Placebo, alcohol and flumazenil provocations: Subjective and objective registrations in psychopharmacological experiments

Lars Saxon
ABSTRACT

Abuse and dependency of alcohol or drugs is a major problem today, with severe implications for society as well as for the individual. Treatment is often characterised by hard work and the road to recovery is (among other problems) paved with acute and protracted withdrawal symptoms as well as relapse. Lately there has been an increased interest, and success, in the pharmacological treatment of dependency of different substances (e.g. acamprosate and naltrexone for alcohol, methadone and buprenorphine for opioids and possibly naltrexone for amphetamine and flumazenil for benzodiazepines).

Essential for the investigation of promising substances are valid and reliable tools for registration of relevant subjective and objective effects during experimental treatment. Therefore, the aim of this work was to find and test suitable tools, and to study different psychopharmacological consequences, in experiments designed to affect acute and/or post-acute effects of benzodiazepines and alcohol.

To study the objectives I was interested in I have performed two studies with intravenous provocations, one with placebo and alcohol and one with flumazenil, resulting in five separate reports on different topics.

In this thesis, I have highlighted the importance of taking gender as well as placebo effects in account when performing pharmacological provocations on subjects treated for benzodiazepine dependence. Further, I have shown that it is possible to register acute tolerance to alcohol with an objective test previously not used in this context. I have also shown that it was possible to measure pharmacologically induced reductions in symptoms of benzodiazepine withdrawal using subjective and objective registrations. In addition, I have found that a reduction of self-rated withdrawal related aggression could be measured after intravenous flumazenil. Finally, I have pointed to the risk for loss of important information when creating indexes of multiple measures.
LIST OF PUBLICATIONS

The present thesis is based on the following papers:


3.3.2.1 Infusions of alcohol ............................................ 18
3.3.3 Assessment.............................................................................................................................. 19
  3.3.3.1 Study II................................................................. 19
  3.3.3.2 Study V...................................................................... 20
3.3.4 Data analysis .......................................................................................................................... 21
  3.3.4.1 Study II................................................................. 21
  3.3.4.2 Study V...................................................................... 22
4 RESULTS AND DISCUSSION........................................................................................................ 23
  4.1 Study I..................................................................................................................................... 23
    4.1.1 Groups at base-line ........................................................................................................... 23
    4.1.2 Effects of placebo on subjective ratings ........................................................................... 23
    4.1.3 Effects of placebo on objective measures ......................................................................... 24
  4.2 Study II .................................................................................................................................... 27
    4.2.1 Alcohol habits.................................................................................................................... 27
    4.2.2 Alcohol concentrations .................................................................................................... 27
    4.2.3 Effect of alcohol on Reaction Time .................................................................................. 28
    4.2.4 Alcohol-related individual differences on performance ............................................... 29
  4.3 Study III .................................................................................................................................. 30
    4.3.1 Effects of flumazenil on subjective ratings ...................................................................... 31
    4.3.2 Effects of flumazenil on objective ratings ........................................................................ 33
  4.4 Study IV................................................................................................................................... 34
  4.5 Study V .................................................................................................................................... 35
    4.5.1 Alcohol concentrations .................................................................................................... 36
    4.5.2 Alcohol concentrations and ratings of mood ..................................................................... 36
    4.5.3 Alcohol habits and ratings of mood .................................................................................. 36
    4.5.4 Alcohol effects on uni- and bipolar ratings of mood ...................................................... 37
5 SUMMARY AND CONCLUSIONS .................................................................................................. 41
  5.1 Summary:.................................................................................................................................. 41
  5.2 Conclusion: ................................................................................................................................ 42
6 ACKNOWLEDGEMENTS............................................................................................................ 43
7 REFERENCES................................................................................................................................. 44
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BAC</td>
<td>Blood alcohol concentrations</td>
</tr>
<tr>
<td>BEC</td>
<td>Breath ethanol concentration</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>HSD</td>
<td>Honestly significantly different</td>
</tr>
<tr>
<td>M</td>
<td>Mean</td>
</tr>
<tr>
<td>MACL</td>
<td>Mood adjective check list</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>PSAP</td>
<td>Point subtraction aggression paradigm</td>
</tr>
<tr>
<td>RT</td>
<td>Reaction Time</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the means</td>
</tr>
<tr>
<td>sMACL</td>
<td>Swedish mood adjective check list</td>
</tr>
<tr>
<td>TBW</td>
<td>Total body water</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

1.1 Subjective and objective registrations in psychopharmacological experiments.

Soon after I had begun to work clinically in the field of alcohol and drug dependency I was introduced to the two-volume work *Actions of alcohol* by Henrik Wallgren and Herbert Barry (1970). In the beginning of the first volume there is a sentence that serves as a good starting point for the work presented here.

“The extensive use and misuse of alcoholic beverages provides a powerful incentive for acquiring accurate knowledge about this drug but also gives rise to obstacles against such knowledge” (Wallgren and Barry, 1970).

Obstacles might be of many different sorts, religious, psychological, medical, political as well as of technical or practical nature related to the tools at hand when doing research. The present work will focus on these last obstacles, namely registrations of subjective and objective effects during provocations with placebo, alcohol and flumazenil. All study of humans (clinical or in research) use some sort of measurement. It may be subjective, by the subject itself or by some external observer, or different objective tools might be used. Of fundamental importance, regardless of if the observation done is subjective or objective, is that the tools we use are valid and reliable. In the work presented here I have used well-established objective tools, e.g. physiological measures and reaction time, as well as validated and newly constructed subjective measures in the study of different effects in psychopharmacological provocations.

1.2 Placebo

Placebo, (literally "I will please"), has been defined as "a substance having no pharmacological effect but given to placate a patient who supposes it to be a medicine." (Webster's new twentieth century dictionary of the English language, 1979) and its use in the treatment and study of somatic and psychological conditions has a long tradition (Strauss and von Ammon Cavanaugh, 1996). In their classic work, Beecher *et al.*, (1953) identified a group of subjects that they labeled "placebo reactors" who consistently had a therapeutic response to placebo. "Placebo reactors" were later described as being more anxious and outgoing, less mature, and more somatically
preoccupied than "placebo non-reactors" (Lasagna et al., 1954). It has since been suggested that certain personalities may be more prone to respond to placebos (Moertel et al., 1976), but the literature is inconsistent regarding who these “placebo reactors” are, or if they have a particular personality type (Hahn, 1985; Shapiro and Shapiro, 1997a). Some features have however been described with some consistency. Responders “tend to be more communicative, more socially responsive, and more trusting and confident in their physicians than non-responders” (Crenshaw Rawlinson, 1985). Frequency of effect varies between 30 and 50% of treated patients (Beecher, 1965; French, 1990). In drug trials for different psychiatric disorders, however, response rates varied between 0 and 51% according to Strauss and von Ammon Cavanaugh (1996). It seems further, that people are not consistent in their placebo responses (Lieberman 1964; Todd 1987; Turner 1994). Jensen and Karoly (1991) found that motivation had a positive influence on the placebo effect and that expectation had a greater effect the first time placebo was given.

1.3 Placebo and gender

Usually, no difference between males and females in frequency of placebo responders is found (Lasagna et al., 1954; Moertel et al., 1976; Spiro, 1986). Gender differences may however be found under special conditions, such as manipulation of expectation (Jensen and Karoly, 1991), or in special populations, such as subgroups of depressive disorders (Bialik et al., 1995; Frank et al., 1999). In their study on the effects of motivation on the placebo effect, Jensen and Karoly (1991) found that their manipulation of expectation "primarily affected females".

1.4 Placebo and benzodiazepine withdrawal

Besides the important role placebo-reactions play in favorable responses to medication (Shapiro and Shapiro, 1997a) there are also placebo-reactions to (actual or supposed) reduction of medication. For instance, it has been shown to be possible to neutralize the effect of an opioid analgesic with placebo if the patients were informed that the injection might contain an opiate antagonist (Butler et al., 1983). Winokur and Rickels (1981) described a case of pseudowithdrawal during unchanged doses of diazepam in a patient who participated in a study of benzodiazepine withdrawal reactions. Tyrer et al. (1983) reported of withdrawal symptoms in about 20% of patients who falsely believed that their dose was gradually reduced. Reactions of pseudowithdrawal highlight the psychological component in dependency and in
withdrawal, and point to the importance of placebo-controlled studies. This also raises questions of whether expectation and placebo-effects influence symptoms of benzodiazepine withdrawal and could have implications for the important psychological parts of the treatment for benzodiazepine dependency (Tyrer et al, 1985; Sanchez-Craig et al, 1986; Crouch et al, 1987).

1.5 Placebo in pharmacological experiments

One of the first controlled clinical trials was performed by Lind in 1747 when he compared the efficacy of lemon juice with that of other remedies in preventing scurvy (Lind, 1753). In the 19th century the use of placebo was introduced when Flint (1863) tested the effectiveness of different drugs for rheumatism by putting patients on a “placeboic remedy” and then observed the course of the disease. The use of a control-group had yet to be introduced so the patients served as their own controls. The first example of randomization might have been when Johannes Fibiger in 1898 “allocated patients by day of admission” (Hrobjartsson et al., 1998). The use of single-blind and double-blind placebo controlled studies was introduced in 1931 (Amberson et al. 1931) and 1933 (Evans and Hoyle, 1933) and the, often described as the, first example of the subsequent “gold standard” (the randomized controlled trial) was published in 1948 (Doll, 1998).

1.6 Placebo and subjective and objective registrations

Almost every possible measure of effect has been used in the direct or indirect (e.g. placebo control) study of placebo effects. In a review-article on placebo compared to no treatment it was suggested that effects could be divided into binary or continuous and that these in turn could be subjective or objective (Hrøbjartsson and Gøtzsche, 2005). Interestingly it was more common to use subjective measures of outcome (102 of 156 studies) and the only significant effect was found for subjective continuous measures.

1.7 Alcohol

Beverages containing alcohol (Arabic: al khul) have been manufactured for consumption as well as for religious and medical purposes since the late Stone Age at least (Patrick, 1952). The most commonly reported reason for consuming alcohol is relaxation (Hall, 1996) and this is in line with reviews over reported psychological benefits associated with moderate alcohol-use that lists effects such as stress reduction,
subjective/mental health, mood enhancement, sociability, cognitive performance etc. (Baum-Baicker, 1985; Peele and Brodsky, 2000).

1.8 Alcohol dependence

The downside of alcohol consumption is among others its well-known dependency-producing properties. Alcoholism was defined as a chronic, relapsing disease by Dr. Magnus Huss in his monograph "Alcoholismus chronicus" (Magnus Huss, 1851). In a 2002 review it was estimated that approximately 700 000 individuals in Sweden had an alcohol consumption high enough to be risky and that 300 000 were alcohol dependent (Andreasson, 2002).

1.9 Alcohol and acute tolerance

That the effects of alcohol, at any given blood alcohol concentration, are greater when the concentration is rising than when the same concentration is reached again as the level falls is well described (Beirness and Vogel-Sprott, 1984; Bennett et al., 1993; Hiltunen and Järbe, 1990). This recovery-effect within a single episode of intoxication (Tabakoff et al., 1986) has been named the Mellanby-effect (Mellanby, 1919) acute tolerance (Bennett et al., 1993), or acute recovery effect (Vogel-Sprott, 1979). Several studies have shown that acute tolerance is influenced by factors such as learning, alcohol dose, drinking history, method of measuring alcohol concentrations, as well as the kind of performance studied (Bennett et al., 1993; Hiltunen, 1997a; Hiltunen, 1997b; Hiltunen and Järbe, 1992; LeBlanc, 1975; Portans et al., 1989; Vogel-Sprott, 1979).

Acute tolerance at a steady state concentration of alcohol has been questioned, although it has been demonstrated in many single-dose studies (Kaplan et al., 1985). An early study (Mirsky et al., 1941), showed that the concentration at which intoxication diminished was higher than the threshold-level for signs of alcohol intoxication. This study was however criticised (Kaplan et al., 1985) for its design, the drinking history of the subjects, and the lack of objective measures.

1.10 Objective registrations of acute tolerance to alcohol

Some support for acute tolerance during steady-state concentration of alcohol has however been found. Loomis and West (1958), found that during repeated alcohol administrations, impairment in simulated driving ability was not changed during the 3 to 5 hours of a maintained alcohol concentration compared to the first hour, although
some of the subjects reported less intoxication. In Loomis and West’s study tests were
carried out on four occasions, and therefore possibly confounded by the repeated
administrations of alcohol. As has been suggested by others, the development of
chronic tolerance could interfere with the expression of acute alcohol tolerance
(Hiltunen, 1997a; Hiltunen, 1997b; Portans et al., 1989). Kaplan et al. (1985) studied
the development of acute tolerance for word recall when the effect of steady-state
concentrations of alcohol was investigated over the course of 6 hours. No effect was
found, however, on measures of standing steadiness, manual tracking, or subjective
measures of sedation and intoxication.

1.11 Benzodiazepines

In 1960 the first benzodiazepine (chlordiazepoxide) was introduced and then
followed by the more (in)famous diazepam in 1963. Binding sites for
benzodiazepines (the GABA/benzodiazepine receptor) were identified in 1977
(Braestrup and Squires). There was a dramatic increase in the use of benzodiazepines
from 1960 to the first half of the 70:s. In 1975 anxiolytics and hypnotics accounted
for 10% of the prescriptions in the USA. Similarly, benzodiazepines rapidly became
popular in Sweden. Between 1991 and 2002 the sales of benzodiazepines increased
by 20% (Centralförbundet för alcohol- och narkotikaupplysning, 2004).

1.12 Benzodiazepine dependence

Dependency producing properties of benzodiazepines was reported one year
after the introduction of the first benzodiazepine (Hollister et al., 1961). This has
since then been supported by studies both in animals (Petursson and Lader 1981a;
Rosenberg and Chiu 1985) and humans (Covi et al., 1973; Lader, 1983; Tyrer et al.,
1981). Benzodiazepine dependency can be divided in three types. 1) Dependency on
high doses with tolerance may develop in patients who consume larger than
therapeutic doses over a long period of time, often several years (Lader, 1983;
Petursson and Lader, 1981b). 2) Dependency on low doses may appear after
consumption of therapeutic doses during a usual treatment period (Covi et al., 1973;
dependency can cause anxiety and/or depression without physical symptoms and
1.13 Benzodiazepine withdrawal

In 1973 Covi et al. described withdrawal reactions after discontinuation of treatment with chlordiazepoxide. A number of symptoms may appear during benzodiazepine withdrawal (Ashton, 1991; Marks, 1985). Most symptoms are, however, not specific for benzodiazepine withdrawal, which have caused some authors to emphasise the emergence of new symptoms as indicating a withdrawal syndrome (Owen and Tyrer, 1983; Petursson and Lader, 1981b; Tyrer and Seivewright, 1984). Symptoms of special interest are those that are uncommon in anxiety syndromes and not present prior to the consumption of benzodiazepines. Ashton (1991) suggests that these symptoms include hypersensitivity to sensory stimuli, paresthesias, extreme dysphoria, as well as visual hallucinations, distorted body perception, psychotic reactions, formication, muscle fasciculation and twitches. Furthermore, depersonalisation and derealisation is unusually frequent. In addition, it is difficult, both for patients and clinicians, to differentiate between reappearance of symptoms present before medication and withdrawal symptoms or rebound effects (Petursson and Lader, 1981b).

Treatment of benzodiazepine dependency is usually treated by either an abrupt discontinuation of medication or by tapering of the benzodiazepine dosage. A rapid decrease in dosage has been reported to cause significantly more severe symptoms compared to a gradual decrease (Busto et al., 1986; Sanches-Craig et al., 1987). Abrupt discontinuation of intake of benzodiazepines with short half-lives causes more severe distress than when medication is tapered off. For benzodiazepines with longer half-lives there may be no important difference between the two methods (Schweizer et al., 1990). Tapering, in combination with supportive therapy, usually helps to keep withdrawal distress at a reasonable level, which is vital, as higher levels of withdrawal distress tend to increase the risk for treatment failure and relapse (Bergman et al. 1989). A third treatment-strategy based on flumazenil, a partial benzodiazepine agonist with low intrinsic activity at GABA/benzodiazepine receptors, has been proposed (Savic et al., 1991).

1.14 Subjective and objective registrations of benzodiazepine withdrawal

Self-rating scales have been used with the intent to measure and describe the existence and characteristics of benzodiazepine withdrawal (Busto et al., 1989; Tyrer et al., 1990). The scale constructed by Tyrer et al. (1990) was recently used in a study (Gerra et al., 2002) investigating treatment of benzodiazepine withdrawal with
flumazenil, oxazepam or placebo and could differentiate between ratings made during the different conditions. In the same study blood pressure and heart-rate was monitored and was proved useful in describing changes during the course of treatment.

1.15 Flumazenil

Flumazenil, a partial benzodiazepine agonist with low intrinsic activity at GABA/benzodiazepine receptors, is well-tolerated (Darragh et al., 1983a) and initial studies in animals showed flumazenil to be devoid of intrinsic actions (Costa et al., 1983; Darragh et al., 1983b; Hünkeler et al., 1981) (Figure 1). Subsequent studies in animals and humans have, however, shown flumazenil to have agonist-like and inverse agonist-like effects depending on test-situation and dose. At doses higher than 30 mg effects usually are partially agonistic (File and Pellow, 1986; Higgit et al., 1986; Lloyd et al., 1981) while at doses lower than 30 mg effects were often antagonistic to those produced by benzodiazepines (File and Pellow, 1986). Flumazenil has further been suggested to have agonistic and inverse agonistic properties at the same time (De Vry and Slangen, 1985).

Figure 1. Schematic presentation of some benzodiazepines and their classification.

Reproduced with the kind permission from the authors and the publisher (Litton and Hall Läkartidningen, 1993.

High doses (30-600 mg orally) of flumazenil administered to healthy individuals who have not consumed benzodiazepines may (Higgit et al., 1986) or may not (Darragh et al., 1983b; Lupolover et al., 1984) produce benzodiazepine-like psychomotor or psychotropic effects, whereas effects at lower doses (< 30 mg) are often the opposite to those produced by benzodiazepines. Individuals who are on benzodiazepines might react with withdrawal symptoms if given flumazenil. However, in one study benzodiazepine tolerant patients only reported mild symptoms ("a
shivering sensation") 1-2 minutes after i.v. injection of 1.5 mg flumazenil (Savic et al., 1991).

1.16 Subjective and objective registrations of effects of flumazenil

Different subjective and objective methods have been used in the study of effects of flumazenil. A good example of this is Higgit et al.’s (1986) report on the effect when a number of methods were used. Of special interest here is the use of blood pressure and heart rate as well as self ratings. Systolic and diastolic pressure fell and the heart rate decreased after two doses (30 mg and 100 mg) of flumazenil compared to placebo. Self ratings of mood and bodily symptoms showed that subjects reported more discontentedness, sadness, antagonism and withdrawal during flumazenil than during placebo. Negative mood reactions have also been reported by others (Darragh et al., 1983a; Schöpf et al., 1984). Bodily symptoms that were self rated during flumazenil comprised sweating, shaking, breathing-difficulties, problems in focusing and feelings of being slowed down.

1.17 Flumazenil and benzodiazepine withdrawal

In benzodiazepine-dependent rats, flumazenil induces fewer and less severe withdrawal symptoms than those seen in ordinary withdrawal without flumazenil (File and Baldwin, 1987; McNicholas and Martin, 1982). Periodic administration of flumazenil to benzodiazepine dependent primates has been shown to reduce symptoms during subsequent withdrawal (Gallager et al., 1986). In humans, infusion of 0.2-2.0 mg flumazenil reduced psychological and physiological symptoms that had persisted for months to years after benzodiazepine withdrawal (Lader and Morton, 1992). Thus, responses to flumazenil may have favourable effects in subjects previously treated for benzodiazepine dependency compared to control subjects. Gerra et al., (2002) compared flumazenil to oxazepam tapering and found that flumazenil reduced both withdrawal symptoms and craving.

If shown to be consistent, effects like those mentioned above could be useful in both the diagnosing and treatment of benzodiazepine-dependency. Difficulties in differentiating physical from psychological dependency, as well as withdrawal symptoms from reappearance of original symptoms, when evaluating suspected benzodiazepine dependency might be solved by administration of flumazenil. Further, the suggested capability of flumazenil to affect withdrawal symptoms quantitatively and qualitatively might, as indicated by Lader and Morton (1992), be useful in the
case of treatment-resistant patients. To develop the capacity to endure and cope with somatic and psychological distress in the future is important for patients to experience manageable withdrawal-symptoms during treatment (Sanches-Craig et al., 1987). Flumazenil might, as indicated (McNicholas and Martin, 1982; Savic et al., 1991), facilitate for patients to learn new coping-methods. This, however, lies in the future and of more immediate interest is to increase the knowledge about flumazenil and its effects on protracted symptoms of benzodiazepine withdrawal.

1.18 Benzodiazepines and aggression

Benzodiazepines were initially known for their “taming” properties (Randall, 1960) and moderate to high doses have been shown to reduce aggression in animals while low doses may have an intensifying effect in some situations (Miczek et al., 1994). Paradoxical rage reactions to benzodiazepines are also known to occur in some individuals, both animal and human (DiMascio, 1973).

1.19 Benzodiazepine withdrawal and aggression

Among many other symptoms, benzodiazepine withdrawal has been associated with irritable, hostile and aggressive behaviors in both animals ((Ashton, 1991; Herman et al., 1976; Krsiak et al., 1998; Nath et al., 2000; Votava et al., 2001) and humans (Ashton 1984; Fontain et al., 1984; Hallström and Lader, 1981; Lader, 1984; Lader and Petursson, 1983; Petursson and Lader, 1981b; Owen and Tyrer, 1983). In clinical populations, the incidence of such symptoms ranges from 19 to 75%.

1.20 Flumazenil and aggression

Flumazenil has, in animal studies, been reported to reduce (Allikmets and Rago, 1983; Ostrovskaiia and Molodavkin, 1985; Uhlrova et al., 2004; Ushijima et al., 1984; Vasar et al., 1984), increase (Beck and Cooper, 1986; Rodgers and Waters, 1984) or not/inconclusively affect (Fachinelli et al., 2003; Gourley et al., 2005; Mos et al., 1987; Mos and Olivier, 1986; Polc et al., 1981; Sakaue et al., 2001; Skolnick et al., 1985; Sulcova and Krsiak, 1984) hostility and aggression. Benzodiazepine-naive humans have reported more “antagonism” when given flumazenil compared to placebo (Darragh et al., 1983b; Higgitt et al., 1986). In animals flumazenil has also been shown to decrease the aggression-heightening effects of the GABA_A receptor modulator alcohol (De Almeida et al., 2004; Miczek and Krsiak, 1979; Weerts et al., 1993). One study has investigated the effects of flumazenil on aggressive responding
in humans (Tcheremissine et al., 2005). Doses of flumazenil (2 and 3mg) did not produce statistically significant changes in aggressive responding. There was, however, some individual variation across subjects and this might be related to the previous history of benzodiazepine abuse in the two subjects responding to the provocation.

It has been suggested that consumption of benzodiazepines causes a receptor shift so that agonists become less effective and inverse agonists become more effective (Nutt, 1990). To this theory File and Hitchcott (1990) has added that individual levels of anxiety influence the effect of flumazenil on benzodiazepine withdrawal. They suggested that flumazenil acts as an agonist when anxiety is high and as an inverse agonist when it is low.

1.21 Subjective and objective registrations of effects of flumazenil on aggression during benzodiazepine withdrawal

In the only study of effects of flumazenil on aggression (Tcheremissine et al., 2005) no subjective registrations were recorded. A computerized test (PSAP) that measures individual’ tendency to aggress or avoid provocation was used, but no overall effects of flumazenil was found.

1.22 Mood

Mood has been defined as “a characteristic (habitual or relatively temporary) state of feeling” (Webster's new twentieth century dictionary of the English language, 1979). One line of thinking about mood that has been thoroughly studied since the middle of the last century in the context of exposure of drugs was initiated by Nowlis, and the group around him (Nowlis, 1961; Nowlis and Nowlis, 1956). This work resulted in the mood adjective check list (MACL) for self-reporting. Working on an initial hypothesis of the existence of four fundamental, bipolar, aspects of mood Nowlis ended up with 12 unipolar aspects (Nowlis, 1965). Some of Nowlis assumptions were later questioned (Meddis, 1972; Sjöberg et al., 1979; Svensson, 1977). With the use of a different response scale and a statistical analysis Sjöberg et al., (1979) presented six bipolar aspects of mood (pleasantness/unpleasantness, activation/deactivation, extraversion/introversion, calmness/tension, positive/ negative social orientation and control/lack of control). Of these dimensions of mood pleasantness/unpleasantness, activation/deactivation, calmness/tension are considered to be more basic. This work resulted in the Swedish Mood Adjective Check List (sMACL) (Sjöberg et al., 1979)
consisting of 71 adjectives, which was tested and validated in pharmacological provocations (placebo, alcohol, diazepam and caffeine) (Persson et al., 1980; Svensson et al., 1980).

1.23 Mood and acute alcohol administration

In 1919 Mellanby described that the subjective effects of alcohol differ between increasing and decreasing levels (Mellanby, 1919). Dominantly positive effects have been reported after moderate doses of alcohol (Lindman and Taxell, 1976; Myrsten, 1975) as well as increased positive experiences while negative ones are reduced (Judd, 1977). It has been reported that subjective experiences were of both positive and negative quality while blood alcohol was rising and negative when levels decreased (Babor et al., 1983; Schuckit, 1980).

In addition, subjective effects of alcohol seem to be biphasic in action, being stimulating at low doses and the opposite at high doses (Cameron, 1974; Smith et al., 1975). Further, Persson et al., (1980) studied how three aspects of mood (pleasantness, activation, and calmness) were affected by low to moderate doses of alcohol. At the highest dose (0.85 g/kg body weight), subjects reported increases in all three aspects of mood during the initial phase of intoxication, followed by decreases in self-reported mood, with pleasantness finishing at a base-line level, activation below the base-line and calmness just above the base-line. Lower doses tended to induce more negative feelings. Cameron (1974) also argued that time was an important factor. “We noticed that regardless of actual blood concentrations, subjects reported the same sort of bodily and emotional states at about the same time after the onset of drinking.” In a review of the literature on the biphasic actions of alcohol, Pohorecky (1977) suggested that this effect could be studied in two ways. The first is by comparing the effects of higher and lower doses, and the second is by studying the effects of higher doses over time. It was concluded that both dose and time are important for the biphasic actions of alcohol. Thus, it seems relevant to study the effects during conditions that take in to account both dose and time. One way to do this is to keep subjects steady-state levels of blood alcohol for a reasonable time by intravenous infusion of alcohol.

Intravenous alcohol in the study of mood has a long history, at least since the mid 1930 (Newman, 1935). Reported effects have been positive (Newman, 1935), negative (Warren and Raynes, 1972), mixed (Hartocollis, 1962; Monteiro et al.,
1990) or small (Mayfield and Allen, 1967) with doses of alcohol given intravenously ranging between 0.6 and 0.8 g/kg body weight.

In addition to the above, it seems that the individual’s alcohol habits influence reactions during alcohol intoxication (Morzorati et al., 2002; Persson et al., 1980; Smith et al., 1975).

1.24 Subjective registrations of mood during acute alcohol administration

Different scales have used to measure mood effects of alcohol. One major difference between these scales is how they view mood dimensionally (e.g. bi- or unipolar). Whether mood should generally be regarded and measured as uni- or bipolar has been discussed extensively by Sjöberg et al., (1979). As described above, psychometric support for the bipolar hypothesis was presented by Sjöberg et al., (1979) and the hypothesis is further supported, in the context of alcohol administration by Persson et al., (1980). It has, however, been suggested that it is more useful to use an instrument that measures unipolar experiences when studying effects of alcohol (Earleywine and Erbich, 1996; Earleywine and Martin, 1993; Martin et al., 1993). In their work with the “Biphasic Alcohol Effects Scale” they have shown the importance of the use of a unipolar scale to capture changes in positive and negative experiences associated with different limbs on the blood alcohol curve. Thus it is of interest to investigate if mood best is studied with Sjöberg’s bipolar scale or with its unipolar measures during intravenous alcohol administration.
2 AIMS

2.1 General aims

Essential for the investigation of promising substances are valid and reliable tools for registration of relevant subjective and objective effects during experimental treatment. Therefore, the aim of this work was to find and test suitable tools, and to study different psychopharmacological consequences, in experiments designed to affect acute and/or post-acute effects of benzodiazepines and alcohol.

2.2 Specific aims

Study I To analyze responses to repeated placebo injections in patients treated for benzodiazepine dependency and healthy controls.

Study II To (i) extend the results concerning acute tolerance at steady-state concentrations to include reaction time combined with parallel processing of speed, accuracy and attention, (ii) to compare our earlier results from single dose studies with continuous alcohol administration, and (iii) to evaluate complexity of tests and previous drinking history as predictors of acute tolerance.

Study III To (i) study possible effects of flumazenil when given in cumulative doses to patients treated for benzodiazepine dependency and healthy controls, and (ii) to evaluate if previously benzodiazepine dependent individuals who complain over protracted withdrawal symptoms are helped by flumazenil.

Study IV To analyze the effects of flumazenil on self-rated hostility and aggression in patients previously treated for benzodiazepine dependency and healthy controls.

Study V To (i) study differences between bi- and unipolar subjective effects of intravenous alcohol administration on three aspects of mood (pleasantness, activation, and calmness), and ii) to examine what effect individual alcohol habits had on these variables.
3 MATERIALS AND METHODS

To study the objectives I was interested in two studies were performed resulting in five separate reports (I-V) on different topics.

3.1 Overview of subjects

<table>
<thead>
<tr>
<th>Table 1. Subjects participating in different studies.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
</tr>
<tr>
<td>Study I</td>
</tr>
<tr>
<td>Study II</td>
</tr>
<tr>
<td>Study III</td>
</tr>
<tr>
<td>Study IV</td>
</tr>
<tr>
<td>Study V</td>
</tr>
</tbody>
</table>

3.2 Studies I, III and IV

3.2.1 Subjects

Two groups of 10 individuals were studied. 1) One group of control subjects that consisted of physically and psychiatrically healthy males (n=5) and females (n=5) with an age ranging from 34 to 48 years (mean age 42 years). 2) One group of male (n=5) and female (n=5) patients with a history of benzodiazepine dependence. (Characteristics of this group are shown in original paper III) The majority (8/10) of these individuals had consumed doses that clearly exceeded therapeutic doses (≤30 mg diazepam per day) and had been treated for this by tapering. Patients should have been benzodiazepine free for at least three weeks before the beginning of the study, but still complain about characteristic withdrawal symptoms. One patient had been free from benzodiazepines much longer than the rest (> 5 yrs) but was included in the study because he related his present symptoms to his previous use of benzodiazepines and, when he was evaluated separately, and compared to the patient group as a whole and to the controls, nothing indicated that he should be excluded from the present study.
Potential subjects were excluded if: 1) they used drugs of any kind, 2) they were alcohol dependent, or 3) they had any signs of previous illness or central nervous system damage. All subjects were tested for benzodiazepines and salicylates in their urine and were examined by a physician before each experiment.

Subjects received verbal and written information about the study before giving their consent to participate in the study, which was approved by the local Ethics Committee. Subjects received some financial compensation for participating (i.e. 600 SEK).

### 3.2.2 Procedure

#### 3.2.2.1 Study I:

The provocations in study III and IV was preceded by a habituation period consisting of two single-blind placebo infusions followed by five infusions of cumulative doses of flumazenil or placebo. This habituation period was not included in the analysis of flumazenil effects. Later analysis of this period showed it to be more reactive and interesting than expected.

An indwelling venous catheter was placed on the non-dominant hand. To this 30 cm of catheter tubing was attached, so that infusions could be administered with as little disturbance as possible to the subject. The subjects participated in a study exploring the effects of flumazenil on symptoms of benzodiazepine withdrawal.

#### 3.2.2.2 Study III and IV:

These studies had a "double-blind cross-over" design and every subject experienced one provocation with flumazenil and one with placebo (i.e. the vehicle for flumazenil). The order of flumazenil/placebo was randomised at the pharmacy.

Flumazenil/placebo was injected during one minute, after which the catheter was flushed with 2 ml of saline. A cumulative dose-response curve was created by administering 0.05, 0.10, 0.25, 0.50 and 1.00 mg of flumazenil at 15-minute intervals, with assessments after each dose (total dose = 1.9 mg). This gradual increase of dose is further in accordance with recommendations made by Lader (1996). To avoid carry-over effects the two provocations were separated by at least seven days. As described above both provocations were initiated by two single-blind placebo injections to avoid confusion from "first-infusion" effects, and to make subjects comfortable with the test situation and assessments.
Flumazenil might precipitate seizures and anxiety or panic reactions, and this was one reason behind the use of gradually increasing doses of flumazenil. If any severe reactions had appeared these would have been countered by administration of diazepam.

3.2.3 Assessments

Subjective ratings. A unipolar 90-item scale for self-rating of symptoms commonly associated with benzodiazepine withdrawal (Ashton, 1991; Marks, 1985) and with the use of flumazenil (Emrich et al., 1984; Schöpf et al., 1984) was constructed. For each unipolar item a 100 mm visual analogue scale ranging from "not at all" to "very much" was used. The subjects were accustomed to the instrument by instructions and one set of practice ratings before the experiment began. The instructions included that subjects were to define the subjective meaning of the extremes, that they were not expected to indicate changes, but only what they felt at that moment, and that they should work with some speed. Self-ratings commenced seven minutes after infusion, and were completed in approximately 6 minutes, at each dose level.

Physiological assessments. Blood pressure (auscultatory) and heart rate (palpatory) were registered four minutes after injection. EEG was registered during four-minute periods starting one minute before injection. Subjects were asked to close their eyes, relax and not move during this period.

Figure 2. Schematic description of infusions (assessments was made between infusions) in study I, III and IV.
3.2.4 Data analysis

For data analysis in Study I and III, aggregates were made of items measuring negative or positive psychological items, as well as of somatic items. For the analysis reported in Study IV an aggregate was compiled from six items: “Irritation”, “Temper outbursts you can not control”, “Having urges to break or smash things”, “Wants to shout or throw things”, “That you easily get annoyed or irritated” and “Having urges to beat, injure, or harm someone”.

Group differences, differences between group changes over time (i.e. group x time interaction), over treatment conditions (i.e. group x treatment interaction), and treatment effects over time (i.e. treatment x time interaction), as well as three-way (i.e. group x time x treatment interaction) and four-way (i.e. gender x group x time x treatment interaction) interactions were tested for significance by analysis of variance (ANOVA, a split-plot design; Kirk, 1968). Post hoc analysis for paired comparisons was carried out using Tukey's HSD Test (Kirk, 1968). The level of significance was set at 5% and sequential bonferroni was used to control for possible alpha inflation (Rice, 1989).

3.3 Study II and V

3.3.1 Subjects

Six healthy male volunteers aged 32-39 years of age (M=35 years), weight 59-80 kg (M=71 kg) and height 172-185 cm (M=180 cm) who estimated and reported their alcohol consumption to be below the 40g/day cut-off for harmful consumption (Table 2), participated in the study.

Table 2. The subjects’ recent experience with alcohol.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Questionnaire index</th>
<th>Estimated weekly consumption (g/week)(^1)</th>
<th>Reported weekly consumption (g/week)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.65</td>
<td>66</td>
<td>198.7</td>
</tr>
<tr>
<td>2</td>
<td>0.35</td>
<td>46</td>
<td>51.3</td>
</tr>
<tr>
<td>3</td>
<td>0.38</td>
<td>30</td>
<td>59.0</td>
</tr>
<tr>
<td>4</td>
<td>0.59</td>
<td>148</td>
<td>118.7</td>
</tr>
<tr>
<td>5</td>
<td>0.53</td>
<td>15</td>
<td>50.7</td>
</tr>
<tr>
<td>6</td>
<td>0.24</td>
<td>27</td>
<td>23.7</td>
</tr>
</tbody>
</table>

\[\text{M} = 53.8 \text{ SD} = 46.02 \quad \text{M} = 83.7 \text{ SD} = 58.87\]

\(1\) Based on the questionnaire, \(2\) Recorded consumption (3 previous weeks)
They were informed about the aim and design of the experiment, and they then gave their consent to participate in the study, which was approved by the Local Ethics Committee. The subjects received some financial compensation for participating (i.e. 400 SEK). Before the study, the subjects filled out a questionnaire that covered their drinking history. They were free from alcohol and drugs for at least 7 days prior to the experimental period.

3.3.2 Procedure

3.3.2.1 Infusions of alcohol

In a randomized order 7.5 % w/v ethanol in 0.9 % NaCl-solution and NaCl-solution only was administered on two separate occasions. The experimental conditions were double-blinded as far as possible. The subjects were not informed of the order of conditions, and they were told that both infusions might be of the same kind. The subjects were prepared for the infusion 1 hr in advance, so that they were relaxed at the start of the infusion (approx. 12 AM). A simple lunch was served 80 min. after the start of the infusion.

The amount of ethanol to be administered was calculated based on “total body water” (TBW) according to Watson et al. (1981). Ethanol was infused using a volume-directed infusion pump. Infusions were administered quickly for the first 40 min, followed by a slower rate for the next 100 min to maintain a steady-state concentration of alcohol during 60 min. This resulted in four different phases during the experiment. There was an initial phase (40 min) with increasing blood alcohol concentration, followed by a phase (20 min) during which ethanol was distributed throughout the body. During the “steady-state” phase (60 min), the blood alcohol concentration remains at a constant level. Finally, the alcohol is eliminated from the body at a constant rate. The total experimental period was 220 min.

On average, the total volume of ethanol given was equal to one bottle of wine (75 cl, i.e. 60 g 100 % alcohol) given to a male of 70 kg. No negative reactions were reported.

Breath ethanol concentration (BEC) was measured with an Intoxilyser (CMI inc., Minturn, Colorado, model 4011) every 20 min after the infusion began.
3.3.3 Assessment

3.3.3.1 Study II

Computerized Reaction Time Tests: The Reaction Time (RT) tests from the automated psychological test system (Levander, 1987) were administered at seven occasions (-30, 0, 20, 40, 60, 140 and 200 min). Three parts of the RT tests were selected: 1) a simple visual RT, 2) a two-choice (left-right) visual RT, and 3) a more complex version of two-choice visual RT requiring parallel processing, with randomly presented auditory signals (50% occurrence) for response inhibition.

Items recorded in the simple visual RT test were: maximum RT, mean RT, standard deviation of RT, and total number of responses. To the two-choice version of visual RT test, the following items were added: left hand responses (amount, mean, maximum and SD), right hand responses (amount, mean, maximum and SD), left/right errors, and spurious responses. In the final version of this test (two-choice visual RT with auditory inhibition), the number of times the subject failed to inhibit a response was also recorded. In this part of the RT test the subject is asked not to press the button if an auditory signal is presented simultaneously with the visual stimulus. This test taps psychomotor speed, attention, and accuracy, and it has been suggested to measure preference for speed versus accuracy, since the subject can choose between a rapid response taking the risk of giving incorrect responses, or go for safety and wait and see if there will come an auditory signal or not (af Klinteberg et al., 1990). Failed inhibition has also been regarded as an indicator of motor disinhibition, irrespective of speed (af Klinteberg et al., 1990).

To stabilize the baseline performance of the subjects they trained the RT tests on 5 occasions (2+3+3+3+3 test runs). The mean baseline scores for the last training session, as well as test-retest reliability are presented in Table 3.
Table 3. Mean baseline performance of the subjects during the last training sessions.

<table>
<thead>
<tr>
<th>Test</th>
<th>Variable</th>
<th>Response side</th>
<th>Mean</th>
<th>Std Error</th>
<th>Test-retest reliability(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple RT</td>
<td>Reaction time</td>
<td>preferred hand</td>
<td>312.42</td>
<td>45.79</td>
<td>0.82</td>
</tr>
<tr>
<td>Two-choice RT</td>
<td>Reaction time</td>
<td>left-hand</td>
<td>312.13</td>
<td>32.91</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Reaction time</td>
<td>right-hand</td>
<td>317.57</td>
<td>30.20</td>
<td>0.89</td>
</tr>
<tr>
<td>Two-choice RT</td>
<td>Reaction time</td>
<td>left-hand</td>
<td>323.38</td>
<td>29.86</td>
<td>0.97</td>
</tr>
<tr>
<td>with response</td>
<td>Reaction time</td>
<td>right-hand</td>
<td>408.80</td>
<td>72.59</td>
<td>0.97</td>
</tr>
<tr>
<td>Inhibition</td>
<td>Failed inhibition</td>
<td>------</td>
<td>0.17</td>
<td>0.17</td>
<td>0.46</td>
</tr>
</tbody>
</table>

\(^1\) Based on Pearson correlation coefficient (r) between the two last training sessions.

3.3.3.2 Study V

Subjective ratings: Changes in mood during intravenous administration of alcohol were studied using the “sMACL” (Sjöberg et al., 1979). Persson et al., (1980) have previously reported three aspects of mood (pleasantness, activation and calmness) in the scale to be susceptible to alcohol provocation. These three bipolar aspects of mood are compiled from 38 positive or negative adjectives. When self-rating, a person has four response alternatives to choose from: whether each adjective is “in accordance with how you feel just now”, “somewhat in accordance with how you feel just now”, “not in accordance with how you feel just now”, and finally “definitely not in accordance with how you feel just now”. When scoring, these alternatives are numbered 4 to 1. For negative items, the scale is reversed giving a less negative response a higher value.

As mentioned above, in the original scale, each aspect of mood includes both positive and negative items. Previous research (Martin et al., 1993) in the area of acute alcohol effects indicates that such effects are best studied using separate measures of “stimulant” and “sedative” effects. Therefore, I used Sjöberg’s description of the scale and separated positive from negative items resulting in an additional six measures of mood that were also used in the data analysis (Table 4).
Table 4. Subscales and items of sMACL compared to items of the Biphasic Alcohol Effects Scale (Martin et al., 1993).

<table>
<thead>
<tr>
<th>Swedish Mood Adjective Scale (sMACL)</th>
<th>Biphasic Alcohol Effects Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pleasantness</strong></td>
<td></td>
</tr>
<tr>
<td>Positive items</td>
<td>Positive items</td>
</tr>
<tr>
<td>Satisfied</td>
<td>Concentrated</td>
</tr>
<tr>
<td>Happy</td>
<td>Bright</td>
</tr>
<tr>
<td>Harmonious</td>
<td>Interested</td>
</tr>
<tr>
<td>Optimistic</td>
<td>Enthusiastic</td>
</tr>
<tr>
<td>In a cheerful mood</td>
<td>Alert</td>
</tr>
<tr>
<td>Unconcerned</td>
<td>Attentive</td>
</tr>
<tr>
<td></td>
<td>Active</td>
</tr>
<tr>
<td></td>
<td>Energetic</td>
</tr>
<tr>
<td><strong>Negative items</strong></td>
<td></td>
</tr>
<tr>
<td>Dejected</td>
<td>Weak</td>
</tr>
<tr>
<td>Pessimistic</td>
<td>Passive</td>
</tr>
<tr>
<td>Low-spirited</td>
<td>Un-concentrated</td>
</tr>
<tr>
<td>Unhappy</td>
<td>Feeble</td>
</tr>
<tr>
<td>Worried</td>
<td>Tired</td>
</tr>
<tr>
<td>Resigned</td>
<td>Un-enterprising</td>
</tr>
<tr>
<td></td>
<td>Indolent</td>
</tr>
<tr>
<td></td>
<td>Indifferent</td>
</tr>
<tr>
<td><strong>Activation</strong></td>
<td></td>
</tr>
<tr>
<td>Positive items</td>
<td></td>
</tr>
<tr>
<td>Satisfied</td>
<td></td>
</tr>
<tr>
<td>Happy</td>
<td></td>
</tr>
<tr>
<td>Harmonious</td>
<td></td>
</tr>
<tr>
<td>Optimistic</td>
<td></td>
</tr>
<tr>
<td>In a cheerful mood</td>
<td></td>
</tr>
<tr>
<td>Unconcerned</td>
<td></td>
</tr>
<tr>
<td><strong>Calmness</strong></td>
<td></td>
</tr>
<tr>
<td>Positive items</td>
<td></td>
</tr>
<tr>
<td>Easy</td>
<td></td>
</tr>
<tr>
<td>Relaxed</td>
<td></td>
</tr>
<tr>
<td>Quiescent</td>
<td></td>
</tr>
<tr>
<td>Composed</td>
<td></td>
</tr>
<tr>
<td><strong>Stimulant Subscale</strong></td>
<td></td>
</tr>
<tr>
<td>Energized</td>
<td></td>
</tr>
<tr>
<td>Excited</td>
<td></td>
</tr>
<tr>
<td>Stimulated</td>
<td></td>
</tr>
<tr>
<td>Talkative</td>
<td></td>
</tr>
<tr>
<td>Up</td>
<td></td>
</tr>
<tr>
<td>Vigorous</td>
<td></td>
</tr>
<tr>
<td><strong>Sedative subscale</strong></td>
<td></td>
</tr>
<tr>
<td>Difficulty concentrating</td>
<td></td>
</tr>
<tr>
<td>Down</td>
<td></td>
</tr>
<tr>
<td>Heavy head</td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td></td>
</tr>
<tr>
<td>Sedated</td>
<td></td>
</tr>
<tr>
<td>Slow thoughts</td>
<td></td>
</tr>
<tr>
<td>Sluggish</td>
<td></td>
</tr>
</tbody>
</table>

Mood was self-rated every 20 minutes and approx. 90 seconds was used to fill out the scale. Subjects made practice ratings twice during the two weeks prior to the study, as well as 20 minutes before infusion.

3.3.4 Data analysis
3.3.4.1 Study II

Analysis of variance with both factors as repeated measures, i.e. condition (alcohol and placebo) x time (20, 60 and 140 min), was computed for each of the respective test results (ANOVA, Kirk, 1968). Post-hoc comparisons were carried out using Tukey’s HSD tests (Kirk, 1968). From these analyses it was possible to calculate if there was any effect caused by alcohol (i.e. significant main effect for the drug-factor), as well as if acute tolerance existed for the given measure (i.e. a significant drug x time interaction, showing stable performance during placebo, while the time points differed during alcohol administration). When significant interaction was found, acute tolerance was studied either by comparing the effect of alcohol on performance (at equal concentrations) on the ascending and the descending limb of the alcohol concentration curve (comparison of time points 20 min. vs. 140 min.), or during the
steady-state concentrations of alcohol (comparison of time points 60 min. vs. 140 min.). A "better" performance at the time point 140 min. would indicate the occurrence of acute tolerance in both comparisons.

3.3.4.2 Study V

Pleasantness, activation and calmness, as well as unipolar (positive or negative) indexes of each aspect of mood, were compiled from the 38 items on the scale. Differences between experimental conditions as well as changes over time were tested for significance by analysis of co-variance with the order of the two experiments as a covariate to compensate for effects of habituation (ANOVA, Kirk, 1968). Post-hoc analysis for paired comparisons was carried out using Tukey’s HSD Test (Kirk, 1968). Spearman correlation coefficients were calculated for relationships between changes in mood ratings and changes in level of blood alcohol (Siegel, 1956). The level of significance was set at 5% and sequential Bonferroni correction was used to control for possible alpha inflation (Rice, 1989).
4 RESULTS AND DISCUSSION

4.1 Study I

Effects of placebo injections on symptoms of benzodiazepine withdrawal were investigated in the present study.

4.1.1 Groups at base-line

Reported pre-infusion distress was higher in patients than in controls on both occasions (Fig 3 a-c).

No gender related differences were found in base-line ratings of the aggregate of negative items (t-test, \(p = 0.7\)) or the aggregate of somatic items (t-test, \(p = 0.9\)). Nor was there any difference between female and male patient in the number of weeks free from benzodiazepines before the first provocation (t-test, \(p = 0.3\)).

4.1.2 Effects of placebo on subjective ratings

As can be seen in Fig 3 a-c the groups differed significantly from each other with patients self-rating higher on negative (ANOVA, \(P = 0.001\)) and somatic (ANOVA, \(P = 0.02\)) aggregates and lower on the positive aggregate (ANOVA, \(P = 0.01\)). It is of course impossible to know if these differences were caused by the patient’s previous consumption of benzodiazepines or a constitutively higher level of distress, even though nervous symptoms in childhood have been reported to be common in this group of patients (Allgulander, 1978).

The main finding of this study is not that placebo could reduce subjective symptoms of benzodiazepine withdrawal, but that the four-way interaction between group, gender, occasion and time showed that it did so mainly in female patients (ANOVA, \(P = 0.01\)). Both patients and controls reported stable aggregate of negative items on the second occasion while female patients decreased their ratings (\(p = 0.004\) in the post-hoc test) after the second placebo injection on the first occasion. The picture looked similar for the aggregate of somatic items where female patients decreased their ratings significantly (\(p = 0.004\)) on the first occasion. In addition, female patients rated their somatic symptoms significantly (\(p = 0.009\)) lower than male patients after the first injection (Fig 3c) and did no longer differ significantly from controls.
Further, interactions between gender, occasion and time were seen for the negative aggregate (ANOVA, $P = 0.05$) and for somatic symptoms (ANOVA, $P = 0.03$). These interactions consisted of higher symptom intensity for females on the first occasion compared to the second.

**Figure 3a-c.** Mean ± SEM of aggregates of negative psychological items, positive psychological items and somatic items are presented separately. Figures show female and male patients and controls during the first and second occasion.

### 4.1.3 Effects of placebo on objective measures

The groups differed from each other in heart rate (ANOVA, $P = 0.0006$) and diastolic (ANOVA, $P = 0.04$), but not in systolic blood pressure.

In support of the finding on the self-ratings I found placebo effects on objective measures. For systolic blood pressure there was an interaction between gender and
time (ANOVA, $P = 0.002$) showing that the systolic blood pressure decreased significantly ($p = 0.0003$) for females after the second injection (Fig. 4a) while males remained stable. In addition, among patients it was females who decreased significantly ($p = 0.01$) in their systolic blood pressure. Also for diastolic blood pressure there was an interaction (ANOVA, $P = 0.004$) between gender and time and post-hoc test showed that this effect consisted of females decreasing ($p = 0.04$) after the second injection while males remained stable. Further, regardless of group and gender, diastolic blood pressure decreased significantly ($p = 0.02$) after the first injection on the first occasion. Heart rate decreased over time and this decrease was significant ($p = 0.02$) after the second injection in all four groups.

**Figure 4a-c.** Mean ± SEM of physiological variables are presented over time for female and male patients and controls during the first and second occasion.
Usually, as mentioned above, there are no gender differences in frequency of placebo responders. This makes interpretation of this post-hoc analysis especially difficult, in contrast to a situation with the whole group of patients responding since it is reasonable to assume that expectations differed between controls and patients and that this would explain the lack of response in the control group. This does, however, not explain why female patients responded favorably while male patients did not.

Different factors, such as expectation, the provider, habituation, regression toward the mean and reduction of anxiety are often discussed as involved in placebo effects. The symptom reduction in female patients after the second injection on the first occasion followed by lower symptom intensity at the second occasion could be thought of as habituation to the situation and/or as a placebo effect, or as regression toward the mean. Habituation to placebo effects has been described (Fedele et al., 1989). This does not offer a reasonable explanation for our findings since the observed psychological and physiological effects in our study were seen only among female patient, and not among male patients or control subjects. These arguments can be used for or against the possibility that the observed effects due to regression towards the mean. Another possible explanation would be that our observations are related to the relationship between anxiety and placebo effects (Evans, 1985; Melzack and Wall, 1988; Rachman and Phillips, 1978; Shapiro and Shapiro, 1997b). It has, for example, been possible to reduce anticipatory anxiety with placebo (Reiss, 1980). However, the differences between male and female patients in baseline ratings on aggregates of negative or somatic items were not significant which indicates that this is unlikely the case in the present study.

One way of thinking is that factors such as expectation and motivation have influenced females in our study. Jensen and Karoly (1991) found that these factors had largest effect the first time placebo was given and in our study effects were primarily seen during the first provocation.

Yet another possible explanation would be that benzodiazepine dependent females are a population susceptible to placebo provocation. Reports on pseudowithdrawal do not specify gender so it is not helpful to turn to them.
4.2 Study II

With the present study I sought to extend the knowledge on acute tolerance during increasing and steady-state alcohol concentrations as well as to what extent complexity of tests and normal alcohol habits of the subjects affected the outcome of the tests.

4.2.1 Alcohol habits

Reported alcohol consumption during days 7-28 prior to the provocations is shown in Table 2. Correlation analysis showed that the Questionnaire Index (Table 2) correlated significantly with reported consumption (Spearman, \( r = 0.83, \ p = 0.04 \)) but not with estimated weekly consumption of alcohol \( (r = 0.54, \ p > 0.05) \). Four of the six subjects estimated their weekly consumption lower than the consumption reported prior to the experiment.

4.2.2 Alcohol concentrations

Alcohol concentrations are presented in Fig. 5A. The main effect of the drug factor was highly significant \([\text{ANOVA}, \ P = 0.0001]\). No interaction existed between the drug- and time factors \([\text{ANOVA}, \ P = 0.14]\) indicating that the concentrations of alcohol in breath were similar at the three occasions (20, 60 and 140 min.). Alcohol concentrations at 60 and 140 min were within \( \pm 0.10 \% \).
4.2.3 Effect of alcohol on Reaction Time

To determine potential differences in performance under the influence of alcohol, separate ANOVA’s were also computed for each RT test. When comparing the outcome of the tests at ascending and steady-state alcohol concentrations, acute tolerance was shown for the most demanding test (failed inhibition), where not only a reaction-time, but also parallel processing of speed, accuracy and attention were of
importance for a good performance. However, acute tolerance was not seen during steady-state concentrations of alcohol in any of the tests used.

For the simple visual RT and the two-choice visual RT, neither the main effects, nor the interaction effects were significant. However, for the two-choice visual RT with auditory inhibition, a significant main effect for drug in several measures was observed: mean RT (left hand responses) (ANOVA, $P = 0.02$); mean RT (right hand responses) (ANOVA, $P = 0.02$); max RT (right hand responses) (ANOVA, $P = 0.03$); and failed inhibition (ANOVA, $P = 0.004$). A significant drug x time interaction effect (ANOVA, $P = 0.03$) was found for the failed inhibition measure. Post-hoc tests indicated that the 20 min. condition differed significantly from both the 60 min. ($p = 0.04$) and the 140 min. ($p=0.01$) conditions under the influence of alcohol. The 60 min. condition was however not statistically different from the 140 min (Fig. 5B).

**Figure 6.** Mean ($\pm$SEM) number of failures in failed inhibition test related to the subjects’ previous experience of alcohol (y-axis), are presented over time (x-axis) for the "low" and "high" alcohol-consumers.

![Failed response inhibition](image)

4.2.4 Alcohol-related individual differences on performance

Individual differences in drinking history may interact with acute tolerance and we (Hiltunen, 1997a; Hiltunen, 1997b) as well as others (Portans et al., 1989) have shown that consumption of larger quantities of alcohol weakens, or eliminates, acute
tolerance. Further, when an experimental dose of alcohol exceeds the subjects’ customary experience, acute tolerance is apparent in both light and heavy alcohol consumers (Hiltunen, 1997a; Hiltunen, 1997b). In the present study, I therefore asked if the consistently observed significance of the subject factor on test performance in most of the analyses conducted, especially for the failed inhibition, was related to the subjects’ previous experience of alcohol (Fig. 6). The limited number of subjects did not allow for statistical analyses. However, as can be seen in Fig. 6, the three subjects consuming smaller quantities of alcohol were those who most often failed in the response inhibition test (especially at 20 min). They also showed a greater subsequent improvement in performance, indicating that acute tolerance was more pronounced for them.

There may be several reasons why acute tolerance was not shown in most of the tests even when an alcohol effect was detectable. Maybe that two of the reaction time tests were too elementary. This interpretation is supported by the acute tolerance shown for the most complex task. The main effect of alcohol was also stronger in the failed inhibition test (ANOVA, $P = 0.004$). As indicated in Fig 6, individual differences in customary alcohol consumption could also have caused the absence of acute tolerance.

The markedly recovered performance on failed inhibition at 40 min compared to initial scores at 20 min indicates that acute tolerance may have already developed during the first hour of alcohol intoxication, making further recovery of the ability to accomplish the tests during the following steady-state condition impossible. This rapid development of acute tolerance during the first hour of alcohol intoxication might mean that if it was possible to establish a steady-state alcohol concentration faster (i.e. at 20 min), acute tolerance might have occurred at the onset of steady-state. This interpretation focuses on the problem of infusion rate vs. speed of onset of acute tolerance and raises the need of further studies of this question. Possibly a recently described rapid breath alcohol concentration clamping method could be used in further research to test this hypothesis (O'Connor et al., 1998; Ramchandani et al., 1999a,b).

4.3 Study III

With this study I wanted to investigate the potential usefulness of flumazenil in diagnosing and treating symptoms of benzodiazepine withdrawal, as well as the usefulness of a symptom rating scale.
4.3.1 Effects of flumazenil on subjective ratings

As could be expected, previously detoxified benzodiazepine-dependent subjects reported higher distress than healthy control subjects at the beginning of both experiments. There was an overall difference between the groups, with patients scoring higher on aggregate of negative items (ANOVA, $p < 0.005$) and aggregate of somatic items (ANOVA, $P < 0.05$) and lower on aggregate of positive items (ANOVA, $P < 0.03$) (Fig 7a-c). It is of course not possible to determine if this is an effect of their consumption of benzodiazepines or of a higher level of pre-treatment distress.

Figure 7a-c. Aggregates of negative psychological items, positive psychological items and somatic items are presented separately. Figures show patients and controls during placebo and flumazenil. •••• Patients (flumazenil); •----- patients (placebo); O---O controls (flumazenil); O-----O controls (placebo).

There was a significant (ANOVA, $P < 0.02$) interaction between group, treatment and time showing that both groups were stable during placebo but they closed in on each other during flumazenil (Fig. 7a). The initially significant (Tukey, $p$
< 0.002) difference between patients and controls was not significant above the 0.5 mg dose. The reduction in distress was significant (p < 0.03) above the 1 mg dose. There was also a decrease of symptom intensity for patients and an increase for controls over time (ANOVA, P < 0.05). Except for the differences between groups no effects were found for the aggregate of positive items (Fig. 7b) and the aggregate of somatic items (Fig. 7c). These findings support the preliminary hypothesis, based on studies on animals and humans, that flumazenil can reduce protracted withdrawal symptoms in individuals treated for benzodiazepine dependence. The responses in the opposite direction to flumazenil in patients and controls suggest persisting effects after termination after long-term consumption of benzodiazepines.

The number of patients studied makes it relevant to examine individual reactions on flumazenil. These individual variations are shown in table 5 where ratings at base-line are compared with ratings at the highest dose of flumazenil. At the highest dose level six out of ten patients had lower ratings than at base-line on aggregate of negative items, seven had higher ratings on aggregate of positive items and five had lower ratings on aggregate of somatic items.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Aggregate of negative items</th>
<th>Aggregate of positive items</th>
<th>Aggregate of somatic items</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Highest dose</td>
<td>Baseline</td>
</tr>
<tr>
<td>1</td>
<td>153</td>
<td>216</td>
<td>402</td>
</tr>
<tr>
<td>2</td>
<td>288</td>
<td>269</td>
<td>140</td>
</tr>
<tr>
<td>3</td>
<td>189</td>
<td>211</td>
<td>106</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>14</td>
<td>235</td>
</tr>
<tr>
<td>5</td>
<td>276</td>
<td>92</td>
<td>160</td>
</tr>
<tr>
<td>6</td>
<td>75</td>
<td>139</td>
<td>243</td>
</tr>
<tr>
<td>7</td>
<td>363</td>
<td>308</td>
<td>326</td>
</tr>
<tr>
<td>8</td>
<td>158</td>
<td>43</td>
<td>334</td>
</tr>
<tr>
<td>9</td>
<td>483</td>
<td>130</td>
<td>168</td>
</tr>
<tr>
<td>10</td>
<td>235</td>
<td>237</td>
<td>100</td>
</tr>
</tbody>
</table>

These differences in individual responses to flumazenil could be explained by earlier findings that stronger effects of flumazenil tend to be associated with lower neuroticism in patients (Lader and Morton, 1992). Investigation of personality
characteristics was not the aim of the present study but might warrant exploration in future studies. There is also a possibility that flumazenil acted as an anxiogenic in some of our patients, as reported in a group of patients with panic disorder when given flumazenil (Nutt et al., 1990). The change in negative direction on aggregate of negative items, shown by some of our patients was, however, negligible and might depend on factors such as the gradually increased doses or that our patient group consisted of individuals without any panic disorder. That patients and controls are closing in on each other during flumazenil could be interpreted as a tendency to regress toward a group mean. If this, however, was the case the groups should also been closing in on each other during the placebo condition, which was not the case.

4.3.2 Effects of flumazenil on objective ratings

Of value when interpreting subjective findings is if they can be supported by objective measures. In the present study I registered blood pressure and heart rate and there was a trend with patients having higher values on these measures. There was a significant decrease in systolic (ANOVA, $P < 0.0003$) (Fig. 8a) and diastolic blood pressure (ANOVA, $P < 0.03$) (Fig. 8b) over time. For systolic blood pressure the decrease was larger during flumazenil (ANOVA, $P < 0.03$) and the initially significant (Tukey, $P < 0.02$) difference between treatments was no longer significant above the 0.25 mg dose. This dose-dependent effect is consistent with findings of others (Higgit et al., 1986) after oral flumazenil. The lack of effect on diastolic blood pressure in our study might depend on the doses used and route of administration or by differences in experimental conditions. A time-related reduction could also be seen for heart rate (ANOVA, $P < 0.002$).

Most of the patients included in this study were individuals who had consumed high doses of benzodiazepines. This may have influenced the number and intensity of experienced withdrawal symptoms and might limit the results to individuals dependent on high doses of benzodiazepines. These patients, however, constitute a substantial part of the patients that seek help. The fact that they often have difficulties completing treatment makes the results of this study clinically relevant. For these patients flumazenil, as a substance that reduces symptom intensity, could be a therapeutic option leading to less risk for treatment failure and relapses and making psychosocial interventions possible.
4.4 Study IV

Benzodiazepine withdrawal has been associated with hostile and aggressive behavior, whereas the benzodiazepine antagonist flumazenil has had variable effects on hostility and aggression in animal studies. In this study, items that specifically tap aspects of aggression and hostility in the data collected in study III are analyzed. In this analysis I found that patients treated for benzodiazepine dependency experienced stronger feelings of aggression and hostility than controls. A significant (ANOVA, \( P = 0.03 \)) interaction between group, treatment and dose showed that this self-rated hostility and aggressiveness was reduced by intravenous administration of flumazenil among patients. The controls tended to respond with the opposite effect. Post-hoc tests showed that above the 0.5 mg dose, patient ratings were significantly (Tukey, \( p < 0.05 \)) lower as compared to base-line and their ratings were no longer significantly higher than those of the controls. Flumazenil, thus, seems to have acted as an agonist in the patient group and, possibly, as a weak inverse agonist in the control-group. These results indicate that previously reported aggression-reducing effects of
flumazenil in animals might also be shown in humans. What these effects do not support is the suggestion that consumption of benzodiazepines causes a receptor shift that makes inverse agonists act more effectively (Nutt, 1990).

Obvious limitations of these results are the small number of subjects studied and the post-hoc nature of the analyses performed. The results are, however, interesting and further studies should be carried out to confirm these results.

**Figure 9.** Mean (+SEM) of aggregate of self-rated aggression and hostility.

![Graph showing BAI - Aggregate of items](image)

4.5 **Study V**

In this study, I administered alcohol intravenously to 6 healthy males in order to study possible differences between bi- and unipolar ratings of mood. Mood was studied during three phases of blood alcohol levels (increasing, steady-state and decreasing). Possible effects of alcohol habits on ratings of mood were also of interest.
4.5.1 Alcohol concentrations

Maximum blood alcohol concentrations (BAC) were reached after 40 minutes with a mean of 1.19 % (SD = 0.09) (Table 6). Mean BAC during steady-state was 0.83 % (SD = 0.05), which was slightly higher than the 0.70 % predicted by Watson’s formula (Watson et al., 1981). On the basis of these higher than predicted blood alcohol levels during steady-state, one could argue that Watson’s formula is a somewhat blunt instrument for this calculation. On the other hand, judging from the stability of blood alcohol levels during steady-state, the formula seemed to work fairly well in this regard.

Table 6. Blood alcohol levels (%) at maximum, steady-state and during elimination.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Maximum (at 40 min)</th>
<th>Steady-state (M of 60-140 min)</th>
<th>Elimination (M of 160-220 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.19</td>
<td>0.61 (0.07)</td>
<td>0.28 (0.08)</td>
</tr>
<tr>
<td>2</td>
<td>1.19</td>
<td>0.96 (0.02)</td>
<td>0.75 (0.03)</td>
</tr>
<tr>
<td>3</td>
<td>1.11</td>
<td>0.90 (0.06)</td>
<td>0.69 (0.12)</td>
</tr>
<tr>
<td>4</td>
<td>1.30</td>
<td>0.87 (0.06)</td>
<td>0.61 (0.07)</td>
</tr>
<tr>
<td>5</td>
<td>1.28</td>
<td>0.84 (0.04)</td>
<td>0.66 (0.06)</td>
</tr>
<tr>
<td>6</td>
<td>1.06</td>
<td>0.80 (0.08)</td>
<td>0.51 (0.07)</td>
</tr>
<tr>
<td>Mean</td>
<td>1.19</td>
<td>0.83 (0.05)</td>
<td>0.68 (0.07)</td>
</tr>
</tbody>
</table>

4.5.2 Alcohol concentrations and ratings of mood

After Bonferroni corrections, no significant correlation was found between changes in blood alcohol concentration and changes in mood ratings during any of the three phases. The fact that rising blood alcohol levels and mood was not correlated was somewhat unexpected, but supported by Mayfield and Allen (1967). Others (Cameron, 1974; Warren and Raynes, 1972) have reported that positive effects during rising blood alcohol levels are dose-dependent, i.e. that at higher levels ratings turns from positive to negative. This may have been the case here, reducing initial positive effects during the ascending phase.

4.5.3 Alcohol habits and ratings of mood

No significant correlation was found between reported alcohol consumption for days 7-28 prior to the experiment and changes in mood ratings during any of the three phases. This was contrary to Persson et al. (1980), who found that subjects with
higher consumption reported higher ratings of mood, as well as to Smith et al. (1975), who found habitual alcohol consumption to be negatively correlated with measures of elation during experimental alcohol consumption. This lack of effect was not explained by family history of alcohol problems, a factor suggested by Morzorati et al. (2002) to affect subjective perceptions of alcohol intoxication.

4.5.4 Alcohol effects on uni- and bipolar ratings of mood

Of the original three bipolar aspects of mood reported by Persson et al. (1980) to be of interest in the study of alcohol effects, only calmness was significantly (ANOVA, $P = 0.007$) affected by intravenous alcohol administration in our experiment, with subjects rating higher calmness during alcohol treatment than during placebo (Figure 10a). Post-hoc tests showed ratings of calmness to be higher at time 120, 160, 180, 200 and 220 minutes compared to time 0. Furthermore, calmness was further the only bipolar mood rating that changed significantly (ANOVA, $P = 0.04$) over time, regardless of treatment. Ratings of calmness during the two treatments were almost equal at the end of the two experiments. The difference between alcohol and placebo was that ratings during the alcohol condition increased to approximately the final level during the first 20 minutes, in contrast to the gradual changes during the placebo treatment. This picture is, however, slightly different when the two unipolar measures are considered (Figure 10b-c).

There was a significant difference between the effects of alcohol and placebo on the positive (ANOVA, $P = 0.004$) as well as on the negative (ANOVA, $P = 0.04$) calmness index, with post-hoc tests showing that subjects rated both indexes higher during the alcohol condition than during placebo (Figure 10b and 10c). Further, there was a significant (ANOVA, $P = 0.05$) effect of time on a negative calmness index, and post-hoc tests showed ratings at time 160 and 200 to be higher than at base-line. Time had no significant effect on the positive calmness index. From these results it can be concluded that the effect of alcohol on the total index of calmness was mainly due to an instantaneous reduction of negative aspects of calmness during the first 20 minutes of the experiment in combination with a constant increase during placebo (Figure 10c). It seems that stressful feelings were momentarily reduced by an initial dose of alcohol. This, however, seems to work only to a certain blood alcohol level (20 minutes), after which the effect levels out and remains fairly stable throughout the remaining 200 minutes.
There was no significant difference between alcohol and placebo or effect of time for bipolar pleasantness or activation. However, a tendency ($P = 0.07$) towards higher ratings was seen for pleasantness during the alcohol condition.

In contrast to this, analysis of variance revealed a significant difference between the effects of alcohol and placebo on the positive (ANOVA, $P = 0.002$) as well as on the negative (ANOVA, $P = 0.04$) pleasantness index. Post-hoc tests showed ratings of a positive pleasantness index to be higher during alcohol than during placebo, while the opposite pattern was seen for the negative index.
A significant (ANOVA, \( P = 0.01 \)) difference between effects of alcohol and placebo was found for negative activation index and a post-hoc test showed ratings to be higher during placebo than during alcohol.

Interestingly, our finding that the effect of alcohol on calmness is primarily a result of a reduction of negative experiences is supported by interview data describing negative emotional states (Cummings et al., 1980; Marlatt and Gordon, 1985) as well as negative mood (Litman et al., 1983) as primary factors precipitating relapse into alcohol use. Further, our results are also in line with such effects as “changing experiences in a positive direction” and “reducing tension” that are commonly attributed to alcohol (Brown et al., 1980). Subjects rated the negative index of activation higher during placebo than during alcohol, which seems reasonable considering the well-known sedative effects at high (Cameron, 1974; Smith et al., 1975) and decreasing levels of blood alcohol, where negative experiences has been reported by several authors (Babor, 1983; Freed, 1978). The same arguments as above could be used to explain the finding that the negative index of pleasantness was rated higher during placebo than during alcohol. In addition, the positive index of pleasantness was rated higher during the alcohol condition than during placebo. These effects on negative and positive aspects of pleasantness disappear when the indexes are combined to a total pleasantness index, indicating that positive and negative items are preferably analyzed separately.

These results must of course be regarded as preliminary due to the limited number of subjects studied. A possible confounding factor in our observations might be that subjects reached the extreme end of the rating scale at an early stage of the alcohol provocation, eliminating the possibility of any further increases in that direction (i.e. a ceiling effect). On the other hand, ratings did not decrease during the elimination phase (a phase characterized by negative experiences), which would have been the case if they were returning from the “ceiling”.

Possible explanations for the differences between our results and those of Persson et al. (1980) might be differences in context and experimental procedures. In the Persson et al. (1980) study, several subjects were “assembled in a comfortable room and were allowed to do whatever they wanted” while, in the present study, we
wanted to eliminate social confounders and performed the experiment with one subject at a time in a much more neutral (laboratory) environment. This is supported by others (Ekman et al., 1963; Frankenhaeuser et al., 1962) who reported similar findings when studying emotional reactions to alcohol in both a laboratory setting and a more natural one as well as by Lindman (1982), who reported that solitary drinking failed to induce the euphoric effects found in an earlier social drinking session. Further, environmental factors may influence the effects of alcohol (Holdstock and de Wit, 1998; Lindman et al., 1987), and several authors (Behar, 1983; Kalin, 1972; Marlatt and Rohsenow, 1980; Polivy, 1976) have suggested that alcohol in itself is not enough to cause such effects, i.e. different contextual, cognitive and affective factors are necessary. It is also reasonable to assume that the different modes of alcohol administration (oral vs. intravenous) affected subjective experiences differently.

It is also possible that the explanation for the differences lies in the inclusion of both positive and negative items in the same variable. This may have caused unwanted reductions of effects in the bipolar measures, i.e., that they cancelled out specific negative and positive effects. Biphasic actions of alcohol have been reported to be best studied with rating scales that treat ratings of positive and negative items separately (Martin et al., 1993). The results of our separate analyses support this separation when showing significant differences between alcohol and placebo on five out of six unipolar measures of mood in contrast to one in three of the bipolar measures. May be that strong dominance of either the positive or the negative side of an aspect of mood is a necessary condition for measuring bipolar mood during alcohol intoxication, and that the blood alcohol levels studied here were not sufficient to cause this dominance. One indication of this might be pleasantness, for which there was no significant difference between experimental conditions on the total index but for which significant differences were found between the placebo and alcohol conditions in both the positive and the negative index.
5 SUMMARY AND CONCLUSIONS

5.1 Summary:

In this thesis I have presented studies on subjective and objective effects during experimental treatment. Treatments have consisted of provocations with placebo, alcohol and flumazenil and the general aim of these provocations was to test suitable tools for the investigation of different psychopharmacological consequences in experiments designed to affect acute and/or post-acute effects of benzodiazepines and alcohol. To enable this two studies were performed, resulting in five separate reports (I-V) on different topics.

In study I the focus was on gender-related effect of placebo on withdrawal symptoms in patients treated for benzodiazepine dependence. It was found that symptoms related to benzodiazepine withdrawal could be reduced using placebo injections. This effect could be seen in female, but not in male patients or healthy controls irrespective of sex.

Paper II investigated acute-tolerance to alcohol during intravenous administration of alcohol. Acute tolerance was shown for the most demanding test (failed inhibition), where not only reaction time, but also parallel processing of speed, accuracy and attention were of importance for a good result. Acute tolerance seems to have developed already during the first hour of alcohol intoxication with no acute tolerance seen in any of the tests used during steady-state concentrations.

In study III the effects of the benzodiazepine antagonist flumazenil on withdrawal symptoms in patients treated for benzodiazepine dependence was examined. To do this I constructed a self-rating scale based on experiences presented in paper V. When designing the study, experiences from paper II were incorporated. Findings in this study included that the instrument could differentiate between patients and controls as well as between placebo and flumazenil. It was also found that flumazenil reduced withdrawal symptoms while the trend was the opposite for controls.

In paper (IV) pre-clinical reports that flumazenil can affect hostility and aggression were examined. Analyses of items of hostility and aggression identified that
self-rated aggression and hostility in patients treated for benzodiazepine dependency might be reduced by flumazenil.

The study in paper V aimed to investigate differences in subjective effects of intravenous alcohol administration on mood (pleasantness, activation, and calmness) when analysed as uni- and bipolar measures. Only one (calmness) of the bipolar aspects of mood was significantly affected by intravenous alcohol. In contrast, there were significant differences between alcohol and placebo for five of the six unipolar indexes of mood. The results support the hypothesis that subjective effects of alcohol on mood are preferably studied using self-ratings that allow positive and negative aspects to be analyzed separately.

5.2 Conclusion:

In this thesis, I have highlighted the importance of taking gender as well as placebo effects in account when performing pharmacological provocations on subjects treated for benzodiazepine dependence. Further, I have shown that it is possible to register acute tolerance to alcohol with an objective test previously not used in this context. I have also shown that it was possible to measure pharmacologically induced reductions in symptoms of benzodiazepine withdrawal using subjective and objective registrations. In addition, I have found that a reduction of self-rated withdrawal related aggression could be measured after intravenous flumazenil. Finally, I have pointed to the risk for loss of important information when creating indexes of multiple measures.
6 ACKNOWLEDGEMENTS

I wish to thank all those who have contributed to making this thesis possible:

Associate professor Arto Hiltunen, my supervisor for sharing his scientific knowledge and for his patience, support and warm friendship. Special thanks for the sneaky teaching.

Professor Hans Bergman, my co-supervisor, for the generous sharing of his great knowledge in the field of psychometrics.

Associate professor Stefan Borg, head of the Stockholm Centre for Dependency Disorders, for believing in me through the process and for providing the resources necessary for my scientific work.

To Paula Liljeberg and Nadja Eriksson, heads of the Methadone Maintenance Programme in Stockholm, for making it possible for me to combine clinical work with research.

Stefan Skagerberg who initially introduced me to the field of dependency research.

Professor Paul Hjemdahl for his emphasis on scientific stringency, critical evaluation and the use of fewer words.

Christina Cavalli, Bo Dovborg, Kirsi Laitinen and Eva Lindholm for their assistance during the provocations as well as to Annika Sonnenstein for reading and commenting my thesis.

To all colleagues and patients who have contributed to balance in my professional life.

To my Eva-Lena, Linn and Johan for bringing indispensable balance in my life.
REFERENCES


Becker HK, Keats AS, Mosteller F, Lasagna L (1953) The effectiveness of oral analgesics (morphine, codeine, acetylsalicylic acid) and the problem of placebo “reactors” and ”non-reactors.” *J Pharmacol Exp Ther* 109, 393-400.


File SE, Hitchcott PK (1990) A theory of benzodiazepine dependence that can explain whether flumazenil will enhance or reverse the phenomena. Psychopharmacology (Berl) 101, 525-32.


Frank E, Thase ME, Spanier CA, Reynolds III CF, Kupfer DJ (1999) Gender-Specific Response to Depression Treatment. J Gend Specif Med 2, 40-44.


Mirska IA, Piker P, Rosenbaum M, Lederer H (1941) "Adaptation" of the central nervous system to varying concentrations of alcohol in the blood. *Q J Stud Alcohol* 37, 598-605.


