle oil. Data is displayed as median ± interquartile deviation. *$p < 0.05$.

Alcohol preferences of three voluntary drinking weeks are presented in Table 2. In the first voluntary drinking week (week 4) the AA rats in the BA group had higher (not statistically significant) preference for alcohol than the control group ($p < 0.10$). During second and third drinking weeks, alcohol drinking seemed to be more equal in BA compared with control. However, in the Wistar rats, if anything, the pattern for the preference development was higher in the BA compared to control (although not statistically significant) during the last drinking weeks (experimental weeks 5 and 6).

Table 2. Alcohol preference/week (% of total fluid intake), median ± MAD.

Rat line	Treatment	Week 4	Week 5	Week 6
AA	Controls	32.1 ± 23.3	93.7 ± 2.3	95.0 ± 3.3
AA	BA	63.7 ± 31.1	89.6 ± 4.02	96.0 ± 2.5
p-value		0.10	0.76	0.33
Wistar	Controls	4.5 ± 2.5	7.2 ± 5.61	21.5 ± 13.1
Wistar	BA	5.0 ± 2.1	21.0 ± 9.2	33.8 ± 8.9
p-value		0.94	0.14	0.10

5.3.2. The effect of Benzyl Alcohol on basal testosterone and corticosterone levels

Basal testosterone and corticosterone levels are displayed in Table 3. Control and BA group values are combined because no significant differences (not even trends for difference) were observed for the different treatments. In the AA rats basal testosterone levels were significantly lowered during days 8 ($p < 0.001$) and 22 ($p < 0.01$) compared to day 1. In Wistars basal levels were lowered at day 8 ($p < 0.01$). However, after the washout period (day 22) basal levels were back to original values.

In comparing AA to Wistar rats no differences were found on day 1. However, at day 8 the AA testosterone levels tended to be lower ($p = 0.06$) and at day 22 this difference was significant ($p < 0.001$) in AA compared with Wistars. Regarding the basal corticosterone levels, elevations were observed at day 8 ($p < 0.05$ and $p < 0.01$ compared with day 1 and 22, respectively). A similar pattern (but not significant) was observed for the AA rats. In general, (days 1 and 8) corticosterone levels were lower ($p < 0.001$ and $p < 0.001$) in AA compared with Wistar rats.

Table 3, Basal testosterone and corticosterone levels in first day of experiment (day 1), after 1 week (day 8) and after washout period (day 22), treatment groups combined.

Rat line	Day 1	Day 8	Day 22
Testosterone nmol/L			
AA	4.34 ± 7.05	0.07 ± 1.11***	0.07 ± 1.84**
Wistar	2.79 ± 2.69	0.59 ± 1.2**	2.73 ± 3.51
Line dif.	p = 0.27	p = 0.07	p = 0.00
Corticosterone ng/mL			
AA	83.05 ± 44.19	123.05 ± 56.08	89.95 ± 73.06
Wistar	218.90 ± 74.91	261.00 ± 74.88*	146.00 ± 76.86**
Line dif.	p = 0.00	p = 0.00	p = 0.22

Values are expressed as median ± MAD for control and BA groups combined. *p < 0.05, **p < 0.01, ***p < 0.001 for days 8 and 22 compared with day 1 (Wilcoxon). Line differences at days 1, 8 and 22 are assessed by Kruskal–Wallis test.

5.3.3. Effect of Benzyl Alcohol on alcohol mediated testosterone and corticosterone changes

The alcohol-mediated testosterone level change was measured from basal to 1 and 2 h after alcohol injection after the washout period. The changes in AA (controls vs. BA group) over 1 h were + 0.08 ± 0.24 vs. + 0.20 ± 0.48 nmol/L, and over 2 h+ 0.02 ± 0.51 vs. + 0.08 ± 0.19 nmol/L. The corresponding values for Wistar rats (controls vs. BA group) over 1 h were + 2.07 ± 1.23 vs. + 2.14 ± 1.52 nmol/L and over 2 h − 1.58 ± 2.04 vs. + 0.53 ± 2.86 nmol/L. The differences between the control and BA groups were not significant in either rat line.

The number of AA and Wistar animals with increased or decreased levels of testosterone during alcohol intoxication, is presented in Table 4. No significant effects of BA were observed. However, a significant line difference emerged at 2 h after alcohol administration, with the Wistars displaying less testosterone increases ($p < 0.01$, combined groups) compared with the AA rats.

Table 4. The frequencies of testosterone changes during alcohol intoxication changes are calculated as difference to zero value.

Rat line	Treatment	Increase	Decrease
1 h			
AA	BA	8	2
AA	Control	9	1
AA total		17	3
Wistar	BA	7	3
Wistar	Control	7	2
Wistar total		14	5
2 h			
AA	BA	9	1
AA	Control	8	2
AA total		17	3
Wistar	BA	5	5*
Wistar	Control	3	6*
Wistar total		8	11**

Values express number of individual directional testosterone changes from basal level to 1 and 2 h after alcohol administration. *$p \leq 0.05$ **$p < 0.01$, respectively, for the frequency differences between corresponding Wistar and AA groups 2 h after alcohol administration.

5.4. Corticosteroid modulation of testosterone changes during alcohol intoxication (Study IV)

5.4.1. AA versus ANA rats and F2 populations

According to the present results, the high-drinking AA rats displayed a trend for positive correlation (rho = 0.373, p = 0.073) between testosterone elevations during 1 hour's alcohol intoxication (after a dose of 0.75 g/kg) and the starting point corticosterone levels (Fig. 11). However, non-drinking ANA rats displayed non-significant negative correlation (rho = -0.362, p = 0.107) between decreasing testosterone levels and basal corticosterone levels after 1 hour. The overall correlation difference between the AA and ANA lines was significant after 1 hour (Z = 2.401, p = 0.016). At the times 2 and 3 hours after start or with the dose of 1.5 g/kg no significant correlation differences were found.

The F2 high and low consumption populations did not display significant differences between each other regarding correlations between basal corticosterone levels and subsequent alcohol-mediated testosterone changes. Nevertheless, a non-significant trend for a similar correlational result as shown for the original AA/ANA was observed (results not shown). In addition, a significant negative correlation appeared in the low-drinking population between the mean alcohol drinking on the third week and the testosterone change at one and two hours after the priming 2 g/kg alcohol injection (r = -0.334, p = 0.044 and r = -0.400, p = 0.016, respectively).

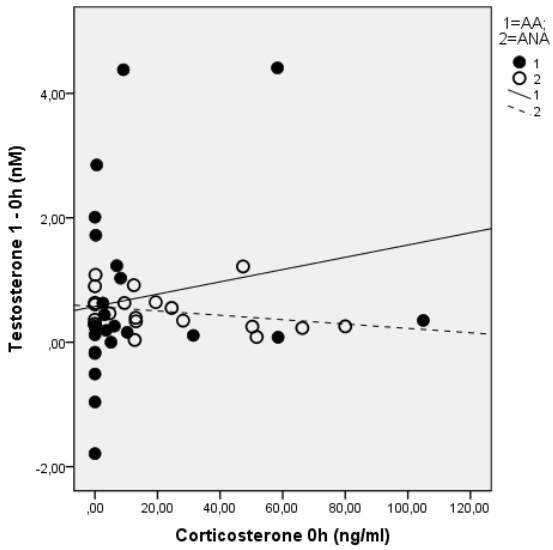

Figure 11. AA and ANA correlations between testosterone change at 1 hours (1h) of alcohol intoxication (by a dose of 0.75 g/kg) and the starting corticosterone level (0h), p = 0.016 for correlation comparisons.

5.4.2. AA versus Wistar rat and the effect of Nandrolone Decanoate

According to the present results, the high-drinking AA rats displayed a trend for a positive correlation (r = 0.431, p = 0.074) between testosterone elevations during 2 hours' alcohol intoxication (after a dose of 1.5 g/kg) and the starting point corticosterone levels (Fig. 12). The low-drinking Wistar rats displayed a non-significant negative correlation (r = -0.154, p = 0.529) between decreasing testosterone levels and basal corticosterone level during the 2 hours. Only a trend for the overall correlation difference between the lines was seen (Z = 1.715, p = 0.086).

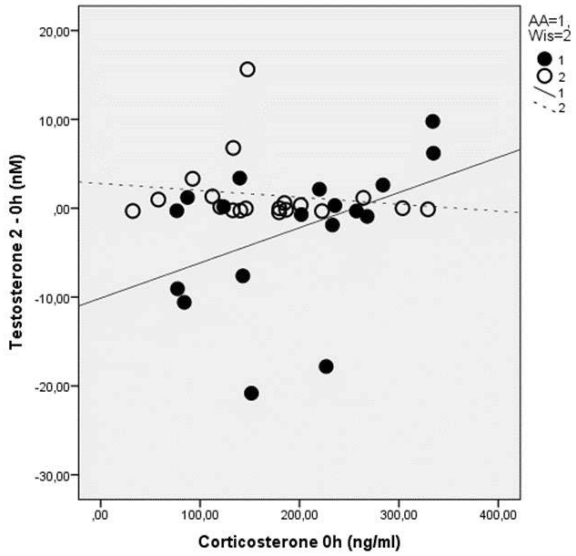

Figure 12. AA and Wistar rat correlations between testosterone change at 2 hours (2h) of alcohol intoxication (by a dose of 1.5 g/kg) and the starting corticosterone level (0h), p = 0.086 for correlation comparisons.

Only a weak tendency (Z = 1.500, p = 0.134) for a correlational difference between the control and ND groups was observed in AA rats (r = 0.431, p = 0.074 for the control and r = 0.070, p = 0.770 for the ND group) at 2 hours' alcohol intoxication (Fig. 13). However, a significant difference in the starting corticosterone levels (0 h) appeared between the control (190.0 ± 18.2 ng/ml) compared to the ND group (87.5 ± 9.1, p = 0.000).

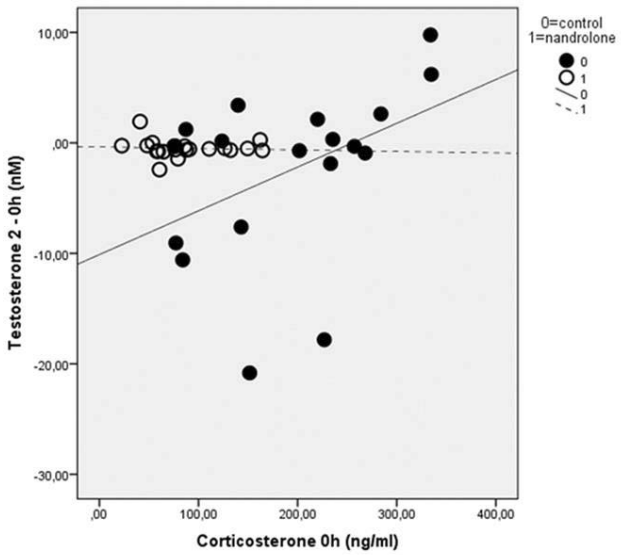

Figure 13. Correlational effect of nandrolone on AA rats between testosterone change at 2 hours (2h) of alcohol intoxication (by a dose of 1.5 g/kg) and the starting corticosterone level (0h), p = 0.134 for correlation comparisons.

The alcohol drinking average of the 3rd drinking week and testosterone change (2 hours after alcohol injection) displayed non-significant positive correlation in experiment 1 (lights on at 6 a.m.) r = 0.469, p = 0.288 for AA and negative correlation r = -0.553, p = 0.155 for Wistars (correlational comparison Z = 1.687, p = 0.092). Also, in experiment 2, corresponding correlations emerged with a non-significant positive r = 0.480, p = 0.276 in AA and negative r = -0.542, p = 0.132 in Wistar rats (correlational comparison Z = 1.751, p = 0.080) (IV, Fig 4). In addition to the correlational difference between AA and Wistar populations, regarding alcohol consumption in 3rd week and testosterone change at 2 hours, a similar significant overall correlational difference emerged (Z = 2.927, p = 0.003) between nandrolone-treated and control AA rats.

5.4.3. AA versus Wistar rats and the effect of Benzyl Alcohol

According to the present results, the high-drinking AA rats displayed a significant positive correlation (r = 0.749, p = 0.013) between testosterone elevations during 2 hours' alcohol intoxication (after a dose of 1.5 g/kg) and the starting point corticosterone levels (Fig. 14). The low-drinking Wistar rats displayed a non-significant negative correlation (r = -0.401, p = 0.284) between decreasing testosterone levels and basal corticosterone level during the 2 hours. A significant overall correlation difference between the lines was observed (Z = 2.508, p = 0.012).

No other significant effects by BA for a correlational difference between the control and BA groups regarding testosterone elevation and corticosterone, were observed in AA and Wistar rats.

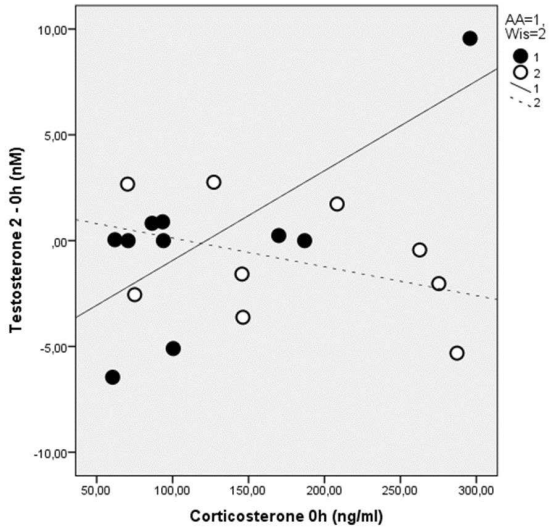

Figure 14. AA and Wistar rat correlations between testosterone change at 2 hours (2h) of alcohol intoxication (by a dose of 1.5 g/kg) and the starting corticosterone level (0h), p = 0.012 for correlation comparisons.

The alcohol drinking average of the 3rd drinking week and testosterone elevation (2 hours after alcohol injection) displayed non-significant positive correlation r = 0.433, p = 0.212 for AA and negative correlation r = -0.539, p = 0.155 for Wistars (correlational comparison Z = 1.917, p = 0.055). At 1 hour from alcohol injection the positive significant correlation was r = 0.638, p = 0.047 for AA and the negative non-significant correlation was -0.206, p = 0.600 (correlational comparison Z = 1.727, p = 0.084). No other effects by BA for a correlational difference between the control and BA groups, regarding alcohol drinking and testosterone elevation, were observed in AA and Wistar rats.

6. DISCUSSION

6.1. F2 (Study I)

AA and ANA rat lines have been bred by selection for high- and low- alcohol preference over several generations (Eriksson, 1968). The success of selective breeding, which has created a number of differential phenotypes, indicates that genetic factors are important determinants of alcohol consumption behavior in these and other similarly selected rodent lines (Colombo et al., 2006; Draski et al., 2001; Eriksson, 1968; Murphy et al., 2002; Quintanilla et al., 2006).

Study I was designed to test the validity of original findings and conclusions regarding the role of endogenous testosterone levels and alcohol-mediated testosterone changes on voluntary alcohol consumption in AA and ANA rats (Apter and Eriksson, 2003; Apter and Eriksson, 2006). By crossbreeding the AA and ANA rats followed by a second randomized breeding a F2 population was derived and used as the experimental tool. Such an experimental F2 design is commonly used as a genetic tool to evaluate earlier initial findings obtained in the original lines.

6.1.1. The role of endogenous testosterone and corticosterone levels

The findings of the F2 experiment are in broad agreement with the differences originally observed by comparing AA and ANA rats. Higher endogenous testosterone levels were found in the high alcohol consumption group of the F2 population compared with the low-consumption group. This positive association between endogenous testosterone levels and alcohol drinking levels (1.2 g/kg/day, range: 1.0-3.5 g/kg for the high and 0.6 g/kg/day, range: 0.3-0.6 g/kg for the low drinkers of the F2 population) in the present study was obtained at even lower average alcohol consumption levels compared with those of the original AA rat line (5-7 g/kg/day) (Sommer et al., 2006). However, significantly higher corticosterone levels in the low-consumption compared with the high-consumption group were detected only at the post-drinking stage. Indeed, it is natural that differences between groups, delimited within the F2 generation are less pronounced than those found between strains produced by selection for many generations. In the alcohol naïve situation there was a trend for similar basal difference, which also supports earlier findings (Apter and Eriksson 2006).

High endogenous testosterone levels have been hypothesized to result from low levels of hypothalamic BEP, which by leading to hypersensitivity of the opiate receptors may result in increased reinforcement by alcohol (Apter and Eriksson 2003). Another explanation for the endogenous association between testosterone and alcohol drinking is that testosterone could independently reinforce traits such as high novelty and sensation seeking (Apter and Eriksson 2003). This hypothesis is supported by earlier data indicating a higher degree of antianxiety and novelty-seeking behavior in AA rats compared with ANA rats (Moller et al., 1997; Salimov, 1999). Human data also support this hypothesis, because elevated endogenous testosterone levels have been correlated with excessive alcohol drinking habits in men in combination with a high degree of antisocial, novelty- and sensation-seeking behaviors (Bergman and Brismar, 1994; Kerschbaum et al., 2006; King et al., 1995; Virkkunen et al., 1994).

Stress and the activated HPA-axis with associated endogenous corticosteroid elevations have also commonly been associated with increased alcohol drinking (Dai et al., 2002; Fahlke and Eriksson, 2000; Pohorecky, 1991). In the present Study I, the low drinkers displayed elevated endogenous corticosterone levels, which suggest that stress is not always a direct causative factor behind the differences in alcohol consumption, neither between the AA and ANA rat lines nor the high and low drinkers of the F2 population. Because stress and elevated corticosterone are known to inhibit testosterone biosynthesis (Hardy et al., 2005; Rivier and Rivest, 1991), it could be that low corticosterone levels have been indirectly selected into the breeding of the AA rat line, due to their association with high testosterone levels which directly predispose for high voluntary consumption of alcohol.

6.1.2. The role of alcohol-mediated testosterone changes

In Study I, there was a significantly higher frequency of alcohol-induced testosterone elevations in high-consumption group compared to low-consumption group. In low consumption group there was high frequency of testosterone decreases from baseline levels. This agrees with previous results from the original AA and ANA rat lines (Apter and Eriksson, 2003). In addition, Study IV confirms the positive correlation between the basal corticosterone level and testosterone elevation in the original AA high-drinking rats during alcohol intoxication. The present results also support the idea that alcohol-induced testosterone elevations increase hypothalamic BEP levels via the feedback regulation, which in turn would promote alcohol reinforcement and drinking. Dopamine actions in nucleus accumbens (NAc) could perhaps play a role in these reactions; dopamine has also been associated with the reinforcing properties of testosterone (Wood, 2004). The reinforcing properties of androgens have also been suggested to be mediated by the metabolites of testosterone acting on GABA(A)/benzodiazepine receptors with connection to the dopamine pathway in NAc (Frye, 2007).

The present results also support the hypothesis of an aversive or negative effect of alcohol-induced testosterone reductions. Such an effect could be detected in the original ANA rat line (Apter and Eriksson, 2003) as well as in the present F2 non-drinking population (Study I). Study IV, confirms these effects, which seem to be due to a negative or absent correlation between the basal corticosterone level and testosterone elevation. The relation between testosterone reductions and the feedback regulation involving endorphin reduction could potentially result in aversive or negative effect of alcohol intake.

6.1.3. The role of alcohol-mediated corticosterone changes

The present F2 results differed from those of previous studies in showing no significant changes in corticosterone after alcohol administration. Apter and Eriksson (2006) found alcohol-mediated corticosterone elevations in both the AA and ANA rat lines, and similar results have been obtained in other studies, where corresponding acute alcohol doses and timing protocols have been applied (Ellis, 1966; Guaza et al., 1983; Orpana et al., 1990; Rivier et al., 1984). One explanation for the present deviating results could be the effect of the earlier alcohol drinking period and HPA habituation, or differences in housing conditions. Tolerance to HPA-activation by chronic alcohol intake has been reported by several researchers (Guaza et al., 1983; Koranyi et al., 1987; Lee and Rivier, 1997; Seeley et al., 1996).

Some of the endogenous and/or alcohol-related line differences in the HPA–HPG axes may be viewed in the light of a transcriptome analysis where increased endogenous gene expression of the corticotropin-releasing factor receptor subtype 2 (CRF2R) was observed in the AA compared with the ANA line (Worst et al., 2005). The increased CRF2R activity has recently been associated with excessive alcohol intake in mice (Lowery and Thiele, 2010) , which is in line with the high drinking of AA rats. On the other hand, increased CRF2R activity has been shown to blunt the HPG axis in rats (Li et al., 2005; Rivier, 2008), which seems to be in conflict with the AA/ANA line differences in endogenous testosterone and corticosterone levels. Further studies are required to settle the complex role of the CRF2R in the relations between alcohol drinking and the HPA–HPG axes.

6.2. Nandrolone decanoate (Study II)

Study II elucidates relations between voluntary alcohol drinking and the HPA and HPG axes by pharmacological manipulation, using the anabolic androgenic steroid nandrolone decanoate (ND). Previous work had indicated increased activation of the HPA-axis (Lindqvist et al., 2002; Matrisciano et al., 2010; Schlussman et al., 2000) and increased voluntary alcohol intake (Johansson et al., 2000) by ND treatment in rats. Based on this, it was hypothesized that ND increases voluntary alcohol intake due to stress-induced enhancement of alcohol-mediated testosterone elevation (Apter and Eriksson, 2003; Apter and Eriksson, 2006).

6.2.1. Effect of nandrolone on voluntary alcohol intake

Johansson et al (2000) found that chronic ND administration increased voluntary alcohol drinking in Wistar rats. The present Study II basically replicated the experimental design of that study with alcohol-preferring AA and Wistar rats. A 2 weeks treatment and one week washout period were followed by a 3 weeks voluntary alcohol drinking period. Unexpectedly, first experiments gave results opposite to those of Johansson et al. (2000): ND treatment *decreased* subsequent voluntary alcohol intake during the 3 weeks drinking period. This effect was more clearly seen in the alcohol-preferring AA rats. A second ND treatment tended to further decrease alcohol consumption. One possible explanation for these contradictory results was the difference of the day and night cycle between the previous (reversed cycle) and the present study (normal cycle).

To test this possibility, experiment 2 was performed with reversed day/night cycle. In all other respects the design was the same as in experiment 1. The results were similar to those in experiment 1 as regards the AA rats: chronic ND treatment led to a clear reduction in alcohol intake. The effects on Wistar rats in experiment 2, however, somewhat resembled those reported by Johansson et al. (2000). Group medians for alcohol consumption were elevated in the last two weeks, but the effect was not statistically significant.

On the whole, the results gave no clear support to the notion that AAS use may be an etiological factor for human excessive alcohol consumption. Earlier reported associations between AAS and alcohol consumption in humans could be indirectly related via common risk factors for both of these behaviors.

6.2.2. The role of nandrolone-affected endogenous testosterone levels

Nandrolone-mediated testosterone elevations were observed in the morning of day 8, in the middle of the first ND treatment period, in both AA rats (in both experiments 1 and 2) and Wistar rats (only in experiment 2). After the two week ND treatment and the subsequent one week washout period, testosterone levels were elevated in Wistars, now in both experiments. In AA rats, only a non-significant trend to a testosterone elevation was observed in experiment 1 after the washout period.

The elevated endogenous testosterone levels are interesting, because the expected effect of the ND treatment would have been a long-lasting feedback inhibition of testosterone synthesis (Minto et al., 1997). This long-lasting testosterone reduction has been explained by the long half-life of the ester release from the intramuscular depot, approximately 6–8 days in humans (Belkien et al., 1985; Wijnand et al., 1985) and 5 days in rats (van der Vies, 1985).

The pharmacokinetics of ND subcutaneously injected (as in the present Study II) is not known to differ significantly compared with intramuscular administration. In an earlier study where ND was administered subcutaneously in rats (Takahashi et al., 2004) testosterone elevations were also indicated, in accordance with the present study.

Overall, it is difficult to draw any firm conclusions on the role of the nandrolone-affected endogenous testosterone levels in the voluntary alcohol consumption.

6.2.3. The role of nandrolone-affected endogenous corticosterone levels

ND treatment has been associated with increased stress and over-activated HPA axis (Allen et al., 2010) and the increased alcohol consumption in the Johansson study was interpreted as the result of a relationship between elevated stress hormones and increased alcohol drinking, as generally found in both humans and rats (Fahlke and Eriksson, 2000; Gianoulakis, 1998; Tanaka, 1998). Recent findings reported by Apter and Eriksson (2006) and in Study I of the present thesis support the hypothesis that HPA axis activation constitutes a situation where the rewarding effects of alcohol are strengthened via enhanced testosterone elevation. Thus, it is plausible that the increased alcohol drinking in rats after sub-chronic ND (Johansson et al. 2000) is explained by the activated HPA axis.

Earlier studies have reported raised corticosterone levels after ND administration (Lindqvist et al., 2002; Matrisciano et al.; 2010 and Schlussman et al., 2000), and increased half-life of cortisol elimination by related AAS (Linet and Lomen, 1971). Study II, however, clearly demonstrated overall reductions in corticosterone concentrations due to subchronic ND treatment. Moreover, these reductions persisted during the washout period. These results are in line with a previous study in rats (Alsiö et al., 2009), and a transient ND-treatment-induced decrease in cortisol levels has also been observed in horses after physical exercise (Hyyppä, 2001). Nevertheless, significant changes in cortisol levels have been found in humans treated with ND (Alen et al., 1987; Bijlsma et al., 1982). The absence of preservative Benzyl Alcohol in the present study compared with Johansson et al. (2000), could partly explain the discrepant results.

6.2.4. The role of nandrolone-affected alcohol-mediated testosterone and corticosterone changes

The hypothesis in Study II, based on previous indications of increased activation of the HPA-axis (Lindqvist et al., 2002; Matrisciano et al., 2010; Schlussman et al., 2000) and voluntary alcohol intake (Johansson et al., 2000) by ND treatment, was that ND would cause stress-induced elevations of alcohol-mediated testosterone levels. However, the results showed quite the opposite. ND treatment led to a significant reduction of alcohol-mediated testosterone increases, as well as to reduced voluntary alcohol drinking. Moreover, effects on corticosterone levels were non-significant. No signs of a direct role of an alcohol-related corticosterone change were observed. Study IV, confirms these effects, which seem to be due to the absence of correlation between the basal corticosterone level and testosterone elevation.

Instead of confirming the positive association (including reinforcement) between alcohol-mediated testosterone elevation and increased alcohol drinking, the results indicate reduced alcohol drinking that could be due to the aversive or negative effect of alcohol-mediated testosterone reduction as displayed by reduced alcohol drinking. However, these results support earlier animal models for this kind of reducing on drinking in the original non-alcohol-drinking ANA rats (Apter and Eriksson, 2006), as well as in the low-consumption group of the F2 population described in Study I.

In addition to testosterone elevation-mediated reinforcement and increased alcohol drinking, the results of study II also support negative reinforcement (here used as opposite to reinforcement) or disinforcing (here perhaps a better expression than negative reinforcement) effect of alcohol-induced testosterone reductions. This could mean that decreased testosterone would lower hypothalamic BEP levels, which consequently could lead to negative reinforcement or disinforcement. The aversive effect by testosterone reduction is also supported by an earlier hypothesis linking testosterone depression to aversive value of social behaviors (Wood, 2004).

6.3. Benzyl alcohol (Study III)

One important methodological difference between Johansson et al (2000) and the present Study II should be acknowledged. In the earlier work the ND product Deca-Durabolin was used, which contains BA as a preservative, but in Study II pure ND was used. It is possible that the treatment with subcutaneous BA, known to be toxic (Kappelgaard et al., 2004; Menon et al., 1984; NTP, 1989), may have been the agent causing an increased stress response, rather than the ND itself. In study III, the effect of BA *per se* on voluntary alcohol drinking was tested, with a study design otherwise replicating the ND study.

The results suggest that the BA as such does increase voluntary alcohol intake. The effect was primarily observed in the low-drinking Wistar rats at the end of the drinking period. Signs of increased voluntary drinking due to BA treatment in the high-drinking AA line were observed only in the first week of drinking. These differences could be explained by the fact that the AA rats are used as a genetic model of excessive drinking and they already have a high alcohol preference, which makes it difficult to further increase the alcohol drinking. The results of Study III support the hypothesis that the 2-week BA treatment accompanying the administration of Deca-Durabolin in the study of Johansson et al. (2000) may have increased voluntary alcohol consumption, which would explain the difference compared with the results of the present Study II.

To our knowledge, there is only one earlier study of the effect of BA on voluntary alcohol drinking in rats (Messiha et al., 1992). These authors found that BA inhibited alcohol drinking. However, they administered one huge BA dose (0.5 g/kg, i.p., compared to our daily dose of 0.03 g/kg) in the middle of a 2-week drinking period. Possible unspecific toxic effects of the high BA dose, as also indicated by simultaneous pronounced body weight reduction (Messiha et al., 1992), make the result difficult to interpret. In the present Study III no significant body weight differences were found between the control and BA groups.

No significant corticosterone or testosterone changes were observed after the BA treatment as compared with the control treatment. However, skin irritation and dermatitis in both rat lines appeared in almost all of the animals already after a few days of subcutaneous BA treatment. These symptoms remained or became worse throughout the BA treatment period; gradual recovery took place during the washout period. To our knowledge such symptoms have not been reported before in rats. However, BA-mediated skin irritation has earlier been observed in mice (Lashmar et al., 1989), guinea pigs (Ishihara et al., 1986; Klecak et al., 1977) and rabbits (Api et al., 2015). Likewise, sensitization and allergic dermatitis have been reported in humans exposed to BA-containing products (Schnuch et al., 2007; Shmunes, 1984; Wilson et al., 1986).

Although actual stress responses, as expressed by corticosterone elevations followed by testosterone reduction, were not observed to be specifically associated with the BA treatment, the rats may conceivably have suffered from mild stress as a result of their skin problems or other undetected symptoms even after the washout period. Earlier stress has commonly been associated with increased alcohol drinking later in both experimental animals (Becker et al., 2011; Pohorecky, 1981; Roman and Nylander, 2005) and humans (Gianoulakis, 1998; Pohorecky, 1991), which could also be the case in the present experimental situation, as well as in the previous study by Johansson et al. (2000). In addition, stress may produce long-lasting adaptations which may reduce possible alcohol-mediated corticosterone changes and which may explain why there were no detectable changes in corticosterone levels in spite of skin symptoms in the treatment group.

One aim of Study III was to find out whether a possible BA-mediated increase in voluntary alcohol drinking might be due to stress-related actions of the HPA and/or HPG axes. Stress and HPA axis activation have previously been found to be connected to alcohol-mediated testosterone elevation followed by increased voluntary alcohol drinking (Apter and Eriksson 2003, 2004, Study I). However, the hormonal data of Study III show no significant BA effects in either rat lines regarding basal corticosterone, testosterone and alcohol-mediated testosterone levels.

BA is known to cause cross-tolerance to other alcohols, as e.g. to alcohol (Khanna et al., 1997). As tolerance has been linked to the propensity to consume more alcohol in both animals (McBride and Li, 1998) and humans (Schuckit, 1994), this might also be at least partly involved in the BA-mediated increased alcohol consumption. Future studies are needed to test this hypothesis.

6.4. Corticosterone, alcohol-mediated testosterone changes and alcohol drinking (Study IV)

Study (IV), have produced correlational evidence for the positive association between basal corticosterone levels and subsequent testosterone elevations during alcohol intoxication in high-drinking AA rats. The combined evidence are depicted in Fig. 11, 12 and 13. Support for these notions are seen also in studies I, II and III, as well is in earlier studies (Apter and Eriksson 2003, 2006).

The fact, that basal corticosterone correlates to subsequent testosterone elevations, means that corticosterone levels could regulate subsequent testosterone elevations, which in turn may cause reinforcement and increased alcohol drinking.

In the low-drinking ANA and Wistar rats no positive correlations were observed between basal corticosterone and alcohol-mediated testosterone elevations. However, indications on negative correlations appeared, significant in the original ANA rats (Fig. 11), and non-significant or absent correlations in the Wistars (Fig. 12 and 13). Altogether, it seems that the loss of alcohol-mediated testosterone elevation could be related to disinforcement and aversion.

Study (IV) also demonstrates that alcohol-mediated testosterone changes, elevation or reduction, are correlated with subsequent voluntary alcohol high (AA) or low (low drinking F2 and Wistars) consumption.

7. CONCLUSIONS

This thesis views the role of corticosteroids and alcohol-mediated testosterone change for alcohol drinking in rats. The methods used varied from F2-population derived from AA and ANA rat lines, pharmacological treatment with the anabolic steroid Nandrolone Decanoate and Benzyl Alcohol and the correlational studies on corticosterone and alcohol-mediated testosterone changes in AA, ANA and Wistar rats.

Stress-related alcohol drinking and its relation to the HPA-axis has abundantly been investigated previously. Future studies should be undertaken to investigate also the combined HPA-HPG-axes and whether the present results also applies to stress and human alcohol drinking.

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

I. The F2 study replicated the original line differences of AA and ANA rats; higher endogenous testosterone levels were detected on high consumption group and higher frequency of alcohol-induced testosterone decreases and lower endogenous testosterone levels were found in low alcohol consumption group compared to high consumption group. The present data suggests there could be connection between elevated testosterone levels and increased alcohol drinking, as well as between alcohol induced testosterone level reduction and decreased alcohol drinking.

II. Chronic pure ND treatment reduces voluntary alcohol drinking in the alcohol-preferring AA rat line. ND treatment led to a significant reduction of alcohol-mediated testosterone increases, as well as to reduced voluntary alcohol drinking. No significant alcohol-mediated ND effects were observed for corticosterone.

III. The results of the BA experiment suggest that the BA has an increasing effect on voluntary alcohol intake, the effect which was primarily observed in the low-drinking Wistar rats. There was no significant BA effects detected in corticosterone or testosterone levels. The higher frequencies of alcohol-mediated testosterone elevations in the high-drinking AA compared with low-drinking Wistar rats were also detected and this could support our previous hypotheses about the role of testosterone in promoting alcohol drinking.

IV. The data suggest a role of corticosteroids in the regulation of alcohol-mediated testosterone elevation, which may promote voluntary high alcohol drinking in AA rats. However, in addition, the negative correlation between basal corticosteroid levels and testosterone reduction may cause disinforcement (aversion), reducing alcohol intake in ANA and Wistar rats. Why the activated HPA-axis seems to be associated to both testosterone elevation and attenuation is not known. This contradiction is an important target for future studies.

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