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STUDIES OF ACAMPROSATE FOR THE TREATMENT OF ALCOHOL DEPENDENCE

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Cover: *Det går lika bra med saft (Eng: It goes equally well with juice)*. Drawing by Ingrid Hammarberg.

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"Alcohol cue- and priming-induced craving"

Me: – Can you describe a typical situation when you experience strong alcohol craving?
Patient: – It's more a time-point than a situation actually.
Me: – Would you elaborate on that?
Patient: – Well, it's on Friday afternoons that I feel the strongest craving. I feel like if I had a war going on inside of me then. One side wants to drink and the other says no.
Me: – Could you describe an occasion when the "don't drink" side wins?
Patient: Well, first of all, if the clock passes six p.m., the battle is won for me – the craving just disappears.
Me: – How can that be, do you think?
Patient: – It's because all the liquor stores close then. The craving can be ever so strong the minutes before, but as soon as the clock turns six, everything is cool… So I spend much of my Friday afternoons nowadays just watching my clock. But I have also tried another strategy.
Me: – What is that?
Patient: – I sometimes think to myself, that if I drink a small glass of wine – just a small glass – I will be able to stop with that. But as soon as I've had the first sip, it's very difficult for me to stop… And then I usually continue for several days or maybe weeks. So, it is mostly about Friday afternoons actually, my difficulties.

– Conversation with a female patient during a relapse prevention session in thesis study I (2005) (author's translation).

ABSTRACT

Alcohol dependence is a widespread psychiatric disorder with a prevalence of 4-6% in the adult population in western countries. Acamprosate (Calcium acetyl homotaurinate) is approved in many countries as a medication for the treatment of alcohol dependence. It is assumed that acamprosate modulates glutamate neurotransmission within the central nervous system (CNS). However, there are still uncertainties concerning some aspects of acamprosate treatment. The aim of this thesis was therefore to investigate the effect of acamprosate on certain correlates of alcohol dependence.

In one experiment, 56 patients were treated for 21 days with acamprosate vs. placebo. A test battery was then administered by which subjective and physiological responses were measured following presentation of alcohol related stimuli (cue-induced craving) and/or following consumption of a small amount of alcohol (priming-induced craving) in alcohol dependent patients. The results showed that acamprosate attenuated priming-induced craving. Furthermore, acamprosate reduced the priming-induced elevation in plasma cortisol levels. There was also a negative correlation between acamprosate plasma levels and alcohol craving following a priming drink. No effect of acamprosate on cue-induced craving was observed. General craving responses and the correlation to alcohol consumption were also examined, together with neuroendocrine responses. The results showed that acamprosate attenuated general craving responses, and a strong correlation was found between craving and alcohol consumption. No treatment effects on neuroendocrine responses were found.

A newly developed liquid chromatography – mass spectrometry (LC – MS) method was used to evaluate the pharmacokinetics of acamprosate in humans. The aim was to verify earlier pharmacokinetic data in addition to investigate the presence of acamprosate in the CNS. In a 21 days open label study, 13 healthy subjects provided plasma samples regularly and on the last day of treatment also a sample of cerebrospinal fluid (CSF). The results showed that steady state plasma levels of acamprosate was achieved within 5 days following start of treatment and remained above level of quantification for 3 days following termination of treatment. Acamprosate levels in the CSF were between 9–33 ng/mL, which is suggested to represent a pharmacologically relevant concentration.

The efficacy of combining acamprosate with psychosocial intervention was investigated in alcohol dependent patients, using an extended psychosocial intervention (EPI) compared to a minimal psychosocial intervention (MPI) for 24 weeks in addition to acamprosate treatment. The results showed no differences between MPI and EPI on any drinking related measure.

In conclusion, a potential mechanism by which acamprosate mediates its therapeutic effect may be by attenuating the urge to drink following an alcohol slip, and this effect may be dose-dependent. In addition, it was found that acamprosate modulates the hypothalamic pituitary adrenocortical (HPA) axis response and crosses the blood-brain barrier in humans. Finally, the results suggest that the addition of an intensive psychosocial treatment to concomitant acamprosate treatment does not add to treatment efficacy.

LIST OF PUBLICATIONS

I. Hammarberg A*, Jayaram-Lindström N, Beck O, Franck J, Reid MS. The Effects of Acamprosate on Alcohol-cue Reactivity and Alcohol Priming in Dependent Patients: A Randomized Controlled Trial. *Psychopharmacology (Berl)* 2009, 205(1):53-62.

II. Hammarberg A*, Nylander I, Zhou Q, Jayaram-Lindström N, Reid MS, Franck J. The Effect of Acamprosate on Alcohol Craving and Correlation with Hypothalamic Pituitary Adrenal (HPA) Axis Hormones and Beta-endorphin. *Submitted*

III. Hammarberg A*, Beck O, Eksborg S, Jayaram-Lindström N, Lindefeldt A, Andersson M, Brundin L, Reid MS, Franck J. Acamprosate Concentrations in Plasma and CSF after Multiple Dosing Measured by LC – MS: A Pharmacokinetic Study in Healthy Volunteers. *Submitted*

IV. Hammarberg A*, Wennberg P, Beck O, Franck J. A Comparison of Two Intensities of Psychosocial Intervention for Alcohol Dependent Patients Treated with Acamprosate. *Alcohol and Alcoholism* 2004, 39(3), 251-255.

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CONTENTS

LIST OF ABBREVIATIONS

5HT	5 hydroxytryptamine
iAMPAR	α-Amino-3-hydroxy-5-methylisoxazole-4-proprionic acid receptor
Ach	Acetylcholine
A.D.	Anno Domini
a.m.	Ante Meridiem
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ACTH	Adrenocorticotropic hormone
AUC	Area Under the Curve
AUDIT	Alcohol Use Disorder Identification Test
BAES	Biphasic Alcohol Effect Scale
B.C.	Before Christ
BID	Bis In Diem
BP	Blood Pressure
CAD	Cumulative Abstinence Duration
CBT	Cognitive Behavioral Therapy
CDT	Carbon Deficiency Transferrin
CIWA-Ar	Clinical Institute Withdrawal Assessment for Alcohol
CM	Contingency Management
CRA	Community Reinforcement Approach
CRF	Corticotropin releasing factor
CSF	Cerebrospinal fluid
DSM-IV	Diagnostic and statistical manual of mental disorders (4^{th} edition)
e.g.	Exempli gratia (for example)
EPI	Extended Psychosocial Intervention
FDA	Federal Drug Agency
fMRI	functional Magnetic Resonance Imaging
GABA	Gamma-aminobutyric Acid
GC – MS	Gas cromatography – mass spectrometry
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GLP	Good Laboratory Practice
GSR	Galvanic Skin Response
HPA	Hypothalamic Pituitary Adrenal
HPLC	High Performance Liquid Chromatography
HR	Heart Rate
i.e.	Id est (that is)
iGluRs	ionotropic glutamate receptors
iNMDAR	ionotropic N-methyl D-aspartate receptor
ITT	Intention To Treat
LC – MS	Liquid chromatography – mass spectrometry
LTP	Long Term Potentiation
mGluRs	metabotropic glutamate receptors
MI	Motivational Interviewing
MPA	Medical Products Agency

MPI	Minimal Psychosocial Intervention
NAcc	Nucleus Accumbens
NNT	Numbers Needed to Treat
OCDS	Obsessive Compulsive Drinking Scale
PACS	Penn Alcohol Craving Scale
PANAS	Positive And Negative Affect Scale
PET	Positron Emission Tomography
p.m.	Post meridiem
RCT	Ramdomized Clinical Trial
SD	Standard Deviation
SEK	Swedish krona
Short-DAQ	Shortened version of the Desire for Alcohol Questionnare
SSRI	Selective serotonin reuptake inhibitor
SCL-90	Symptoms Check List – 90
SEM	Standard Error of the Mean
Temp	Skin temperature
TID	Ter In Diem
TLFB	Timeline follow-back
VAS	Visual Analog Scale
VTA	Venteal tegmental area

Foreword

In 1997, I began my basic training to become a licensed psychotherapist. During this time, I came to work with long-term treatment for substance dependent individuals with concurrent social, economical and often psychiatric problems in a treatment facility within the social services in one of the municipalities of Stockholm. One of my first – and with hindsight naïve – question to the treatment staff I was working with was: "Do you use any theories concerning treatment as a framework for your work with the patients?" The question was met with great surprise, and the answer: "We take the best parts from each theory and kind of blend them". I was surprised with the total lack of cost-benefit analyses of the often very expensive treatments provided. The lack of critical evaluation of treatments for substance dependence was widespread at that time-point.

Since then, there has been what I consider to be a substantial development within the field of treatment for substance dependence. Systematic knowledge has been gathered about which treatments are efficacious (SBU 2001). Evidence based treatments are increasingly requested, not least by politicians who wants to make sure that tax payers' money is used with care, and by patients themselves – anxious to receive the best available treatment.

However, there is still a widespread skepticism among treatment providers concerning well documented treatment methods. For example, despite the overwhelming support for methadone and/or buprenorphine as pharmacological treatments for opiate dependence, a recent national survey among Swedish social welfare secretaries (who administer a large part of the funding for treatment) showed that a majority still advocate psychosocial treatment as first line treatment (unpublished data). Further, a poll established that unspecified counseling is the most common psychosocial treatment for alcohol dependence in Sweden, despite a broad arsenal of evidence based psychosocial treatments available.

During lectures for different groups of staff working in this field, I have received several examples of a moralistically colored view on individuals suffering from dependence disorders, a view that is also reflected in a resistance against evidence based psychosocial and pharmacological treatments. Two examples of this are the following citations: "I think it is only a lack of will if you cannot resist the urge to drink. Alcoholics do not dare to stop – They are afraid!" (Anonymous 2007, author's translation), and "It must be painful to stop drinking. That is how you learn to stop. That's why I don't like chemical solutions to alcohol problems" (Anonymous 2009, author's translation). It is hard to imagine a patient suffering from e.g. hypertension or diabetes who would accept to be treated with methods not proven efficacious, not due to lack of availability, but because the treatment staff do not wish to provide them.

It is my hope that the work presented within the framework of this thesis will contribute to establish evidence based psychosocial and pharmacological treatments as something naturally provided for individuals suffering from alcohol dependence.

1 INTRODUCTION

1.1 OVERVIEW OF THE THESIS

Acamprosate (Calcium acetyl homotaurinate) has been used for the treatment of alcohol dependence since the mid 1980s (Lhuintre, Daoust et al. 1985), and was approved by Medical Products Agency (MPA) in Sweden in 1996. Although still utilized to a very small degree in the treatment of alcohol dependence (not published data from the Swedish National Board of Health and Welfare, 2007), acamprosate represents a new generation of treatment alternatives by targeting neurobiological consequences induced by long-term alcohol consumption.

The efficacy of acamprosate in reducing alcohol consumption has been evaluated in more than 20 clinical trials (Mann, Lehert et al. 2004). However, relatively few studies have aimed at illuminating the potential mechanisms behind acamprosate action in humans, e.g. if factors known to maintain alcohol dependence are modulated by acamprosate treatment.

In study I of this thesis, a laboratory based approach was applied to study if acamprosate modulates craving responses induced by sensory stimuli known to be related to alcohol consumption (cue-induced craving) and/or consumption of a small amount of alcohol (alcohol priming-induced craving) in alcohol dependent patients.

It is assumed that craving may predispose a relapse to heavy drinking in dependent patients (Drummond 2001). In study II, it was examined if a short-term treatment with acamprosate modulates general craving responses and the correlation to alcohol consumption. In addition, the neuroendocrine responses to acamprosate treatment were investigated.

Few studies have examined the pharmacokinetic properties of acamprosate in humans, and the methods used may not have been optimized specifically for acamprosate. In study III, liquid cromatography – mass spectrometry (LC – MS) was used to verify previous results on acamprosate pharmacokinetics in humans. In addition to this, the presence of acamprosate in cerebrospinal fluid (CSF) was investigated.

The question whether the addition of a psychosocial intervention improves the efficacy of acamprosate treatment has been debated and previous research show inconclusive results. In study IV, the effect on alcohol consumption of two intensities of psychosocial interventions (minimal psychosocial intervention (MPI) vs. extended psychosocial intervention (EPI)), in combination with acamprosate, was evaluated.

1.2 BACKGROUND

1.2.1 Historical Perspective on the Use of Alcohol

The alcohol contained in alcoholic beverages is called ethanol (C_2H_5OH) (Ethanol is hereafter referred to as alcohol). Alcohol is a psychoactive substance commonly used worldwide. It is estimated that 90% of the adult population in western countries consumes alcohol with some regularity (WHO 2008). Alcoholic beverages were produced in Mesopotamia as long as 10.000 years ago (Rudgley 1994), probably originally in the form of a mildly alcoholic thick brew of beer consisting of ungerminated grains and a small quantity of natural sugars. Wine making was probably invented in what is present day Armenia at some point between 6000 and 4000 B.C. (Patrick 1952; Courtwright 2001). The oldest evidence of a distillation process to

hard liquor comes from Babylonia (approximately 2nd millennium B.C.) and the technique was brought to Europe in the 11th century A.D. In Nordic countries, artifacts related to alcoholic beverages have been found in Iron Age graves (1st century A.D.) (Rudgley 1994). Already in the earliest of human writings, the significance of alcoholic beverages is clear. For example, wine drinking is mentioned more than 200 times in the Bible and in the epic Greek poems of Homer – the Iliad and the Odyssey – wine drinking fulfils many symbolic and ritual functions.

Historically, most societies have restricted alcohol consumption in order to reduce severe consequences (1998). Both informal and legal means have been mobilized for this purpose. The former may be social pressure regulating which alcohol related behaviors that are, and which are not, sanctioned in a specific context. For example, drinking alone is widely considered not to be an appropriate behavior. The latter would be governmental legislations used in order to force down alcohol consumption or its undesirable consequences. For example, the 18th amendment to the American constitution (the Volstead act) was passed in 1919 as a response to increased alcohol consumption. Ten years of prohibition followed in which federal prohibition agents worked to enforce the law. Governmental regulations in the alcohol field often face difficulties. Reflecting this was that many people reacted to prohibition by producing alcohol themselves or by smuggling it. Increased crime and violence was often the result (Kobler 1973; Everest 1978). Another example of governmental regulation was when British pubs in 1916 were enforced to close between 11 p.m. and 11 a.m. The reason was to try to have munitions workers drink less alcohol and as a secondary effect work harder during the First World War.

In Sweden, the widespread alcohol abuse and its negative consequences were increasingly recognized during the latter part of the 19th century. New legislations were implemented in which production, distribution and consumption were put under stricter control (Edman 2004). Other forces were also striving for a reduction in alcohol consumption. The temperance movement grew stronger during this time and also within the labor movement, temperance was advocated. Other important initiatives were that the first legislations concerning treatment of alcohol dependence were enforced during this time (SFS 1841; SFS 1913), and several treatment facilities for dependent patients emerged (Prestjan 2007). Further, between 1914 and 1955, Sweden practiced a system with a ration book for each adult citizen (the Bratt system, named after its inventor Ivan Bratt), which limited the amount of alcohol that was possible to buy. There was a continuous decline in consumption during the first half of the 20th century. After this, the consumption has gradually increased again. The yearly consumption in 2007 was approximately 10 liters of pure alcohol per capita, corresponding to approximately 100 bottles of wine (75cl) per year (CAN 2008). The present consumption of alcohol beverages in Sweden matches the level a century ago.

1.2.2 Consequences of Alcohol Consumption

Consequences from alcohol consumption are vast and difficult to evaluate. From a global perspective, close to 4% of the disease burden is attributed to the consequences of alcohol consumption (WHO 2008). The most common consequences of alcohol consumption are liver cirrhosis, neuropsychiatric diseases, cancer, cardiovascular diseases, intentional and non-intentional injuries. In Sweden, approximately 3000 – 4000 individuals are estimated to decease every year in alcohol related injuries, accidents, or diseases (Jarl, Johansson et al. 2006). Alcohol is also highly involved in many cases in emergency wards, primary health care and psychiatric care, adding up in huge costs in the form of e.g. somatic and psychiatric

treatments, loss of production, sick pay and social insurances. Although moderate alcohol consumption probably has beneficial health consequences, a recent study estimated the annual net cost of alcohol in Sweden to 20.9 billion SEK (Jarl, Johansson et al. 2006).

1.2.3 Mechanism of Action

1.2.3.1 Metabolism

Alcohol is a small molecule almost solely metabolized in the liver. Alcohol is first oxidized to acetaldehyde via the enzyme alcohol dehydrogenase. In the next step, the enzyme acetaldehyde dehydrogenase oxidizes acetaldehyde into acetic acid. The end product is then carbon oxide and water. Alcohol metabolism follows zero-order kinetics which means that the capacity of the liver to eliminate alcohol reaches its maximum fast. The half life of alcohol in the body is then dependent on the amount consumed. Approximately 0.1 gram alcohol per kilo bodyweight and hour is metabolized by the liver, leaving the remaining alcohol to continue to circulate throughout the body.

1.2.3.2 Alcohol and Interaction with Neurotransmitters

Alcohol is highly soluble in both water and lipid and readily crosses the blood-brain barrier into the central nervous system (CNS) within minutes following alcohol intake. There is no specific receptor for the alcohol molecule. Instead, alcohol interacts with almost all neural circuits in the CNS, making it difficult to dissect the precise neurobiological basis for its various psychoactive effects. Alcohol interactions with all these systems together define the full profile of short- and long-term consequences of alcohol consumption (Harris 1999; Clapp, Bhave et al. 2008). Below, some of the interactions between alcohol and relevant neurotransmitter systems are described (See also Figure 1).

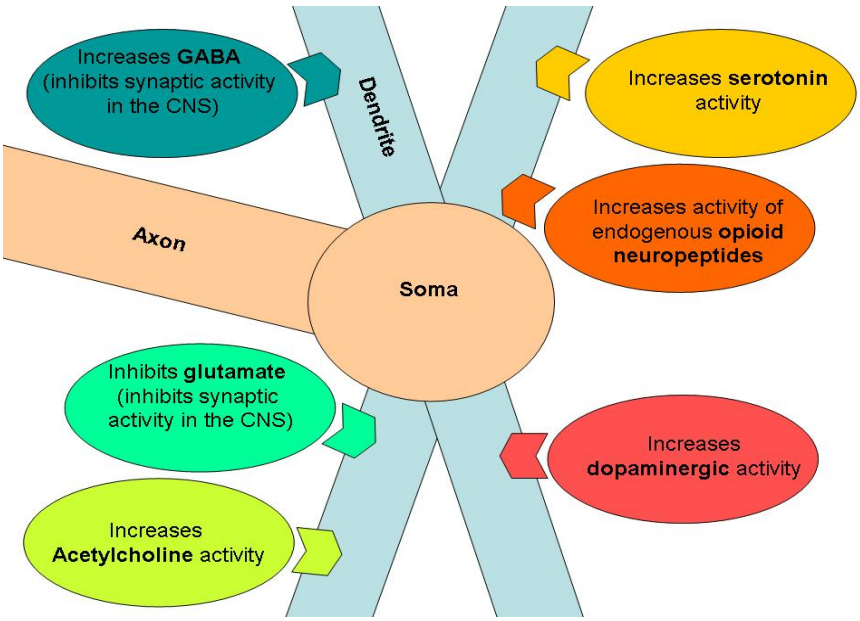

Figure 1. Schematic overview of some of the acute effects of alcohol on relevant neurotransmitters in the CNS.

1.2.3.2.1 Dopamine System

Dopamine has important functions in the brain, including cognition, voluntary movement, motivation and reward. One important dopaminergic neurocircuit is the mesolimbic dopamine system, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) with further projections to the limbic system and the orbitofrontal cortex (Koob 2000). This system is believed to be critically involved in the acquisition and maintenance of behaviors. The dopamine system is activated both in response to natural rewards (food, water, sexual behavior) as well as to addictive drugs, and is sometimes referred to as the brain reward circuitry. It has been shown that alcohol consumption stimulates dopamine release in various species (Bradberry 2002). Further, it has been suggested that the dopamine system may be critical in developing and maintaining alcohol dependence (Gonzales, Job et al. 2004; Di Chiara and Bassareo 2007).

1.2.3.2.2 Glutamate System

Glutamate is the major excitatory neurotransmitter in the CNS mediating approximately 70% of synaptic transmission. Glutamate projections are present in numerous circuitries in CNS, e.g. the mesolimbic dopamine system (Omelchenko and Sesack 2007; Geisler and Wise 2008), hippocampus and frontal cortex (Gass and Olive 2008). Alcohol inhibits glutamate action on subtypes of glutamate receptors which both in vitro (Kumari and Ticku 2000) and in vivo for different brain regions, such as the hippocampus (Simson, Criswell et al. 1993), cerebral cortex, NAcc, amygdala and VTA (Hoffman 2003) (for a more detailed discussion on the glutamate system, see section 1.5.1.1).

1.2.3.2.3 Gamma-aminobutyric Acid (GABA) System

GABA is another widespread neurotransmitter in the brain, and has an inhibitory effect on synapses. Alcohol facilitates GABA-ergic transmission, mainly through a potentiation of $GABA_A$ receptor function (Davies 2003), hereby decreasing brain activity. Most probable, this potentiation of $GABA_A$ receptors produces the anxiolytic and sedative effects of alcohol (Johnston 2005).

1.2.3.2.4 Opioid System

The endogenous opioid system is involved in pain and emotion regulation and also modulates the activity of the mesolimbic dopamine system (Oswald and Wand 2004). The opioid peptides β-endorphin, enkephalin and dynorphin are ligands with different affinities to the opioid receptors mu (μ), delta (δ) and kappa (κ) which are present both in the CNS and in the peripheral nervous system. Alcohol increases the release of endorphins and enkephalins in different brain regions, including the VTA (Marinelli, Quirion et al. 2004; Marinelli, Bai et al. 2005), an action probably responsible for some of the reinforcing effects of alcohol.

1.2.3.2.5 Serotonin System

The brain serotonin system is involved in several vital functions like attention, motivation, and emotions. An increased level of serotonin has been observed following alcohol administration in rats (Yan 1999) and in humans (Lovinger 1997). It has been proposed that the serotonin system may be related to the reinforcing effects of alcohol through connection with the mesolimbic dopamine system (Ciccocioppo 1999).

1.2.3.2.6 Nicotinergic System

The cholinergic system has important functions, both in the peripheral nervous system and within the CNS. For example, the neurotransmitter acetylcholine (Ach) is a major neurotransmitter in the autonomic nervous system. It has been shown that alcohol can activate the brain reward system through nicotinic ACh receptors. For example, studies in rats have shown that ACh levels in VTA and dopamine levels in the NAcc are increased with alcohol consumption (Larsson, Edstrom et al. 2005). The ACh system has been suggested to be involved in maintaining addictive behavior e.g. by modulating dopamine release following presentation of alcohol cues (Lof, Olausson et al. 2007).

1.2.3.2.7 The Hypothalamic Pituitary Adrenal (HPA) Axis

In addition to the effect on central neurotransmitters, alcohol also exerts its effects via the HPA axis. Following a physiological and/or psychosocial stressor, an HPA axis response is initiated in order to adapt the body to a changed environment. Corticotropin releasing factor (CRF) is released from the paraventrical nucleus of hypothalamus into blood vessels with afferents to the anterior pituitary gland where it binds to CRF_1 receptors. This triggers a release of adrenocorticotropic hormone (ACTH). ACTH thereby stimulates cortisol release from the adrenal gland (Turnbull and Rivier 1997; Koob 2008). Cortisol increases blood pressure and blood sugar, and reduces immune responses. Acute alcohol consumption increases activity in HPA axis hormones (Rivier and Lee 1996) while a chronic alcohol consumption results in a dysregulated stress system (Adinoff, Junghanns et al. 2005; Adinoff, Krebaum et al. 2005).

In thesis studies I – III, the effect of acamprosate treatment on HPA axis hormones was investigated.

1.3 ALCOHOL DEPENDENCE

1.3.1 Clinical Features

Modern classification systems clearly differentiate between use, abuse and dependence of alcohol. Alcohol use refers to consumption of alcoholic beverages for non-medical purposes, e.g. a glass of wine for a Friday dinner. The most common reported reason for alcohol use is recreational, for example relaxation, mood-enhancement and increased sociability (Baum-Baicker 1985; Peele and Brodsky 2000).

According to the fourth edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (APA 2004), alcohol abuse refers to repeated adverse social and legal consequences caused by the use of alcohol, e.g. failure to manage work, school or family related functions or driving under the influence of alcohol. The distinction between alcohol abuse and dependence has been debated over the years. According to DSM-IV, alcohol dependence is defined in terms of physiological, behavioral and psychosocial symptoms in contrast to the more pronounced social and legal oriented criteria of substance abuse. Further, people suffering from alcohol abuse may be able to return to a non-problematic alcohol use, while alcohol dependence is generally considered to be a persistent state.

In Table 1, the seven criteria for alcohol dependence according to DSM-IV are displayed, that together summarize a state in which alcohol has become a dominating element in an

Table 1. DSM IV criteria for alcohol dependence.

A maladaptive pattern of alcohol use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period.
(1) Tolerance, as defined by either of the following:
(a) A need for markedly increased amounts of alcohol to achieve intoxication or desired effect.
(b) Markedly diminished effect with continued use of the same amount of alcohol.
(2) Withdrawal, as manifested by either of the following:
(a) The characteristic withdrawal syndrome for alcohol
(b) Alcohol (or a closely related substance) is taken to relieve or avoid withdrawal symptoms
(3) Alcohol is often taken in larger amounts or over a longer period than was intended
(4) There is a persistent desire or unsuccessful efforts to cut down or control alcohol use
(5) A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects
(6) Important social, occupational, or recreational activities are given up or reduced because of alcohol
(7) Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol

individual's life. Historically, there has been a distinction between physical and psychological dependence, which is also reflected in the DSM-IV criteria. The first two criteria can be seen as expressions of a physical dependence and are essentially an effect of the chronic exposure of alcohol to the brain and body.

The third criterion is often referred to as "loss of control" over alcohol use, and may be exemplified with an individual consuming a small dose of alcohol (i.e. a priming dose) which eventually leads to a heavy drinking episode. The fourth criterion may be exemplified with an individual who despite several treatment efforts or own attempts to refrain from alcohol, still fails to achieve abstinence. Criteria three and four may be considered as clinical reflections of the concept of "craving", with a hard to resist urge to consume alcohol as a dominant feature. Criterion 5 and 6 refers to different social and relational consequences of alcohol consumption, e.g. failure to maintain family or work related commitments. The last criterion may be illustrated by an individual who continues to consume alcohol despite liver cirrhosis or some other major somatic or psychiatric condition.

The basic assumption of the studies within this thesis concerning alcohol dependence is a model in which a transition from recreational to compulsive use occurs through alcohol-induced changes in the brain. Long-term exposure to alcohol persistently alters the activity in the above described neurotransmitter systems, resulting in the physiological and psychosocial symptoms captured in DSM-IV.

A model that describes such a transition suggests that in addition to an initially positive reinforcing effect of a drug, an opposing negative force is initiated. The underlying goal of this reaction is to maintain homeostasis within brain systems. However, the opposing process persists even after the initially positive effect has diminished, with the difference that it is now unopposed. The consequence is that the affective baseline is shifted over time, resulting in an escalating aversive set point, a phenomenon called allostasis (Koob and Le Moal 2008). The opponent process can be either "within" or "between" neural systems. The former refers to changes within the neurocircuits responsible for the rewarding effects of alcohol, e.g. the mesolimbic dopamine system. The latter phenomenon refers to e.g. recruitment of the stress system, such as increased CRF levels following alcohol consumption (Koob and Le Moal 2005).

The progressed state of allostasis is clearly reflected in the DSM-IV criteria for alcohol dependence. For example, the first criterion refers to a progressively diminishing response to alcohol, i.e. a change of set-point. The acute alcohol withdrawal state due to a hyper-active glutamate system, captured in criterion 2, is another example (see section 1.5.1.1.1). Consequences of allostasis in the protracted withdrawal state can be reflected in conditioned responses as increased susceptibility to cues that elicit craving responses, making it difficult to avoid relapses (criteria 3 and 4).

1.3.2 Prevalence of Alcohol Dependence

The prevalence of alcohol dependence has been estimated to 4-6% of the adult population in western countries (Grant 1997), a figure which corresponds to recent Swedish studies (Andreasson 2002). The lifetime prevalence of alcohol dependence is estimated to be around 15% (Ojesjo, Hagnell et al. 1982; Ojesjo, Hagnell et al. 2000) while the percentage of individuals in Sweden with risky alcohol consumption in 2005 (as measured by those who score above cut-off level for hazardous drinking in alcohol use disorder identification test (AUDIT)) was 14% (Kallmen, Wennberg et al. 2007). In sum, alcohol abuse and dependence may be regarded as a major public health problem with approximately twice the prevalence of that of major depression or anxiety disorders (Hasin, Goodwin et al. 2005).

1.4 TREATMENT OF ALCOHOL DEPENDENCE

1.4.1 Brief Historical Background

Treatments for alcohol dependence have differed depending on a number of factors, e.g. by historical time point, by country or region and by the view embodied by the treatment provider on the etiology of alcohol dependence. A comprehensive historical review of the field is beyond the scope of this thesis, and below follows a brief overview from a Swedish viewpoint.

Sweden was early out concerning diagnostics and treatment for alcohol dependence. In the latter half of the 19th century, a small body of idealistically dedicated physicians took some important initiatives for the treatment of the widespread alcohol dependence disorder. In his thesis "Alcoholismus Chronicus – ett Bidrag till Dyskrasiernas Kännedom: Enligt Egen och Andras Erfarenhet" (Eng: "Alcoholismus Chronicus, or Chronic Alcohol Illness. A Contribution to the Study of Dyscrasias Based on my Personal Experience and the Experience of Others"), Magnus Huss made the first major contribution to a careful

diagnosis of alcohol dependence (Huss 1849). His work also proved as inspiration for practitioners to begin to work with this patient group. These early initiatives comprised mainly voluntary treatments in institutions or hospitals for middle class, male individuals. Nursing care, outdoor activities, relaxation and abstinence requirement were the core treatment components (Prestjan 2007).

With the state-controlled institutional care that developed from the 1910s and onward, alcohol dependence treatment drastically changed character. Alcohol dependence was no longer viewed as a medical problem but instead as a social and moral one. In addition, the alcohol dependence diagnosis was reserved for the working class (Edman 2004; Edman and Hamran 2007). Alcohol dependence was henceforth treated with discipline and rationing. Individual needs were subordinate to the goal of rehabilitating the individual back to society by educative measures, hard labor and coercive measures.

The more medically oriented view of alcohol dependence was for a long time hampered by the lack of a medication that targeted clinically relevant symptoms. With the invention of disulfiram (Antabuse) in the 1950s, the situation changed. Since disulfiram interfere with alcohol metabolism (see section 1.4.4.2.1), the actual alcohol consumption could be challenged for the first time. Disulfiram fitted well in to the general treatment ideology which advocated total abstinence.

The next major change within the field occurred by the end of the 1970s when the 12-step treatment made a major breakthrough in Sweden. The method was founded in the US in the 1930s (Alcoholics-Anonymous 2001), and have for a long time been the most widespread treatment alternative in Sweden. Patients were most often referred to a rehabilitation clinic for four weeks or longer, after which followed one year of aftercare. Today, 12-step treatments are most often provided as non-institutional care.

Since the beginning of the 1990s, new treatment alternatives have been developed both concerning psychosocial and pharmacological treatments (Miller and Hester 1989; Heilig and Egli 2006), although the possibility for alcohol dependence patients to have access to these treatments still differs considerably (2008).

Treatment infrastructure is multifaceted. Specialized treatments for alcohol related conditions are provided by the state (e.g. treatments of overdose or withdrawal symptoms), while municipalities fund and provide much of the long-term treatments. In addition, there is a large sector of foundations, self-help groups and private companies that offer treatment for alcohol dependence.

1.4.2 Treatment of Alcohol Overdose

Initial symptoms of alcohol overdose are usually nausea and vomiting, followed by inability to stand, absent reflexes, difficulties in awakening, slow and shallow or irregular breathing and pale skin with ascending levels of blood alcohol. Alcohol overdose may be a life threatening condition due to respiratory depression. Careful monitoring of oxygen saturation and vital signs is necessary in treating alcohol overdose. Further, patients may need intravenous hydration and/or respiratory support with mechanical ventilation in order to prevent respiratory depression.

1.4.3 Treatment of Alcohol Withdrawal

Abrupt termination of extensive and prolonged alcohol consumption regularly leads to symptoms ranging from mild tremors, sweating and anxiety, to severe convulsions and delirium tremens which are life-threatening conditions (Hall and Zador 1997; Becker 2008). Based on clinical symptoms, consumption patterns and history of previous withdrawal episodes, the need for alcohol withdrawal treatment is assessed. Severe symptoms often require pharmacological treatment in order to reduce the risk for severe brain damage or death. Recommended treatment for alcohol withdrawal is benzodiazepines (Becker 2008). Thiamine treatment for the Wernicke-Korsakoff syndrome may also be warranted. Treatment of alcohol withdrawal can relieve a patient's discomfort in an acute phase, reducing the risk of relapse into renewed alcohol consumption. Alcohol withdrawal treatment may also offer an opportunity to involve a patient in long-term treatment for alcohol dependence.

1.4.4 Long Term Treatment of Alcohol Dependence

The goal of long term treatment for alcohol dependence is debated, and different views are often expressed. Proponents for some treatment directions advocate total sobriety, while others support a view of reduced alcohol consumption as the primary treatment goal. As described in an earlier section, alcohol dependence is a chronic relapsing disorder, among others characterized by obsessive heavy drinking. Most treatment orientations with this definition as a starting point advocate relapse prevention and/or reduction of heavy drinking episodes as main therapeutic objectives.

Treatments may be divided into psychosocial and pharmacological alternatives.

1.4.4.1 Psychosocial Treatments of Alcohol Dependence

Efficacious psychosocial treatments seem to have some common ingredients (Berglund, Thelander et al. 2003). Focus of treatment should be on modifying maladaptive thoughts and behaviors related to alcohol (treating assumed anticipatory reasons for drinking has not proven efficacious for alcohol dependence symptoms). Treatments should ideally be guided by some sort of guidelines or manual which enhances compliance in execution of practice by the therapist. In addition to this, treatments should be easily accessible, be executed in a non-confrontational style and be provided for a sufficiently long time period to have an effect. Regardless of these common factors, psychosocial approaches vary considerably e.g. in the length of time, intensity and setting (group or individually oriented).

Motivational Interviewing (MI) is based on cognitive and social psychological principles, with the aim to evoke patients' own motivation for change, and to consolidate a personal decision and plan for change (DiClemente, Bellino et al. 1999). MI has proved to be efficacious for the treatment of alcohol dependence in several trials (1997).

Cognitive Behavioral Therapy (CBT) is often executed within the framework of an individually or group centered relapse prevention course. Among other defining principles, much effort is placed on identifying positive and negative reinforcers of alcohol dependence and to replace these with non-alcohol related alternatives. CBT is a well documented psychosocial treatment method for alcohol dependence (1997; Anton, O'Malley et al. 2006).

Contingency management (CM) imply attenuating alcohol consumption by providing tangible reinforcers for abstinence in the form of vouchers for goods or services (e.g. a movie ticket)

(Higgins and Petry 1999). CM has shown to be efficacious for the treatment of stimulant abuse, while research has been scant in the alcohol field. However, existing studies show that CM reduces alcohol consumption in dependent patients, indicating that CM may be a promising treatment alternative in the future (Miller 1975; Petry, Martin et al. 2000).

Community Reinforcement Approach (CRA) is an approach that combines elements of CBT and MI and also recruit the patients' family and network (Smith, Meyers et al. 2001). The goal of CRA is to rearrange as many as possible aspects of an individual's environment or "community" so that a drug-free life-style is more rewarding than one dominated by alcohol (Hunt and Azrin 1973). Although CRA has proven to be efficacious in several trials (Smith, Meyers et al. 1998), the method is hitherto offered to a limited extent in Sweden.

12-step treatment originates from the Alcoholic Anonymous movement and lacks an explicit theoretical framework. Treatment is based on the assumption that a patient needs to reach a state of insight in how the consequences of alcohol dependence have affected oneself and others (Alcoholics-Anonymous 2001). Alcohol related thoughts and behaviors needs to be replaced. Sobriety is most often required and a spiritual dimension is an important element of the treatment. 12-step treatment has proven efficacious in several trials (1997; Winters, Stinchfield et al. 2000).

Brief interventions for alcohol dependence have shown that substantial improvements in alcohol related symptoms can be achieved with relatively small efforts (Heather 1995; Moyer, Finney et al. 2002). Early studies showed that initial screening and a few brief counseling sessions decreased alcohol consumption persistently (Edwards, Orford et al. 1977; Chick, Ritson et al. 1988). There is a great variability in definitions of brief interventions in the literature (Heather 1995; Moyer, Finney et al. 2002). One defining property of brief intervention is its length. One single intervention session has been termed as "minimal intervention", up to three sessions as a "brief intervention", five to seven sessions as "moderate" and eight or more sessions as "intensive" or "extended" interventions (Babor 1994; Chick 2004). Interventions may be categorized differently depending on its intensity. For example, if an intervention is provided with low intensity during a long time interval, up to four sessions may be regarded as a minimal intervention (Chick 2004). Other characteristics is whether brief intervention is delivered by health care personnel vs. a specialist in the area of substance dependence (Moyer, Finney et al. 2002). The former being "primary care" brief intervention which is relatively short and less structured, typically given in primary care settings for non-treatment seeking individuals (Wallace, Cutler et al. 1988). The latter type of brief intervention is provided to treatment seeking patients and are usually longer, more structured and delivered by a specialist (Drummond, Thom et al. 1990). A brief intervention is most often performed according to the principles of MI in a non-judgmental and non-confrontational style. The technique attempts to increase a person's awareness of the potential problems caused by alcohol, and the risks faced with a continued consumption. Continuous feed-back and skills training may also be part of the treatment. The efficacy of brief intervention has been evaluated in several studies in different settings, e.g. among university students (Marlatt, Baer et al. 1998; Stahlbrandt, Johnsson et al. 2007), in emergency care settings (Heather 2007) and for drunk drivers (Nilssen 2004; Dauer, Rubio et al. 2006).

In study IV in this thesis, two intensities of psychosocial interventions as concomitant treatment with acamprosate were compared. The first treatment was MPI which consisted of in

all four sessions with a trained physician during 24 weeks. The second treatment was EPI which consisted of 10 – 15 visits to a trained nurse in addition to the visits to the physician.

1.4.4.2 Pharmacological Treatments of Alcohol Dependence

1.4.4.2.1 Disulfiram – A Medication Acting on the Metabolism of Alcohol

For a long time, the only available pharmacological treatment alternative for alcohol dependence was disulfiram which has been used clinically since the late 1940s. The mechanism of action of disulfiram is by inhibiting the degradation of alcohol in the liver by inhibiting acetaldehyde dehydrogenase. Increasing levels of acetaldehyde following alcohol consumption leads to unpleasant symptoms, e.g. flushing, shortness of breath, tachycardia, headache and nausea. Anticipation of these symptoms may prevent the individual from drinking alcohol. In systematic reviews, disulfiram has weak evidence for long term treatment, as compared to placebo (Berglund, Thelander et al. 2003). When administered under supervision, disulfiram is more effective than when no supervision occurs. Taken together with known – although rare – side effects such as depression, hepatotoxicity and psychotic reactions, disulfiram is by some practitioners considered to be an outdated treatment.

Research in the neurobiology of alcohol dependence has led to the development of new pharmacological treatment alternatives for long-term treatment of alcohol dependence (Heilig and Egli 2006; Tambour and Quertemont 2007) among which a few have qualified for phase III clinical trials, i.e. trials in patient populations.

1.4.4.2.2 Medications Acting on GABA-ergic Neurotransmission

The $GABA_B$-receptor agonist baclofen is used for the treatment of muscle spasticity. Baclofen reduces the firing of dopamine neurons (Cott, Carlsson et al. 1976) and reduces alcohol consumption in rats (Daoust, Saligaut et al. 1987). Clinical trials have given preliminary evidence for that baclofen is efficacious in increasing abstinence rates for alcohol dependent patients (Addolorato, Caputo et al. 2002; Flannery, Garbutt et al. 2004; Addolorato, Leggio et al. 2007). An adequately sized randomized clinical trial (RCT) with sufficient duration is needed to evaluate if this promising medication holds as a treatment alternative.

The $GABA_A$-receptor agonist and glutamatergic antagonist topiramate is used for the treatment of epileptic seizures and has also been evaluated as a medication for alcohol dependence. Pre-clinical evidence is scarce concerning the mechanism of topiramate, but a balancing effect between a hyper-glutamatergic and hypo-GABA-ergic state in acute and protracted alcohol withdrawal state has been proposed (Heilig and Egli 2006). Topiramate has shown to reduce heavy drinking for alcohol dependent patients (Johnson, Ait-Daoud et al. 2003; Johnson, Rosenthal et al. 2007). However, the safety and tolerability of topiramate has been debated, especially if compared to the medications in use today (acamprosate and naltrexone, see below). Taken together with the uncertainties concerning the mechanism of action, further research is rendered for topiramate to be a recognized treatment for alcohol dependence.

1.4.4.2.3 Medication Acting on Serotonin Neurotransmission

A number of trials have investigated the effect of selective serotonin reuptake inhibitors (SSRIs) (e.g. fluoxetine, citalopram and sertraline) for alcohol dependent patients. The hypothesis has been that a stabilizing effect on a dysregulated serotonin system in dependent patients may be beneficial in reducing craving and alcohol consumption. However, clinical

trials have been inconclusive and generally generated negative results except for the subgroup of alcohol dependent patients with co-morbid depression (Kranzler, Burleson et al. 1995; Cornelius, Salloum et al. 1997).

Another hypothesis involving the serotonin system has been a dampening of the reinforcing effects of alcohol by blocking $5HT_3$ receptors in VTA by the antagonist ondansetron, leading to suppression of alcohol-induced dopamine activity in NAcc (Johnson 2008). Studies have shown that ondansetron reduces voluntary alcohol intake in rats and mice (Tomkins, Le et al. 1995). In human laboratory studies, ondansetron has shown to decrease alcohol preference and desire to drink in healthy volunteers (Johnson, Campling et al. 1993; Swift, Davidson et al. 1996). A double blind placebo controlled trial showed an effect of ondansetron treatment on alcohol consumption for dependent patients (Johnson, Roache et al. 2000), a result most pronounced for a subgroup of patients with an early onset of alcohol dependence. There are currently ongoing RCT:s further investigating the efficacy of ondansetron for the treatment of alcohol dependence.

1.4.4.2.4 Medication Acting on Nicotinergic Ach Neurotransmission

Nicotinic receptors have been shown to mediate dopaminergic effects in an alcohol cue paradigm for rats (Lof, Olausson et al. 2007). The compound varenicline acts as a partial agonist at the $\alpha_4\beta_2$ nicotinic receptor and a full receptor agonist at the α_7 nicotinic receptors and is approved in Sweden for smoking cessation (Champix). Varenicline reduces alcohol drinking in rodent models (Steensland, Simms et al. 2007). In addition, one clinical trial has shown that varenicline reduces self-administration of alcohol and craving in heavy drinking smokers (McKee, Harrison et al. 2009). Clinical trials are currently underway to explore this promising pharmacotherapeutic agent for alcohol dependent patients.

1.4.4.2.5 Medication Acting on CRF Neurotransmission

The neurotransmitter CRF mediates stress responses and has shown to be involved in alcohol withdrawal responses (Heilig and Egli 2006; Heilig and Koob 2007; Koob 2008). Further, alcohol withdrawal produces anxiety like states with elevated levels of CRF, further emphasizing the connection between alcohol and stress (Zorrilla, Valdez et al. 2001). As could be expected from this correlation, CRF antagonists reduce anxiety and alcohol consumption as shown in rats (Lodge and Lawrence 2003). To date, there have been no clinical trials of CRF antagonists.

1.4.4.2.6 Medication Acting on Opioid Neurotransmission

The endogenous opioid system has been shown to be involved in the reinforcing effects of alcohol. Drugs modulating the activity in this system decreases volitional ethanol intake in rat models (Volpicelli, Davis et al. 1986; Franck, Lindholm et al. 1998; Lindholm, Werme et al. 2001). In numerous clinical trials, the nonselective opioid receptor antagonist naltrexone has proven a robust effect in improving abstinence rates and attenuation of heavy drinking and other alcohol related measures (O'Malley, Jaffe et al. 1992; Volpicelli, Alterman et al. 1992; Anton, O'Malley et al. 2006). Human laboratory studies have shown that naltrexone attenuates cue-induced alcohol craving (Myrick, Anton et al. 2008) and priming-induced alcohol seeking behavior and craving (McCaul, Wand et al. 2000; O'Malley, Krishnan-Sarin et al. 2002; Anton, Drobes et al. 2004; Drobes, Anton et al. 2004) which may be part of the explanation for the efficacy of naltrexone.

Further, the nonselective opioid antagonist nalmefene has proven promising results for the treatment of alcohol dependence and may be a future treatment candidate (Mason, Salvato et al. 1999).

1.5 ACAMPROSATE

For the moment, two treatment alternatives beside disulfiram are approved by the Swedish MPA (as well as the US Food and Drug Administration (FDA)) for the treatment of alcohol dependence. Naltrexone was approved in the US in 1992 and in Sweden in 2000. Acamprosate was approved in Sweden 1996 and in the US in 2007.

1.5.1 Effects of Acamprosate on Neurotransmitter Systems

Acamprosate has a complex pharmacodynamic profile, and the precise mechanism of action in the CNS has been debated. At the time the first clinical trial of acamprosate in dependent patients appeared in Lancet 1985 (Lhuintre, Daoust et al. 1985), it was believed that acamprosate acted as a GABA-receptor agonist (Boismare, Daoust et al. 1984). However, most evidence supports that acamprosate works mainly as a modulator of glutamatergic neurotransmission (De Witte, Littleton et al. 2005).

1.5.1.1 Glutamatergic Neurotransmission

When glutamate is released into the synaptic cleft, it can bind to two independent families of glutamate receptors. *Ionotropic* glutamate receptors (iGluRs) comprise N-methyl-D-aspartate receptors (iNMDAR), kainate and α-Amino-3-hydroxy-5-methylisoxazole-4-proprionic acid receptors (iAMPAR) located at the postsynaptic spine (Clapp, Bhave et al. 2008; Gass and Olive 2008). The iNMDAR receptor is an ion channel, blocked by magnesium ions in its resting state. If the neuron is depolarized simultaneously as glutamate is present, the receptor is activated. This double function makes the iNMDAR vital in learning, memory and neuronal plasticity.

Metabotropic glutamate receptors (mGluRs) constitute three groups of receptors (mGluR I-III), each consisting of several subunits. mGluRs are predominantly located pre-synaptically and acts as modulators of glutamatergic transmission (Clapp, Bhave et al. 2008).

As mentioned earlier, glutamate receptors are present in numerous neurocircuits of the brain. Of special importance for the development of alcohol dependence are glutamatergic afferents from the prefrontal cortex, amygdala and hippocampus to the NAcc, i.e. the brain reward circuitry. For example, it is known that alterations in glutamate activity affects dopamine release in NAcc (Imperato, Honore et al. 1990).

1.5.1.1.1 Effects of Acute and Chronic Alcohol on Glutamatergic Neurotransmission

Alcohol inhibits glutamatergic neurotransmission action (Kumari and Ticku 2000). Among glutamate receptors, the iNMDAR seems to have the highest affinity for alcohol (Lovinger, White et al. 1989; Tsai, Gastfriend et al. 1995) and is responsible for the immediate effects of alcohol on glutamate transmission (Krystal, Petrakis et al. 2003). That alcohol attenuates iNMDA invoked activity has been shown also in vivo for e.g. cerebral cortex, NAcc, amygdala and VTA (Hoffman 2003). Further, exposure to alcohol negatively affects induction of long-term potentiation (LTP), a critical component in memory and learning (Blitzer, Gil et al. 1990). There is little evidence that alcohol has any effects on the metabotropic glutamate receptors (De Witte, Littleton et al. 2005).

With chronic or intermittent alcohol consumption, the glutamate system adapts to the suppressing effects of alcohol by an upregulation of iNMDARs glutamatergic receptors (Henniger, Wotjak et al. 2003; Nelson, Ur et al. 2005), a process which may constitute an example of alcohol tolerance development. As a result, the brain is left in a hyperglutamatergic state following acute withdrawal from alcohol consumption (Tsai and Coyle 1998; De Witte, Pinto et al. 2003) giving rise to an aversive state characterized by symptoms ranging from sweating, tremor, anxiety to seizures and delirium tremens (for clinical features and treatment of alcohol withdrawal, see section 1.4.3).

1.5.1.2 Effects of Acamprosate on a Hyperglutamatergic System in the Acute and Protracted Withdrawal-state

Early electrophysiological studies have shown that acamprosate inhibits glutamatergic mediated postsynaptic potentials in iNMDAR in vitro (Zeise, Kasparov et al. 1993). The precise mechanism behind how acamprosate inhibits iNMDAR activity is still unknown. Studies have suggested that acamprosate allosterically inhibits iNMDARs (Rammes, Mahal et al. 2001) e.g. by acamprosate binding to the binding site of other amino acids on the iNMDAR (Naassila, Hammoumi et al. 1998). It has further been suggested that acamprosate may work as a partial agonist on the iNMDAR, i.e. rather as a stabilizer on the receptor than as a pure antagonist, with agonistic properties with low receptor activity (in a non alcohol-withdrawal brain) and antagonistic properties with high receptor activity (in alcohol withdrawal, i.e. a hyperglutamatergic state) (al Qatari, Bouchenafa et al. 1998).

Apart from the suggested interaction with iGluRs, it has been shown that acamprosate acts as an antagonist at the mGluR5 receptor (Harris, Prendergast et al. 2002).

Studies have shown that acamprosate normalizes a hyperglutamatergic state caused by extensive alcohol consumption. In a pivotal experiment, extracellular levels of glutamate in NAcc were compared between acamprosate vs. untreated rats in acute withdrawal from alcohol (Dahchour, De Witte et al. 1998) (See Figure 2).

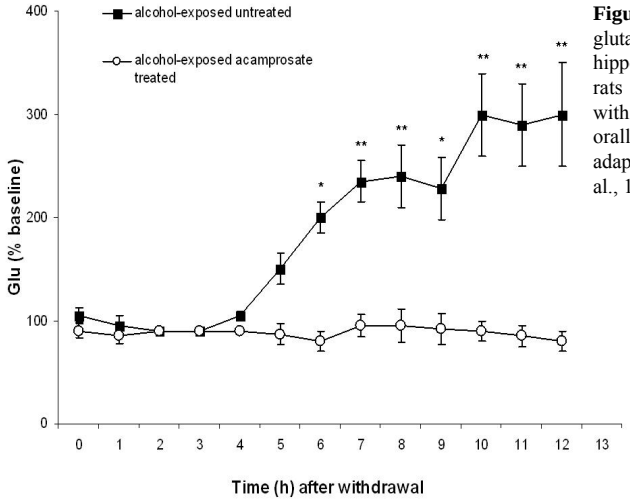

Figure 2. Changes in extracellular glutamate concentrations in the hippocampus of alcohol-dependent rats undergoing withdrawal, treated with acamprosate (white) (400 mg/kg orally) or vehicle (black). Figure adapted after Dahchour, De Witte et al., 1998. *p < 0.05, **p < 0.001

In this experiment, acamprosate completely abolished the increase in glutamate levels observed for untreated rats. In addition to this, the inhibitory effect of acamprosate on withdrawal-invoked glutamate release has shown to be maintained over repeated cycles of alcohol exposure and withdrawal (Dahchour and De Witte 2003). Further, acamprosate prevents behavioral manifestations of withdrawal in rats (Dahchour and De Witte 1999).

Further evidence for the effect of acamprosate to reduce a hyper-glutamatergic state in the brain was found in a recent study on mice null-mutated of the clock gene *per*2. These mice showed a hyper-glutamatergic state due to the attenuated expression of the glutamate transporter (Spanagel, Pendyala et al. 2005). The mutated mice also showed elevated alcohol consumption compared to controls. Acamprosate treatment normalized the elevated glutamate levels in NAcc as well as alcohol consumption.

These pre-clinical findings of an interaction between acamprosate and a hyperexcitability state have also been corroborated by studies in humans. In a sample of alcohol dependent subjects, acamprosate treatment initiated 8 days before withdrawal and continued during 15 days into withdrawal, reduced arousal levels in the brain in alcohol withdrawal state as measured by magnetoencephalography (Boeijinga, Parot et al. 2004). However, few clinical trials have specifically examined if acamprosate treatment during early withdrawal phase have any beneficial effect compared to placebo. The only RCT in which treatment was initiated before withdrawal, failed to show a beneficial effect of acamprosate treatment (Kampman, Pettinati et al. 2009).

1.5.1.3 Effects of Acamprosate on Dopaminergic Activity

There are some evidence supporting that acamprosate may modulate the reinforcing effects of alcohol. For example, pre-treatment with acamprosate abolished the alcohol-induced elevation in extracellular dopamine levels in NAcc in rats (Olive, Nannini et al. 2002). Indirect measures of an interaction between acamprosate and the mesolimbic dopamine system was shown by increased dopamine transporter density and decreased dopamine receptor density in NAcc, alongside a reduced alcohol consumption with acamprosate treatment in rats (Cowen, Adams et al. 2005). It is suggested that attenuation of ethanol induced dopamine activity by acamprosate is mediated by glutamate neurons co-localized with dopamine neurons in the NAcc (Cano-Cebrian, Zornoza-Sabina et al. 2003; Cowen, Adams et al. 2005).

1.5.1.4 Effects of Acamprosate on Neuroendocrine Responses

1.5.1.4.1 Acamprosate and β-endorphin

Acamprosate has been shown to affect the endogenous opioid system. In two separate studies, acamprosate was shown to increase basal β-endorphin plasma levels for rats in withdrawal (Zalewska-Kaszubska, Cwiek et al. 2005), and to inhibit the alcohol-induced increase in β-endorphin levels visible in untreated rats (Zalewska-Kaszubska, Gorska et al. 2008). These results were corroborated in a clinical trial which showed elevated plasma levels of β-endorphin in alcohol dependent patients treated with acamprosate compared to placebo (Kiefer, Jahn et al. 2006). Interestingly, this elevation was most marked for patients with a high alcohol intake before inclusion.

1.5.1.4.2 Acamprosate and HPA axis Activity

In a study examining plasma levels of HPA axis hormones in acamprosate treated patients, a trend was found for cortisol levels to be elevated compared to placebo (Kiefer, Jahn et al. 2006). The result may reflect a restoration by acamprosate of a dysregulated HPA axis following alcohol withdrawal.

1.5.2 Effects of Acamprosate on Alcohol Related Measures

1.5.2.1 Pre-clinical studies

Pre-clinical studies have demonstrated acamprosate's effects in a number of different behavioral paradigms such as a decrease in self-administration in a free choice paradigm following alcohol deprivation (Spanagel, Holter et al. 1996); a decrease in operant self-administration behavior (Holter, Landgraf et al. 1997; Bachteler, Economidou et al. 2005); a reduction of cue-induced reinstatement of alcohol seeking behavior (Bachteler, Economidou et al. 2005) and an elimination of conditioned abstinence behavior (Cole, Littleton et al. 2000).

1.5.2.2 Clinical Trials

The efficacy of acamprosate in modulating alcohol consumption has been evaluated in almost 20 randomized placebo controlled trials ranging from 90 days up to one year and comprising approximately 6000 patients. Trials have predominantly been European, but recently American (Anton, O'Malley et al. 2006; Mason, Goodman et al. 2006), Asian (Namkoong, Lee et al. 2003), Brazilian (Baltieri and De Andrade 2004) and Australian (Morley, Teesson et al. 2006) studies were published.

The primary outcome measure has varied between studies and in the following, results for acamprosate are reported without taking into consideration which variable was chosen as primary outcome measure for each respective study. Acamprosate increases *continuous abstinence rates*, commonly regarded as the most rigid outcome measure (Paille, Guelfi et al. 1995; Sass, Soyka et al. 1996; Whitworth, Fischer et al. 1996; Pelc, Verbanck et al. 1997; Poldrugo 1997; Besson, Aeby et al. 1998; Gual and Lehert 2001; Kiefer, Jahn et al. 2003; Baltieri and De Andrade 2004).

Acamprosate increases the *cumulative abstinence duration* (CAD) (Sass, Soyka et al. 1996; Whitworth, Fischer et al. 1996; Pelc, Verbanck et al. 1997; Poldrugo 1997; Besson, Aeby et al. 1998; Tempesta, Janiri et al. 2000; Gual and Lehert 2001; Kiefer, Jahn et al. 2003), *time to first drink* (Pelc, Verbanck et al. 1997; Tempesta, Janiri et al. 2000), *time to first relapse* to heavy drinking (Sass, Soyka et al. 1996; Gual and Lehert 2001; Kiefer, Jahn et al. 2003), and also shortens *duration of relapse* (Sass, Soyka et al. 1996; Tempesta, Janiri et al. 2000; Gual and Lehert 2001). Acamprosate also improves *abstinence,* categorically measured as no drinking episodes since last visit to the clinic (Poldrugo 1997; Besson, Aeby et al. 1998; Baltieri and De Andrade 2004).

In addition to these outcome measures purely focusing on drinking behavior, some studies have shown that acamprosate improves *retention in treatment*, which is considered to be an important factor for successful outcome in treatment (Paille, Guelfi et al. 1995; Sass, Soyka et al. 1996; Poldrugo 1997). However, other studies failed to find a corresponding effect (Besson, Aeby et al. 1998; Kiefer, Jahn et al. 2003). Lastly,

most clinical trials have used *biological markers* of alcohol consumption, e.g. liver enzymes and/or carbohydrate deficiency transferrin (CDT) as secondary measures. However, only few studies found an effect of acamprosate treatment in these measures (Pelc, Verbanck et al. 1997; Besson, Aeby et al. 1998).

Some studies have failed to show an effect of acamprosate compared to placebo (Roussaux, Hers et al. 1996; Chick, Howlett et al. 2000; Namkoong, Lee et al. 2003; Anton, O'Malley et al. 2006; Morley, Teesson et al. 2006), and reasons for this have been discussed. Factors as deviant populations compared to previous trials (Roussaux, Hers et al. 1996; Namkoong, Lee et al. 2003; Anton, O'Malley et al. 2006), too high level of medical management creating ceiling effects (Anton, O'Malley et al. 2006), as well as type II statistical errors (Chick, Howlett et al. 2000) have been discussed.

The efficacy of acamprosate in treatment of alcohol dependence has been evaluated in reviews and meta-analyses (Bouza, Angeles et al. 2004; Mann, Lehert et al. 2004). Mann, Lehert et al. (2004) included 17 acamprosate studies with in all more than 4000 alcohol dependent patients in a meta-analysis. The result showed that following 6 months of treatment, 36.1% of the acamprosate treated patients were still abstinent, while the same figure for placebo patients were 23.4%. Also for CAD and retention in treatment, there was a statistically significant difference in favor of acamprosate treatment compared to placebo. The number needed to treat (NNT) – which refers to the number of patients who need to be treated in order to prevent one additional bad outcome (i.e. relapse) – were 7.8 for acamprosate, which is comparable to e.g. naltrexone treatment for alcohol dependence (Srisurapanont and Jarusuraisin 2005), SSRI treatment for depression (Moncrieff 2001), and much more efficacious than e.g. pharmacotherapy for hypertension.

1.5.2.2.1 Acamprosate in Combination with Naltrexone

Two studies have examined if a combined treatment with acamprosate and naltrexone is more efficacious than treatment with the two medications alone (Kiefer, Jahn et al. 2003; Anton, O'Malley et al. 2006). In the first trial, Kiefer Jahn et al. (2003) compared four treatment arms: naltrexone, acamprosate, naltrexone + acamprosate and placebo with continuous abstinence rates as primary outcome measure. The results showed that all three treatment arms fared better than placebo. In addition to this, there was a beneficial effect of naltrexone + acamprosate compared to acamprosate alone, but not compared to naltrexone alone. In the large US "COMBINE" study (Anton, O'Malley et al. 2006), no beneficial effects were found with a combination of acamprosate + naltrexone compared to acamprosate alone (or over any other treatment condition).

1.5.2.3 Acamprosate as a Medication for Reducing Alcohol Craving

It has been assumed that acamprosate attenuates alcohol consumption by reducing conditioned craving responses. However, only in a few of the previously mentioned clinical trials, acamprosate treated patients showed a decrease in self-reported craving compared to placebo (Pelc, Verbanck et al. 1997; Chick, Howlett et al. 2000), while the other trials found no such effect. In addition, the study by Chick, Howlett et al. (2000) showed a deviating pattern of results. In this study, acamprosate treated patients reported a decrease in craving while no effect on alcohol consumption was found.

An explanation for these conflicting findings may be that acamprosate does not work at all by attenuating craving responses, but by some other mechanism as suggested by Tempesta, Janiri et al. 2000. Another possibility is that the instruments used to measure craving were insufficient in some respect to capture craving responses. The instruments used in clinical trials of acamprosate have been either the Obsessive Compulsive Drinking Scale (OCDS) (Anton, Moak et al. 1995), the Penn Alcohol Craving Scale (PACS) (Flannery, Volpicelli et al. 1999) and/or some kind of visual analogue scale (VAS). In these self-report questionnaires, patients are asked to retrospectively indicate in what degree they have experienced an urge to consume alcohol during the last week or even longer time period. However, there are firm indications that craving responses may last only for a couple of minutes and that intensities of responses vary a lot during a day (Rohsenow and Monti 1999; Drummond 2001; Ooteman, Koeter et al. 2006).

Hence, if acamprosate modulates craving, this effect might have gone undetected due to methodological reasons.

1.5.2.4 Laboratory Based Models for the Study of Treatment Effects on Craving

1.5.2.4.1 Conditioned Responses to Alcohol Consumption

Several models of alcohol craving propose that subjective and physiological responses related to alcohol may be conditioned to previously alcohol related situations or stimuli (in the following referred to as "cues") (Rohsenow and Monti 1999). Two mechanisms have been proposed for this effect to occur. First, according to the *conditioned withdrawal model,* it is assumed that cues become conditioned to the negative state of alcohol withdrawal (Ludwig, Wikler et al. 1974). For example, a low blood sugar level or environmental context such as the room in which previous withdrawal sessions were experienced, may in themselves elicit a withdrawal state. Alcohol craving would be the result for the individual in order to be alleviated from the negative state (negative reinforcement). Second, according to the *conditioned appetitive model* (Stewart, de Wit et al. 1984; Niaura, Rohsenow et al. 1988; Niaura 2000) a corresponding conditioning process may occur in which cues related to the positive effects of alcohol may elicit alcohol craving, e.g. the taste or smell of a preferred beverage (positive reinforcement).

Both conditioning models assume that the risk for relapse to heavy drinking will be elevated either by a subjectively experienced craving or by automated responses if an alcohol dependent patient is exposed to alcohol related cues (Rohsenow and Monti 1999; Drummond 2001).

In laboratory based research models, conditioned craving responses have shown to be elicited by either environmental cues (Cooney, Litt et al. 1997; Ooteman, Koeter et al. 2007), stress (Cooney, Litt et al. 1997; Litt, Cooney et al. 2000; Sinha 2009; Sinha, Fox et al. 2009) or a priming dose of alcohol (Kaplan, Meyer et al. 1983; Cooney, Litt et al. 1997; Davidson, Palfai et al. 1999; O'Malley, Krishnan-Sarin et al. 2002), each factor strongly contributing to the maintenance of alcohol dependence. It has also been shown that cue-, stress- and priming-related craving responses are markedly increased for dependent individuals compared to social drinkers (Sinha, Fox et al. 2009).

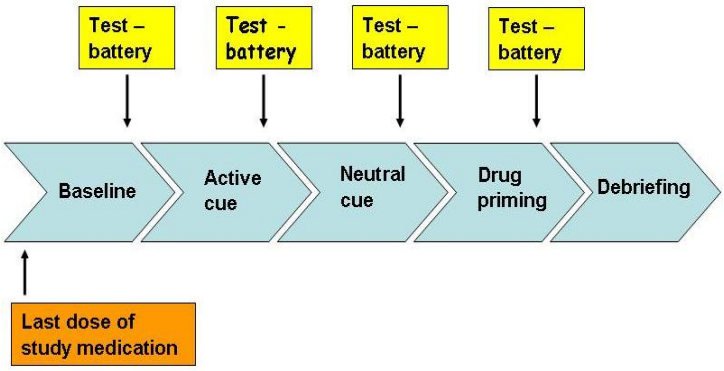

Figure 3. Schematic representation of a test-day in a human laboratory study of drug cue- and priming-responses. The procedure differs according to research question. In this example, subjects have been pre-treated with a study medication, and ingest their last medication dose at the clinic on the morning of the test-day. Baseline session aims at establishing a relaxing environment for the subjects, in order to provide basal subjective and physiological measures. Active cues may comprise visual, auditory, olfactory and tactile stimuli related to drug consumption (e.g. drug paraphernalia or bottles of alcoholic beverages). Neutral cues are presented in a contextually similar way but comprise stimuli unrelated to drug consumption (e.g. non-alcoholic beverages). Cue conditions are presented in a counterbalanced order in order to avoid effects of order of presentation. Cue presentations may be followed by a drug priming session in which subjects consume a small amount of a drug. The test-day ends with a debriefing session in which experiences during the tests are evaluated. The focus for this session is to reduce or eliminate residual subjective feelings of craving following cue- or priming-sessions. The sessions are separated by a 15 – 30 minute's pause. At the end of each session, a test battery of subjective (e.g. scales measuring craving or mood) and physiological (e.g. blood pressure (BP), heart rate (HR), galvanic skin response (GSR)) measures is administered. The test battery may also include positron emission tomography (PET) or functional Magnetic Resonance Imaging (fMRI) techniques in order to correlate subjective craving with brain activity.

Our research group has developed a laboratory based model suitable to study treatment effects on cue-, stress- and priming-induced responses, such as subjectively experienced craving, physiological, biological and behavioral correlates of alcohol dependence. The paradigm has been used to study different aspects of substance dependence, e.g. subjective and physiological responses to amphetamine and alcohol cues and priming (Jayaram-Lindstrom, Wennberg et al. 2004; Jayaram-Lindstrom, Konstenius et al. 2008; Hammarberg, Jayaram-Lindstrom et al. 2009). In Figure 3, a schematic representation of the procedure of a laboratory study test-day is displayed.

In study I, a paradigm was designed in which both cue- and priming-induced craving responses were studied during the same experimental occasion for a patient, in order to minimize intra-individual variation (Hammarberg, Jayaram-Lindstrom et al. 2009).

1.5.2.4.2 Acamprosate and Laboratory Studies of Cue- and Priming-induced Responses

Only few studies have used a cue-induced craving paradigm to study the effects of acamprosate treatment. One open label study gave preliminary support for an effect of acamprosate on cue-induced craving (Weinstein, Feldtkeller et al. 2003). In a placebo controlled trial, Ooteman, Koeter et al. (2007) examined the response to olfactory cues before and after three weeks of acamprosate, naltrexone or placebo treatment for dependent patients. Acamprosate failed to show any effects on subjectively

experienced craving compared to placebo. However, a decreased peak HR frequency following cue exposure was interpreted by the authors as a modulation of withdrawal related responses by acamprosate.

The effect of acamprosate treatment on alcohol priming-induced craving in dependent patients has not been studied. In a study by Brasser, McCaul et al. (2004), it was found that 7 days of acamprosate treatment with either 0 (placebo), 2 or 4 grams per day in heavy drinking healthy volunteers did not alter subjective effects of administration of 0, 0.5 or 1.0 grams of alcohol/kg bodyweight. The results from this study are difficult to interpret for several reasons. Most important is that acamprosate effects are probably notable only in a dysregulated glutamate system which is probably not the case for heavy drinkers unless they are suffering from alcohol dependence (De Witte, Littleton et al. 2005). Further, subjects were offered a non-preferred beverage in a fixed dosage regimen, which limits the external validity of the results.

In summary, acamprosate is considered to be an efficacious treatment for alcohol dependence. However, as pointed out by some authors (Johnson 2008), few studies have examined how acamprosate treatment interacts with core phenomena of alcohol dependence. In study I, it was investigated if acamprosate attenuates cue- and/or alcohol priming-induced responses, applying a laboratory based design (Hammarberg, Jayaram-Lindstrom et al. 2009).

1.5.3 Pharmacokinetic Properties of Acamprosate

Few studies on the pharmacokinetic properties of acamprosate have been published and early reviews mainly covered unpublished internal Lipha company reports (Saivin, Hulot et al. 1998; Zornoza, Cano et al. 2003). Only two recent studies have examined pharmacokinetic properties of acamprosate in humans (Mason, Goodman et al. 2002; Johnson, O'Malley et al. 2003).

Acamprosate is dissociated into acetyl homotaurine and calcium in plasma. Poor drug permeation across intestinal mucosa has been proposed as the main reason to the low bioavailability (i.e. the proportion of a medication that reaches systemic circulation) of 11$\pm$1% for oral administration of tablets (Fourtillan 1990; Zornoza, Cano-Cebrian et al. 2004).

Steady state of acamprosate in plasma is reported to be achieved in 5 – 7 days in humans. Acamprosate mean elimination half-life ($t_{1/2}$) has been determined to 3.2$\pm$ 0.2h for infusion and 32.7 $\pm$ 4.3h for oral administration (1995). Acamprosate is eliminated probably mainly by renal excretion. Approximately 95% of the administered dose is excreted unchanged into the urine suggesting that acamprosate is not metabolized (Zornoza, Cano et al. 2003). The distribution of acamprosate into the CNS compartment has not been studied in humans, although studies indicate that acamprosate crosses the blood-brain barrier in rats (Durbin and Belleville 1995; Burattini, McGeehan et al. 2008).

Acamprosate metabolism has shown to be unaffected by gender, age, hepatic insufficiency, alcohol consumption, and other psychotropic drugs (Saivin, Hulot et al.

1998; Zornoza, Cano et al. 2003). In contrast, renal insufficiency increases elimination time of acamprosate and food decreases acamprosate absorption (Saivin, Hulot et al. 1998). Concurrent treatment with naltrexone elevates acamprosate plasma levels compared to acamprosate alone (Mason, Goodman et al. 2002; Johnson, O'Malley et al. 2003). A proposed explanation for this phenomenon is that naltrexone – by blocking endogenous opioid transmission in the gut – stimulates gastric motility which in turn may enhance the absorption of acamprosate.

Previous pharmacokinetic studies of acamprosate have been based on two different methods, High-Performance Liquid Chromatography (HPLC) (Chabenat, Ladure et al. 1987) and Gas Chromatography-Mass Spectrometry (GC – MS) (Girault, Gobin et al. 1990). Both methods require extraction and derivatization procedures that could potentially affect accuracy in measurement. With the advent of the LC – MS method there is a new possibility for developing a more direct method for acamprosate measurement in human specimens. Recent studies have used LC – MS/MS for studies in beagle dogs (Rhee, Park et al. 2008) and in rats (Burattini, McGeehan et al. 2008), but to our knowledge no LC – MS method has yet been used in studies of acamprosate in humans.

A validation of previous results for important pharmacokinetic parameters of acamprosate is warranted. Acamprosate presence in human CSF in relation to plasma levels is an important issue, both in terms of expectancies concerning treatment efficacy, but also for evaluation of treatment compliance.

Thesis study III examines pharmacokinetic properties of acamprosate applying LC – MS as method of measurement.

1.5.4 Safety Profile

The side effect profile of acamprosate is considerably mild. Clinical trials of acamprosate consistently report that only digestive system events, e.g. diarrhea and abdominal pain, show a higher prevalence compared to placebo. Other reported side-effects are headache, vomiting, decreased libido and memory impairment, although these problems were equally common in placebo treated patients and may be regarded as consequences of excessive alcohol consumption. In studies using a daily dosing regimen of 3g compared to the usual 2g, no correlation was found between plasma levels of acamprosate and side-effects (Pelc, Verbanck et al. 1997; Johnson, O'Malley et al. 2003; Mason, Goodman et al. 2006). A dose of 4g/day, however resulted in more frequent cases of gastrointestinal upset compared to 2g/day (Brasser, McCaul et al. 2004). The reported side effects generally resolve spontaneously shortly after initiation of treatment.

1.5.5 Psychosocial Treatment in Conjunction to Acamprosate

The general recommendation for pharmacotherapeutic treatments of alcohol dependence is that prescription is made in combination with some form of psychosocial intervention. Emotional support, solving psychological or social problems and increasing medication compliance are some reasons mentioned for psychosocial treatments in general (Garbutt, West et al. 1999; Weiss and Kueppenbender 2006).

In a study examining the efficacy of different psychosocial interventions in conjunction to acamprosate treatment, 248 alcohol dependent patients were treated for 28 weeks with acamprosate, and randomized to either 1) Minimal intervention including in all three 20 minutes visits to a physician within the first 4 weeks of treatment, or 2) Minimal intervention + CBT at seven times for one hour each during week 2 – 8. The results showed no differences in CAD, continuous abstinence rates, time to first relapse or any other secondary measure between the treatment groups (De Wildt, Schippers et al. 2002).

Poor compliance to medication treatment is a problem for treatment of alcohol dependence. Different methods for increasing compliance to treatment have been developed (Starosta, Leeman et al. 2006). Reid, Teesson et al. (2005) investigated if a compliance enhancement therapy would be effective in increasing treatment adherence for acamprosate treatment of alcohol dependence. 20 patients receiving only medical management were compared with 20 patients receiving medical management + 6 sessions of compliance therapy in conjunction with 16 weeks of acamprosate treatment (Reid, Teesson et al. 2005). No differences between groups were found with regard to medication compliance, alcohol consumption measures (time to first drink, time to first relapse, relapse duration), or any other secondary measure.

A study by Pelc, Hanak et al. (2005) examined the beneficial effect of an intense follow-up of patients by a community based nurse compared to a low-intensive physician-only follow up for 100 alcohol dependent patients receiving 26 weeks of acamprosate treatment. Using a non-blind randomized design, CAD was longer for patients receiving a more intensive psychosocial support (Pelc, Hanak et al. 2005).

In the multi-center COMBINE study (Anton, O'Malley et al. 2006), the question of increased efficacy with combinations of psychosocial and medical treatments (acamprosate and naltrexone) was specifically addressed. Acamprosate showed no beneficial effect over placebo, irrespective if combined with CBT or not (In fact, treatment effects were small for all treatment conditions possibly due to ceiling effects caused by intense and repeated assessments and medical management for all patients).

In summary, the addition of psychosocial support to acamprosate treatment needs to be further examined in order for firm conclusions to be drawn concerning improved efficacy. In study IV, two intensities of psychosocial support in conjunction with acamprosate treatment were compared (Hammarberg, Wennberg et al. 2004).

2 AIMS OF THE THESIS

2.1 GENERAL AIM OF THE THESIS

The general aim of the thesis was to examine psychological, behavioral and physiological correlates of alcohol dependence in relation to acamprosate treatment.

2.2 SPECIFIC AIMS OF THE THESIS

The specific aims of the thesis was to investigate:

In study I:
- If acamprosate attenuates alcohol cue-induced craving for dependent patients
- If acamprosate attenuates alcohol priming-induced craving for dependent patients

In study II:
- If a short term acamprosate treatment attenuates general craving for dependent patients
- If a short term acamprosate treatment alters neuroendocrine measures for dependent patients

In study III:
- If plasma concentrations of acamprosate can be accurately measured with a new technique (LC – MS)
- If pharmacokinetic results agree with earlier studies
- If acamprosate can be measured in human CSF

In study IV:
- If EPI is superior to MPI in reducing relapse to drinking in acamprosate treated patients

The presentation of the four studies will focus on the main results. For details concerning procedure and non-significant findings, the reader is kindly referred to each paper. All studies were conducted at the Stockholm Centre for Dependency Disorders (Beroendecentrum Stockholm) in Stockholm, Sweden. The studies were approved by the Regional Ethical Review Board in Stockholm and the MPA and were conducted in accordance with Good Clinical Practice (GCP), Good Laboratory Practice (GLP) and the Declaration of Helsinki.

3 MATERIALS AND METHODS

3.1 STUDY I – ALCOHOL CUE- AND PRIMING-INDUCED CRAVING

3.1.1 Subjects

Fifty-six treatment-seeking alcohol dependent individuals were recruited via advertisements in a local newspaper. Inclusion criteria were: 1) a male or a non-pregnant/non-nursing female between 18 and 65 years of age; 2) a goal of controlled drinking; 3) an intact sense of smell; 4) fulfilling the criteria for alcohol dependence according to DSM-IV; 5) willingness to give informed consent and comply with study procedures; 6) having consumed alcohol on a minimum of 15 of the last 90 days. Exclusion criteria were: 1) seeking complete alcohol abstinence; 2) a diagnosis of an Axis I psychiatric disorder according to DSM-IV criteria (including all forms of substance dependence other than nicotine and alcohol); 3) current use of psychoactive medications to manage schizophrenia, bipolar disorder, or major depression; 4) inpatient alcohol detoxification within the last 4 days; 5) acamprosate medication during the last 12 months; 6) use of illegal drugs during the course of the study.

3.1.2 Procedure

3.1.2.1 Treatment Period

The study used a randomized, double blind, single-site, placebo-controlled design. Patients were assigned to 21 days of either oral acamprosate (1998 mg/day) or placebo. Each patient received a medication box containing 150 tablets (of 333 mg acamprosate or identical placebo) at the start of the trial, with instructions to intake 6 tablets per day (2 tablets, TID). During treatment, patients made a weekly visit to the clinic in which they met the study nurse for urine and blood tests and the study coordinator for relapse prevention and filling out questionnaires. Patients were instructed to refrain from drinking during study. CDT was collected at day 1 and 22, as well as drinking measures collected by Time-Line Follow Back (TLFB) (Sobell and Sobell 1992).

3.1.2.2 Laboratory Sessions

All subjects arrived at the clinic at 9:00 a.m. with the instruction to ingest their morning dose of acamprosate 90 min prior to arriving. Patients were instructed not to use any alcohol 24 hr prior to testing, and use of tobacco was not allowed after 9:00 a.m. Abstinence requirement was checked for by urine screen for drugs of abuse (qualitative analysis), alcohol breathalyzer tests and by using the revised Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar) scale (Sullivan, Sykora et al. 1989).

At 10:00 a.m. a venous catheter was inserted in the arm of the patients' non-writing hand to collect blood samples for measuring cortisol levels. Patients were then offered a standardized light lunch. Patients were also reminded that they would be offered an alcoholic drink on the test-day.

At 10:30 a.m. cue-reactivity tests began. Each test session (Alcohol Cue, Non-Alcohol Cue, and Priming Drink) was separated by a 15 minute pause. Alcohol Cue vs. Non-Alcohol Cue sessions were presented in a randomized, counterbalanced order to control

for order effects. Through the whole test-day, patients were seated in a comfortable sofa in a quiet room at the clinic.

3.1.2.2.1 Alcohol Cue Session

A tray containing several different bottles of alcohol in the manufacturers bottle (including an assortment of vodka, whiskey, gin, beer and wine), was placed on a table directly in front of the patients who were instructed to handle the bottles and to describe the taste of each beverage. Patients then indicated their most preferred beverage. A 1.5 minute alcohol cue video containing a complex mixture of auditory and visual cues specific to drinking alcohol, e.g. socializing in a bar and pouring and drinking close-ups, was then presented. Following the video, patients opened the bottle of the most preferred beverage and poured a medium size quantity (3 – 4 cl.) in the drinking glass, and sniffed the content. Patients then described a situation in which they most prefer to consume the beverage and then placed the glass on the table.

3.1.2.2.2 Non-Alcohol Cue Session

The procedure was contextually similar to the alcohol cue session apart from that the cues consisted of several different bottles of fruit juice, cans of soda, and cartons of milk, in the manufacturers´ container. A 1.5 minute video consisting of similar items placed on a table and being handled and drunk by an individual were presented to the patients.

3.1.2.2.3 Priming Dose Session

The patients chose an alcoholic beverage from the tray presented in the alcohol cue session (wine, beer, spirits) and were then provided with a sample of the preferred brand in a glass at a standardized alcohol dose (0.20 gr. alcohol/kg bodyweight). Hence, the amount of beverage a patient consumed differed due to differences in beverage of choice and the weight of the patients. However, the amount of pure ethanol per kg bodyweight offered was the same for each patient. The patients were instructed to consume the amount they preferred, and that any beverage remaining after 15 minutes would be collected by the study coordinator.

3.1.2.2.4 Alcohol Choice Paradigm

In an alcohol choice paradigm (Griffiths, Troisi et al. 1993), patients identified the crossover value above which a monetary reward would be preferred over another drink of alcohol, equivalent to what they had just consumed. The study-coordinator asked the patients to make a hypothetical choice between having another alcoholic drink or to earn 5 SEK (approximately 75 cent). This procedure continued until a patient chose the monetary reward over the drink (the cross-over value) or maximum 120 SEK (approximately $17).

3.1.3 Measurements

3.1.3.1 Assessment Timing

Subjective and physiological measurements were collected at specific time-points during the test-day: at the end of the Baseline session, following the Alcohol Cue session, In Between Cue session, following the Non-Alcohol Cue session, following the Pre-Alcohol Priming session, 15 minutes Post-Alcohol Priming session and 30 minutes Post-Alcohol Priming session (i.e. the patients filled out questionnaires and provided physiological and biological samples 15 and 30 minutes post finishing the last sip of the priming drink).

3.1.3.2 Subjective Measures

Alcohol craving was measured by a shortened Swedish version of the Desire for Alcohol Questionnaire (Short-DAQ) (Love, James et al. 1998) containing the following 8 items: 1) I could fancy a drink right now; 2) I would worry less if I had a drink right now; 3) To drink alcohol right now would be satisfying; 4) If I started to drink right now I would be able to stop drinking (reversed item); 5) I would accept a drink right now if I was offered; 6) If I drank alcohol right now I would feel less tense; 7) To drink alcohol right now would be nice; 8) I would easily be able to control my drinking if I had a drink right now (reversed item). Patients indicated their responses on an 8 point likert scale ranging from "Do not agree at all" to "Fully agree". Alcohol craving was also measured by a Visual Analog Scale (VAS) (Reid, Flammino et al. 2006) comprising the item "How much would you like to have an alcoholic drink right now?". Withdrawal effects were measured by a VAS containing 5 items. Subjective mood was assessed by the Positive And Negative Affect Scale PANAS (Watson, Clark et al. 1988), where patients indicated the extent to which 20 adjectives matched with their current mood on a scale ranging from 1 to 5. In the last three sessions, patients also filled out the Biphasic Alcohol Effect Scale (BAES) (Martin, Earleywine et al. 1993), containing 14 items measuring excitatory and sedative effects of alcohol consumption.

3.1.3.3 Physiological Measures

GSR and skin temperature (Temp) were recorded using fingertip electrodes connected to a PC computer via a RS232 belt pack/interface unit (JoR AB, Uppsala, Sweden). Responses were measured during 3 minutes in direct conjunction to the sessions. HR and blood pressure (BP) (systolic/diastolic) were recorded manually after the patients had filled out their subjective questionnaires.

3.1.3.4 Biological Measures

Blood samples for the measurements of plasma cortisol were collected following subjective and physiological measures in direct conjunction to the sessions.

3.1.3.5 Drinking Measures

Total amount of alcohol consumed was determined by measuring the amount of beverage left in the drinking glass in the alcohol priming session. Blood samples for determining blood alcohol levels were collected 15 and 30 minutes following finishing the priming drink.

3.1.4 Statistical Analysis

The primary hypothesis of the study was that acamprosate attenuates cue-induced and/or priming-induced craving for alcohol. The two primary outcome measures in the study were 1) the composite score on Short-DAQ, operationally defined as the mean value of the 8 items, and 2) the score on the VAS alcohol craving item. Primary outcome measures were analyzed in two separate repeated measures of ANOVAs comparing Short-DAQ and VAS first in a 2 (Non-Alcohol Cue session, Alcohol Cue session) by 2 (acamprosate, placebo) model of cue exposure effects and second in a 3 (Pre-Alcohol Priming session, 15 minutes Post-Alcohol Priming session, 30 minutes Post-Alcohol Priming session) by 2 (acamprosate, placebo) model of alcohol priming effects. The analysis was done according to completer's principle, i.e. only patients who participated in 21 days of treatment, and also completed all laboratory sessions on the test-day were included in the analysis.

Secondary outcome measures in the study were subjectively experienced withdrawal symptoms, mood and physiological measures (HR, BP, GSR, Temp and plasma-cortisol) on corresponding time-points, and were analyzed by repeated measures of ANOVA. For GSR and Temp, the mean value of 3 minutes recording was used in the analysis. The response on the alcohol choice procedure was analyzed using a one-way ANOVA where the cross-over level was compared between the two treatment groups.

Compliance to acamprosate medication was controlled by investigating urine samples obtained at day 7, 14 and 22 of treatment, and venous plasma samples at day 22 by LC – MS. Pearson's correlation analyses were executed to test correlations between plasma concentration of acamprosate and cue- and priming induced alcohol craving at day 22.

3.2 STUDY II – CRAVING AND NEUROENDOCRINE MEASURES

3.2.1 Subjects
The subjects were identical to study I.

3.2.2 Procedure
The procedure was identical to study I.

3.2.3 Measurements

3.2.3.1 Subjective Measures
At day 1 and 22, alcohol craving was measured by OCDS (Anton, Moak et al. 1995), a measure of general craving experiences based on 14 items rating intensity of desire to drink, obsessive-compulsive aspects of drinking, control over drinking, thought patterns and alcohol consumption. Alcohol consumption was measured by TLFB (Sobell and Sobell 1992) which assesses consumption of any substance of abuse for a specified preceding time period.

3.2.3.2 Biological Measures
At 09:00 a.m. at day 1 and 22, plasma samples for the measurement of cortisol, ACTH and β-endorphin were collected.

3.2.4 Statistical Analysis
Main outcome measure was the difference in OCDS index score – defined as the mean value of the 14 items – between day 1 and 22 (i.e. an index score ranging between 0 and 4). OCDS index scores were analyzed in a repeated measures of ANOVA in a 2 (day 1, day 22) by 2 (acamprosate, placebo) model. Secondary analyses included the difference in neuroendocrine levels between day 1 and 22, and the difference in alcohol use during 90 days prior to inclusion and during treatment, analyzed in a similar 2 X 2 repeated measures of ANOVA. The correlation between OCDS, TLFB and biological measures was analyzed by a Pearson's correlation analysis.

3.3 STUDY III – PHARMACOKINETICS OF ACAMPROSATE

3.3.1 Subjects
Thirteen healthy subjects were recruited by flyers on the Karolinska Institutet campus and by word of mouth. Inclusion criteria were: 1) male or non-pregnant/non-nursing female between

18 and 65 years of age; 2) subject having a goal of abstinence during the study; 3) bodyweight between 65 – 95 kg; 4) willingness to give informed consent and comply with study procedures. Exclusion criteria were: 1) renal insufficiency or any other serious medical condition; 2) use of more than 10 standard units (1 unit=12 grams of alcohol) per week over the last year; 2) evidence of any Axis I psychiatric disorder according to DSM-IV criteria (including all forms of substance dependence) as determined by a clinical interview; 3) current regular use of any concomitant medication; 4) previously treated with acamprosate; 5) use of any illegal drugs during the last 30 days; 6) use of any illegal drugs during the course of the study.

3.3.2 Procedure

The study used an open label, single-site design in which subjects were assigned to 21 days of oral acamprosate treatment (999 mg day 1, 1998 mg/day the following 20 days, and 999 mg the last day of treatment).

3.3.2.1 Study-day 1

Subjects received instructions to fast from midnight before each study day, and to not use any alcohol or drugs of abuse before or during the study. Abstinence was verified by self report on alcohol and drug use since the last visit to the clinic. At 9:00 a.m., a venous catheter was inserted in the subjects' left arm and a baseline plasma sample was drawn. At 9:15 a.m. the subjects received an oral dose of acamprosate (999 mg) with 200 mL of water. A blood sample for pharmacokinetic analysis was then collected every hour for the first four hrs and then every second hour until 10 hrs following medication (in all 8 samples during study day 1). Subjects were served a standardized lunch consisting of a ham pie, two slices of dark bread and an orange at 11:30 a.m. and a light meal consisting of a fruit, a sandwich and a yogurt at 3:30 p.m. At 7:15 p.m., the catheter was withdrawn, and subjects left the clinic.

3.3.2.2 Study-days 2, 7 and 14

At study-day 2 subjects came to the clinic at 9:00 a.m. (with instructions to fast from midnight) and at 9:15 a.m., a plasma sample was drawn to determine acamprosate plasma levels 24 hrs following first acamprosate intake. Subjects then received 125 acamprosate tablets in a box and were informed to take 6 tablets per day (3 tablets BID, 30 min before breakfast in the morning and 30 min before dinner in the evening) during the following 21 days. In all, this sums up to 42 doses of acamprosate (i.e. 1998 mg of acamprosate per day except day 1 and 22 where dosage was 999 mg) with the first dose (999 mg) ingested while still at the clinic. The study medication was provided from Apoteket AB.

On study-days 7 and 14, the subjects returned to the clinic. At 9:00 a.m. a plasma sample was drawn. Subjects ingested their morning dose of acamprosate at 9:15 a.m. and provided plasma samples at 10:15 a.m. and 12:15 p.m. Vital signs, adverse events, potential medication side effects, use of any concomitant medications, nicotine or illegal substances were also assessed. A standardized lunch was served at 11:30 a.m.

3.3.2.3 Study-day 22 and 23

The procedure was identical to study-day 1 and 2 except for patients who accepted to take part in the lumbar puncture procedure. 4 mL of CSF was collected in a sterile tube at 1:30 p.m. at day 22.

3.3.3 Measurements

3.3.3.1 Biological Laboratory Measurements

At screening, serum creatinine was measured to screen for renal insufficiency. Liver transaminases (aspartate aminotransferase (AST), alanine aminotransferase (ALT), and Gamma-glutamyl transferase (GGT)) were determined to rule out liver insufficiency. CRF, ACTH, and cortisol were analyzed from the plasma sample drawn at 9:00 a.m. at day 1 and 22.

3.3.3.1.1 LC – MS Analysis

An aliquot of 0.2 mL of plasma or CSF was prepared for analysis and a volume of 10 μL was injected into an Agilent 1100 MSD LC – MS system. For details concerning analysis, see study III. Calibrator samples were prepared in human blank serum from stock solutions. The concentrations were from 50 – 1000 ng/mL for plasma and CSF.

3.3.4 Pharmacokinetic Evaluation

Plasma concentration-time data from the participants were analyzed by the one-compartment model with a first order absorption phase including a lag time. In total, 42 doses were given for each subject during the study period with 23 – 24 concentration-time data points. Descriptive pharmacokinetic analysis included: area under the plasma concentration-time curve over one dosing interval (AUC); maximum plasma concentration (C_{max}) and time to reach maximum concentration (T_{max}) (estimated from the first single dose administration); elimination half life ($t_{1/2}$) and degree of fluctuation at steady state.

3.4 STUDY IV – PSYCHOSOCIAL INTERVENTIONS

3.4.1 Subjects

Treatment seeking patients who visited the clinic due to alcohol problems were consecutively screened for participation in the study. Inclusion criteria were: (1) men or women 18 – 70 years; (2) a diagnosis of alcohol dependence according to DSM-IV criteria; (3) consumed alcohol on a minimum of 15 out of 90 days prior to screening; (4) residence in Stockholm County (i.e. not homeless); (5) willingness to give informed consent; (6) willingness to stay in treatment for 6 months. Exclusion criteria were: (1) any other substance dependence according to DSM-IV except for nicotine; (2) previous treatment with acamprosate or naltrexone; (3) renal insufficiency or other major somatic disease; (4) any other Axis I DSM-IV diagnosis than alcohol dependence; (5) participated in another clinical study in the last 30 days; (6) pregnancy or lactating for female patients; (7) signs of alcohol withdrawal.

3.4.2 Procedure

3.4.2.1 Procedure Common to All Patients

Patients were required to be abstinent from alcohol during the time between screening and inclusion (checked by self-report and breathalyzer) and were then randomized to receive either MPI or EPI.

In addition to the screening and inclusion visits, patients met the psychiatrist on two occasions during the trial (at weeks 12 and 24). During all four visits, patients received general guidelines and feedback on their drinking behavior and how to cope with high risk situations.

At week 0, all patients were offered acamprosate on a prescription covering 12 weeks at a daily dose of 1998 mg (333 mg tablets; three tablets BID) a treatment all patients accepted. All patients who returned to clinic at week 12 were given a second prescription of acamprosate for the remaining study period. All patients were charged the standard patient's fee and paid for medication.

3.4.2.2 Procedure for Patients Receiving EPI

Patients randomized to EPI met a psychiatric nurse for a minimum of 10 and a maximum of 15 sessions during the trial, in addition to the visits to the psychiatrist. The patients were trained to develop and use behavioral and cognitive skills to deal with high risk situations in line with a manual for relapse prevention (Kadden, Carroll et al. 1992). Each visit lasted between 30 – 40 min.

3.4.3 Measurements

3.4.3.1 Subjective Measurements

TLFB (Sobell and Sobell 1992) was filled out for measures of alcohol use 90 days before inclusion and during the study (at week 0, 12 and 24). Psychiatric symptoms were assessed at weeks 0 and 24 by Symptoms Check List 90 (SCL 90). Alcohol craving was measured by OCDS (Anton, Moak et al. 1995) at week 0, 12 and 24.

3.4.3.2 Biological Measures

Blood and urine samples were obtained at week 12 and 24 for analysis of safety measures (CDT, AST and ALT) and compliance to study medication.

3.4.4 Statistical Analysis

The first primary outcome variable was defined as the percentage of days with heavy drinking (≥60 g pure ethanol for men and ≥48 g for women), as measured by TLFB. The percentage of heavy drinking days 90 days before and during study was calculated for the patients. Heavy drinking was analyzed in a 2 (MPI, EPI) X 3 (week 0, 12 and 24) repeated measurement ANOVA.

The second primary outcome variable was the percentage of days with any drinking which was analyzed in a similar repeated measurement ANOVA.

As a secondary outcome variable, the time to first drink was compared between the two groups by a one-way analysis of variance.

Analyses were executed according to the completer's principle. Patients were defined as completers if they visited the psychiatrist on all four occasions (week -1, 0, 12 and 24). Completion of EPI for a patient was defined as visiting the nurse on a minimum of 10 occasions. Compliance to acamprosate medication was measured by the presence of

acamprosate in urine by negative ion liquid chromatography – mass spectrometry (qualitative analysis).

An intention to treat (ITT) analysis was performed comparing the two groups with respect to successful treatment, defined as a patient who completed the whole treatment and reduced his or her alcohol consumption by 50% with respect to either the number of drinking days or number of days with heavy drinking using CHI-square analysis.

Lastly, OCDS craving scores were analyzed in a 3 (week 0, 12 and 24) X 2 (EPI, MPI) repeated measurement ANOVA.

4 RESULTS

4.1 STUDY I – ALCOHOL CUE- AND PRIMING-INDUCED CRAVING

In study I, it was investigated if acamprosate attenuated alcohol cue- and/or priming induced craving in alcohol dependent patients.

4.1.1 Primary Outcome Measures

In Figure 4, the Short–DAQ scores collected during cue exposure and alcohol priming tests are displayed. As can be seen, patients reported higher scores following Alcohol Cue session compared to Non-Alcohol Cue session, $F(1,40)=22.46$, $p<0,05$, but no effect of acamprosate treatment was found. Patients also reported higher scores in 15 minutes Post-Alcohol Priming session compared to Pre-Alcohol Priming session, $F(2,39)=12.43$, $p<0.05$.

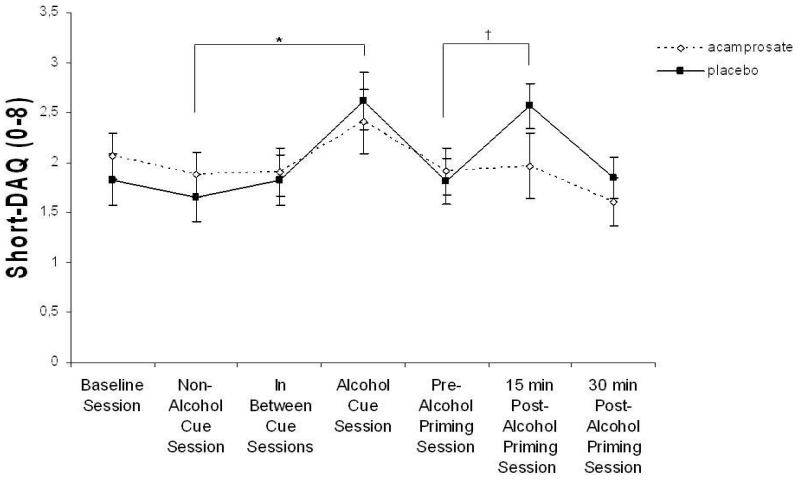

Figure 4. Mean craving (Standard error of the mean (SEM)) scores on Short-DAQ for acamprosate and placebo treated patients on different time-points during test-day. * represents a significant main effect between Non-Alcohol Cue Session and Alcohol Cue Session ($p<0.05$). † represents an interaction effect in that only placebo treated patients reported an increase in craving following an alcohol priming dose ($p<0.05$).

However, this main effect was qualified by a medication interaction effect $F(2,39)=4.34$, $p>0.05$, which is clearly visible in Figure 4. Post-hoc contrast revealed that only placebo treated patients showed an increase in Short-DAQ score in 15 minutes Post-Alcohol Priming session, $F(1,40)=6.96$, $p<0.05$.

VAS-scores of craving showed similar results (Figure 5). Patients reported higher VAS-scores following Alcohol Cue session compared to Non-Alcohol Cue session, $F(1,39)=39.00$, $p<0.05$, but no effect of acamprosate treatment was found. Patients also reported higher VAS-scores in 15 minutes Post-Alcohol Priming session compared to Pre-Alcohol Priming session $F(2,36)=12.65$, $p<0.05$. As for Short-DAQ, this main effect was qualified by a medication interaction effect $F(2,36)=3.16$, $p<0.05$. As can be seen in Figure 5, only placebo treated patients showed an increase in VAS-scores in 15 minutes Post-Alcohol Priming session, $F(1,37)=4.07$, $p<0.05$ (post hoc contrasts).

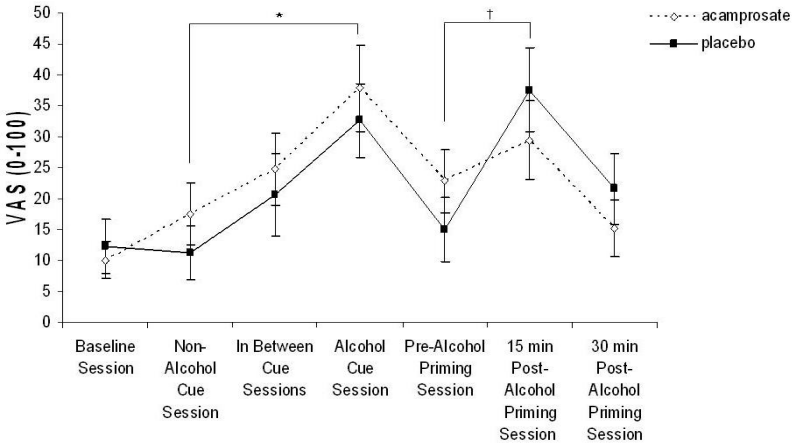

Figure 5. Mean craving scores (SEM) assessed with VAS for acamprosate and placebo treated patients on different time-points during test-day. * represents a significant main effect between Non-Alcohol Cue Session and Alcohol Cue Session ($p<0.05$). † represents an interaction effect in that only placebo treated patients reported an increase in craving following an alcohol priming dose ($p<0.05$).

4.1.2 Secondary Subjective Outcome Measures

No results for secondary subjective measures (PANAS, VAS for withdrawal symptoms, choice model) showed any effects related to acamprosate medication.

4.1.3 Secondary Physiological Measures

The results for GSR and Temp varied as expected by alcohol cue-exposure and priming (an increase in GSR and a concurrent decrease in Temp). However, no effect related to acamprosate treatment was visible. Measures of BP (systolic/diastolic) and pulse revealed no effects of cue presentation or of alcohol priming, and hence no effect of acamprosate treatment was noted.

In contrast, there was an effect of acamprosate treatment on plasma-cortisol levels 15 minutes Post-Alcohol Priming session and 30 minutes Post-Alcohol Priming session compared to Pre-Alcohol Priming session $F(2,32)=3.27$, $p<0.05$ (Figure 6). As can be seen, only placebo patients increased in plasma-cortisol levels following alcohol priming.

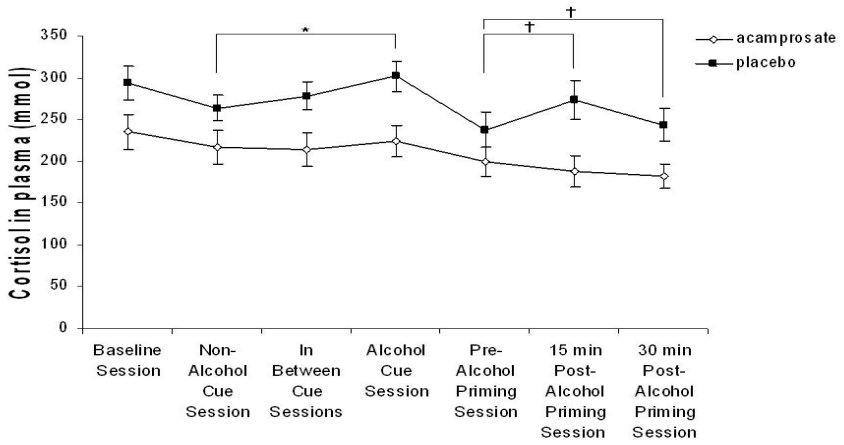

Figure 6. Mean plasma-cortisol levels (in mmol) (SEM) for acamprosate and placebo treated patients on different time-points of measurement during test-day. * represents a significant main effect between Non-Alcohol Cue Session and Alcohol Cue Session ($p<0.05$). † represents an interaction effect in that only acamprosate treated patients reported an increase in cortisol levels following an alcohol priming dose ($p<0.05$).

4.1.4 Alcohol Drinking Control Measures

There were no differences between acamprosate and placebo treated patients in the amount of alcohol consumed in the priming session (87.4% (SD=19.2) for acamprosate and 98.8 (SD=5.6) for placebo patients) or in blood alcohol levels following alcohol priming (2.23 mmol/L (SD=2.1) for acamprosate and 2.78 mmol/L (SD=2.47) for placebo treated patients).

4.1.5 Acamprosate Compliance

Measures of compliance to acamprosate treatment showed that at days 7, 14 and 22 of treatment, all completers showed presence of acamprosate in urine (range 15 – 510 ng/ml, m=171, SD=117). All completers showed presence of acamprosate in plasma at day 22, (m=835ng/ml, SD=487).

4.1.6 Plasma Levels of Acamprosate Correlated to Craving

The results showed a negative correlation between levels of acamprosate in plasma at day 22 of treatment and subjectively experienced craving (Short-DAQ) in 15 minutes Post-Alcohol Priming session ($r=-0.48$, $p<.05$) which is displayed in Figure 7.

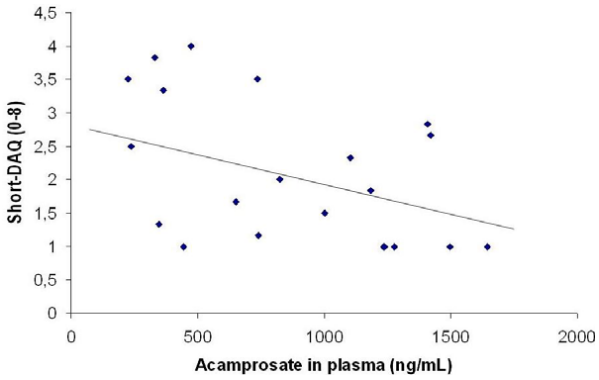

Figure 7. Correlation between subjectively experienced craving following a priming drink and plasma levels of acamprosate (n=20).

Corroborating this result, there was a trend for a similar negative correlation between acamprosate plasma levels and the VAS for craving in the 15 minutes Post-Alcohol Priming session (r=-0.31, p=0.092).

4.2 STUDY II – ALCOHOL CRAVING AND NEUROENDOCRINE MEASURES

In study II, acamprosate effects on alcohol craving and neuroendocrine measures were investigated.

There was a significant overall reduction in OCDS scores between day 1 and day 22, $F(1, 46)=87.7$, $p>0.05$ for both treatment groups (Table 2). Further, an interaction effect of treatment showed that acamprosate treated patients reduced their self reported craving (from 1.72 to 0.65) more than placebo treated patients (from 1.60 to 0.88), $F(1,46)=2.87$, $p<0.05$.

Table 2 also shows an overall reduction in alcohol consumption during study compared to the 90 days prior to inclusion, $F(1,48)=229.48$; $p<0.05$, with no significant differences between treatment groups. There were no differences in the plasma concentration of cortisol, ACTH or β-endorphin, either over time or between treatment groups.

Table 2. Levels of β-endorphin, ACTH, cortisol, and self-reported craving for alcohol (OCDS) are shown for Day 1 and 22, respectively. Heavy drinking (>60 grams of alcohol per day) is expressed as a percentage of 90 days prior to inclusion and of 22 days of treatment. The values represent the mean (SD within brackets).

	OCDS index (0 – 4)		Beta-endorphin		ACTH		Cortisol		Heavy drinking (%)	
	Day 1	Day 22	Day 1	Day 22	Day 1	Day 22	Day 1	Day 22	90 days prior to inclusion	During study
Acamprosate	1.72 (0.49)	0.65* (0.50)	65.7 (52.5)	43.9 (19.5)	0.98 (0.66)	1.40 (1.28)	391.5 (143.9)	316.7 (109.9)	53.1 (21.0)	3.8† (5.8)
Placebo	1.60 (0.28)	0.88* (0.63)	44.9 (21.9)	49.3 (26.7)	0.97 (0.53)	1.15 (0.73)	386.4 (161.3)	322.1 (123.1)	49.5 (20.7)	6.3† (7.2)

* The difference between Day 1 and Day 22 was significant ($p<0.05$). The difference was greater for acamprosate treated patients compared to placebo. † Difference between pre-treatment and during study was significant ($p<0.05$).

Pearson's correlation analysis showed that OCDS scores on day 22 were strongly correlated with heavy drinking during the study, r=0.69, p<0.05 (Table 3). No other correlations were found (including neuroendocrine measures – not displayed).

Table 3. Pearson's correlation analysis for alcohol craving (OCDS) and drinking measures (TLFB) (N=48).

	HD[a] during study	HD 90 days prior to study	OCDS index day 1	OCDS index day 22
HD[a] during study	1			
HD[a] 90 days prior to study	0.12	1		
OCDS index day 1	0.05	0.25	1	
OCDS index day 22	0.69*	0.08	0.22	1

*p< 0.05 level (2-tailed).
[a] HD=Heavy drinking

4.3 STUDY III – PHARMACOKINETICS OF ACAMPROSATE

4.3.1 Development and Validation of the LC – MS Method

A precise and accurate determination of acamprosate levels in plasma was enabled. The limit of detection was 9 ng/mL and the limit of quantification was 33 ng/mL. The accuracy in quantification was high as estimated from quality control samples run together with study samples. Calibration curves were linear over the measuring range 50 – 1000 ng/mL (Figure 8).

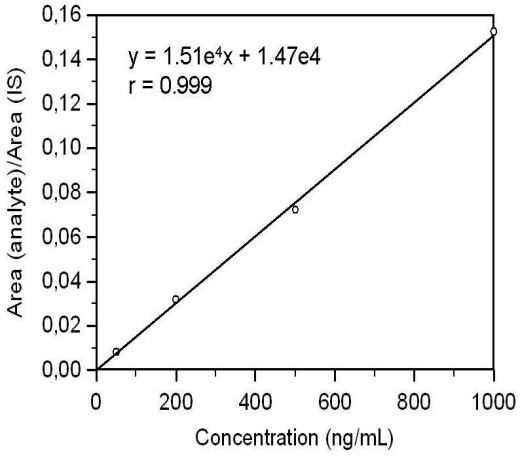

Figure 8. Calibration graph prepared in human plasma showing the linear response in the measuring range 50 – 1000 ng/mL (IS=Internal standard).

A chromatogram obtained from the analysis of an authentic study sample is presented in Figure 9.

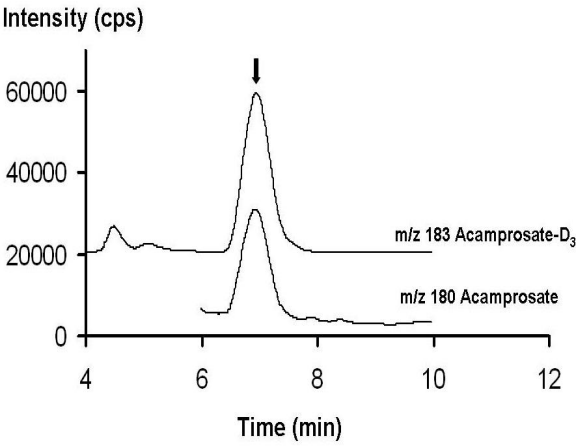

Figure 9. Chromatogram obtained from the LC – MS analysis of acamprosate in a study sample (454 ng/mL) (cps= counts per second). Chromatographic interference was not observed in any sample.

4.3.2 Pharmacokinetic Variables

Results from the pharmacokinetic modeling are shown in Figure 10. Steady state of acamprosate plasma levels were approximately reached 5 days following start of administration with a degree of fluctuation within the range 760 to 915 ng/mL. The plasma concentration was below limit of quantification (33 ng/mL) 4.5 days after administration of the last dose.

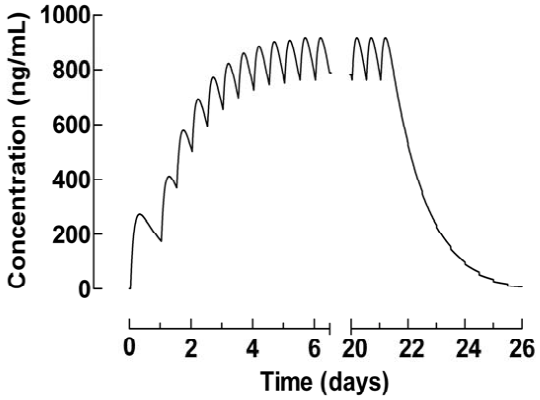

Figure 10. Simulated plasma concentration time curve during the entire study period using all (256) plasma concentration data from all thirteen healthy volunteers.

Pharmacokinetic parameters for acamprosate are displayed in Table 4. Note that pharmacokinetic data are based on the first dose of acamprosate (i.e. drug naïve subjects at day 1).

Table 4. Pharmacokinetic parameters of acamprosate and demographics of subjects.

	Mean	SD	Median	range	Coefficient of Variation (%)[a]
Height (cm)	177	9	177	170 – 200	4.85
Weight (kg)	74.6	10.1	74	62 – 95	13.60
BMI	23.7	1.6	23.70	20.7 – 26.8	6.9
Age	26	4	26	20 – 34	15
AUC[b] (ng/mL/h)	10120	3022	8824	6960 –16378	29.9
T½[c]	20.4	7.6	21.7	9.9 – 30.4	37.32
Tlag (hrs)[d]	1.6	1.2	1.9	0 – 3.1	79.4
Tmax[e]	8.95	3.3	7.7	4.5 – 15.2	36.9
Cmax[f]	272.7	69	286.8	141.9 – 419	25.3
Cmax/mg/kg	20.4	5.9	19.75	9.1 – 31.0	28.8
AUC/mg/kg	752.8	234.5	670.6	463.5 –1174.2	31.2

[a] SD/Mean
[b] Area Under the Curve
[c] Half life of acamprosate
[d] Time to detection of acamprosate in plasma from drug naïve state
[e] Time to maximum plasma concentration
[f] Maximum plasma concentration

Acamprosate concentrations in CSF were analyzed from seven of the subjects. Acamprosate was detected in all samples but the levels were below the limit of quantification, i.e. between 9 – 33 ng/mL. An attempt to improve sensitivity confirmed the presence of acamprosate in CSF but failed to enable the quantification (see main article).

4.4 STUDY IV – PSYCHOSOCIAL INTERVENTIONS

In this study, drinking patterns were compared for acamprosate treated patients receiving either MPI or EPI.

4.4.1 Primary Outcome Measures

The results showed that patients generally decreased in the percentage of heavy drinking days during treatment compared to 90 days before inclusion $F(2,68)=49.4$, $p<0.05$, with no significant difference between treatment groups $F(1,34)=3.6$, $p=0.07$ (Table 5).

Table 5. Alcohol drinking patterns over time for EPI and MPI patients. Results are shown for completers (the three columns to the left) and for non-completers (the three columns to the right). Non-completers are patients who did not visit the psychiatrist at week 12 and/or at week 24. Note that the range of *n* varies for non-completers because fewer patients gave consumption data for 24 weeks than for week 0 and week 12 respectively.

	EPI (completers; n = 22)	MPI (completers; n = 20)	Total (completers; n = 42	EPI (including non-completers; n = 22–29)	MPI (including non-completers; n = 20–32)	Total (including non-completers; n = 42–61)
Prior to inclusion						
Days with heavy drinking (%)	45.6	54.5	50.1	50.9	59.6	55.5
Days with drinking (%)	56.2	60.0	58.1	61.1	66.3	63.9
Week 0–12						
Days with heavy drinking (%)	10.6	18.3	14.2	15.8	20.8	18.2
Days with drinking (%)	21.1	28.3	24.4	28.0	34.1	30.9
Time to first drink (days)	32.7	25.8	29.3	29.6	26.9	28.2
Week 13–24						
Days with heavy drinking (%)	20.2	24.6	22.4	22.6	29.4	26.1
Days with drinking (%)	33.1	31.1	32.1	38.4	38.5	38.4

The same pattern was shown for the second primary outcome measure "percentage of days with any drinking", with a general reduction of alcohol consumption over time $F(2,68)=36.1$, $p<0.05$, but no effect of psychosocial treatment. Further, the results showed no difference between the two groups in time to first drink.

4.4.2 Secondary Outcome Measures

Table 5 show results where non-completers are included (the three right columns), i.e. patients who did not visit the psychiatrist at week 12 and/or week 24 ($n = 19$). Just as in the analysis of completers only, there were no differences between the two groups in alcohol consumption when non-completers were included.

The ITT analysis on successful outcome showed that 26 of the 70 subjects (37%) were successfully treated according to definition with no difference between the EPI and the MPI groups (Table 6).

Table 6. Distribution of patients in MPI and EPI according to successful outcome of treatment (defined as a 50% reduction in the number of heavy drinking days or a 50% reduction in the number of days with any drinking days as well as completion of the whole study).

	BPI (n=34)	MPI (n=36)
Successful treatment	44% (n=15)	31% (n=11)
Not successful treatment	56% (n=19)	69% (n=25)

In the analysis of OCDS craving scores, a general reduction in craving was found between week 0 and 24 from 1.9 to 1.0 (SD=0.54) $F(2,31)=16.8$, $p<0.05$, but no effect of treatment (not reported in main article).

Analysis of compliance to acamprosate treatment revealed no differences between treatment groups as measured by presence of acamprosate in urine at week 12 and 24 (Table 7).

Table 7. Results for compliance to acamprosate for patients in EPI and MPI. The figures show the number of patients who showed positive or negative tests for acamprosate in the urine at week 12 and 24.

	EPI		MPI	
	Pos	Neg	Pos	Neg
Week 12	24	3	24	4
Week 24	17	3	17	5

5 DISCUSSION

5.1 MAIN FINDINGS ON ALCOHOL CUE- AND PRIMING-INDUCED CRAVING

In study I, it was shown that pretreatment with acamprosate attenuated priming-induced craving in alcohol dependence patients. This suggests that one therapeutic mechanism of acamprosate may consist of the modulation of craving responses, thereby preventing a slip from becoming a full relapse. To our knowledge, this effect has not previously been demonstrated. It is in agreement with previously published results from some clinical trials where it was found, as a secondary outcome, that acamprosate shortened the duration of relapse (Sass, Soyka et al. 1996; Tempesta, Janiri et al. 2000; Gual and Lehert 2001). In addition, a meta-analysis of relapse patterns comparing acamprosate treatment with placebo showed that among those who relapsed to alcohol abuse, acamprosate treated patients reduced their quantity and frequency of drinking compared to placebo (Chick, Lehert et al. 2003). Together, these results are consistent with the main finding of the present laboratory study and imply that acamprosate may attenuate the rewarding effects of alcohol. The precise mechanism behind acamprosate's modulation of the reinforcing effects of alcohol remains to be fully understood. However, studies in rats points to possible explanations. Several research groups have shown that acamprosate modulates dopamine release following alcohol administration (Olive, Nannini et al. 2002; Cano-Cebrian, Zornoza-Sabina et al. 2003; Cowen, Adams et al. 2005), a response known to be involved in the reinforcing effects of alcohol (Di Chiara and Bassareo 2007). The results of the present laboratory experiment may be a clinical illustration of this phenomenon.

Study I showed that the degree of alcohol priming-induced craving may correlate with the acamprosate plasma concentrations. This result is in line with results from clinical trials that have provided preliminary evidence for a dose-response effect of acamprosate (Paille, Guelfi et al. 1995; Pelc, Verbanck et al. 1997; Mason, Goodman et al. 2006). A laboratory based model to study dose-response effects of acamprosate in dependent patients is warranted to further investigate this effect.

Placebo treated patients showed elevated HPA axis activity following a priming drink, while no such response was found for acamprosate treated patients. This result further corroborates the main finding of study I, i.e. that acamprosate acts as a modulator on alcohol priming-induced craving. It remains to be elucidated, however, if the co-existent modulation of HPA axis activity and attenuated craving responses by acamprosate represent a causal relationship or if it is an epiphenomenon reflecting some underlying factor.

No effects of acamprosate treatment on cue induced craving was found, corroborating the only previous randomized controlled study (Ooteman, Koeter et al. 2007). However, when aiming for a naturalistic laboratory study, a possible weakness of the design was exposed in that it did not control for the effect of acamprosate on taste cue-induced craving. The taste of alcohol has been shown to elicit activity in the mesolimbic dopamine system alongside a concurrent increase in craving (Filbey, Claus et al. 2008). A design with intravenous alcohol administration (Zimmermann, Mick et al. 2008) would control for the taste factor. However, the naturalistic design would then be hampered. A post hoc analysis showed a correlation between blood alcohol levels 15 minutes post alcohol priming and Short-DAQ responses at the corresponding time-point ($r=0.41$, $p<0.05$). If the treatment effect noted in study I would reflect taste cue-induced craving, then this correlation would probably not have been found. In

addition, when analyzing treatment groups separately it was found that only placebo treated patients showed a significant correlation between blood alcohol levels and craving ($r=0.51$, $p<.05$) while acamprosate treated patients showed no correlation. In summary, this finding further supports the hypothesis that acamprosate attenuates alcohol priming-induction of craving.

5.2 MAIN FINDINGS ON CRAVING AND NEUROENDOCRINE MEASURES

Study II showed that three weeks of standard acamprosate treatment reduced alcohol craving in alcohol dependent patients. Further, the correlation between OCDS scores following 21 days of treatment and the self-reported alcohol use during the trial corroborates the hypothesis that craving for alcohol predicts alcohol consumption (Verheul, Lehert et al. 2005). Contrary to the expectations however, no effect of acamprosate was found on HPA axis hormones or β-endorphin. Neither did we find any correlation between neuroendocrine responses and alcohol craving or consumption.

There are some limitations to the study that could have influenced the likelihood of detecting any treatment effects on HPA axis responses or β-endorphin. The relatively short treatment time is one, as previous studies have lasted for longer periods of time (Kiefer, Jahn et al. 2006; Kiefer, Jahn et al. 2006). Initial effects of acamprosate on craving and alcohol consumption measures may be related to other mechanisms such as an altered glutamatergic (Dahchour, De Witte et al. 1998) or dopaminergic (Olive, Nannini et al. 2002) state. Further, an earlier study showed that acamprosate's effects on β-endorphin levels were most marked in patients with high alcohol intake (Kiefer, Jahn et al. 2006). In the present study, no such effect was found, suggesting that patients were more homogenous in this respect.

In conclusion, the study strengthens the hypothesis that acamprosate reduces alcohol consumption through modulation of craving responses.

5.3 MAIN FINDINGS ON THE PHARMACOKINETICS OF ACAMPROSATE

Study III showed that LC – MS can be used for pharmacokinetic studies of acamprosate. Methodological advantages for LC – MS compared to previous LC and GC – MS detection methods (Chabenat, Ladure et al. 1987; Girault, Gobin et al. 1990) suggests that the present method should be considered for clinical laboratory use, e.g. to improve medication compliance monitoring.

With regard to pharmacokinetic measures in plasma, the results largely agreed with previous research (Saivin, Hulot et al. 1998; Mason, Goodman et al. 2002; Johnson, O'Malley et al. 2003; Zornoza, Cano et al. 2003). Differences may be attributed to dosing regimen, treatment duration, timing of plasma sampling and study population.

The finding that plasma levels of acamprosate seem to remain stable for some time post last dose has not been reported previously. Hence, treatment efficacy may not be dramatically affected if a patient temporarily misses to take the medication. However, our results raise questions concerning individualization of treatment. Although the variation is comparable to e.g. naltrexone, there still are considerable individual differences. Drawing on the finding in study I of a plasma level-dependent craving response in acamprosate treatment, it may be important to maintain a stable acamprosate dosing (Paille, Guelfi et al.

1995; Pelc, Verbanck et al. 1997; Mason, Goodman et al. 2006; Hammarberg, Jayaram-Lindstrom et al. 2009).

It may be argued from the results that the time lag for treatment efficacy noted in acamprosate studies (Sass, Soyka et al. 1996) may depend on pharmacodynamic rather than pharmacokinetic properties of acamprosate. First, there is a delay in time to reach steady state plasma levels and in the next step a delay for acamprosate to enter the brain compartment to a sufficiently high degree. Our results concerning the levels of acamprosate in CSF verifies that acamprosate crosses the blood brain barrier in humans, and that the levels of acamprosate in CSF is comparable to what has been found to have an effect in the brain following systemic administration in rats (Burattini, McGeehan et al. 2008; Ericson 2009). This result indicates that acamprosate exert its effect within the CNS. However, a peripheral mechanism for acamprosate may not be excluded. For example, as previously mentioned, studies in rats and humans have shown that acamprosate modulates neuroendocrine responses, which may indicate an effect of acamprosate on pituitary or adrenal gland secretion (Zalewska-Kaszubska, Cwiek et al. 2005; Kiefer, Jahn et al. 2006; Kiefer, Jahn et al. 2006; Zalewska-Kaszubska, Gorska et al. 2008).

5.4 MAIN FINDINGS ON PSYCHOSOCIAL INTERVENTIONS

The results did not support that EPI would be more effective than MPI, in combination with 24 weeks of acamprosate treatment, in reducing heavy drinking, any drinking, time to first drink, or with regards to definition of a successful treatment outcome.

There are a number of potential pitfalls in evaluating studies of psychosocial interventions in conjunction with a pharmacological treatment. One is the large variation in the number of sessions provided for the patients in different studies, making direct comparisons difficult. Another is a variation in quality concerning the non-active treatment arm. In study IV, the fact that the physician was an experienced specialist within the field might have compromised the results by contributing to a high degree of effectiveness of the MPI, hence reducing the differences between the treatment arms. Further, the concomitant pharmacological treatment (acamprosate in this case) may be too efficacious for additional treatment effects to emerge. To control for this latter confounding factor, a placebo arm for acamprosate treatment is warranted, although outside the scope of the aims of study IV.

Together with previous research, the evidence for an additional effect of an intensive psychosocial intervention to acamprosate treatment is weak (De Wildt, Schippers et al. 2002; Pelc, Hanak et al. 2005; Reid, Teesson et al. 2005). The results are in line with the findings of e.g. the COMBINE study (Anton, O'Malley et al. 2006) in that the combination of psychosocial and pharmacological treatments did not improve the treatment effect. The finding of study IV is important, not least on ethical and economical grounds. Arguably, a less expensive treatment, which saves time and resources both for the individual and for the treatment provider, and which infringes less on the individual's integrity, should be first line treatment when there are no differences in efficacy measures.

6 CONCLUSIONS

Study I:
- Acamprosate attenuated alcohol priming-induced craving, suggesting a reduction of the reinforcing effects of alcohol
- The efficacy of acamprosate to attenuate alcohol-priming induced craving varied with plasma levels
- Acamprosate modulates HPA axis responses following alcohol-priming

Study II:
- Acamprosate reduced general alcohol craving
- Craving correlated with alcohol consumption
- No effects of acamprosate were found in any of the neuroendocrine measures

Study III:
- LC – MS is a valid and reliable method for pharmacokinetic measurement of acamprosate
- Steady state plasma levels of acamprosate is reached within 5 days
- Acamprosate crosses the blood-brain barrier and the concentrations estimated in CSF may be considered as pharmacologically relevant

Study IV:
- EPI showed no beneficial effect in reducing alcohol consumption compared to MPI for acamprosate treated patients

7 ACKNOWLEDGEMENTS

"If I would do it all over again?
Yes, why not, why not –
I would do it slightly different"
(Freddie Mercury)

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