FLUMAZENIL IN ALCOHOL WITHDRAWAL: A DOUBLE-BLIND PLACEBO-CONTROLLED STUDY

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Abstract — The purpose of the present study was to study γ-aminobutyric acid (GABA)-A receptor function in alcohol-dependent subjects during withdrawal, using the benzodiazepine antagonist flumazenil. In particular, we wanted to examine the hypotheses that an endogenous inverse agonist ligand at the GABA-A benzodiazepine receptor (GBzR) is active during withdrawal (in which case flumazenil should be anxiolytic), or whether chronic alcohol intake results in a shift in sensitivity of the receptor in the inverse agonist direction (in which case flumazenil should be anxiogenic). Results from 15 alcohol-dependent subjects in a double-blind placebo-controlled cross-over study showed that flumazenil was neither anxiolytic nor anxiogenic, although withdrawal scores were reduced during the course of the study. The fact that flumazenil was not anxiogenic, as it is in panic disorder, suggests that the GBzR is functioning differently in these two clinically similar conditions.

INTRODUCTION

Alcohol withdrawal may contribute to the pathophysiology of alcohol dependence by producing long-lasting alterations in brain receptors and neurotransmitter function. It is thus important to study brain function during withdrawal. Until recently, it was thought that alcohol exerted its effects through non-specific actions on cell membranes, but it is now clear that alcohol acts on many neurotransmitter systems within the brain, including γ-aminobutyric acid (GABA), N-methyl-D-aspartate (NMDA), noradrenergic, dopaminergic, serotonergic and opioid pathways (Nutt and Peters, 1994). It is likely that some or all of these neurotransmitters will have a role in the pathophysiology of withdrawal (Glue and Nutt, 1990). The GABA-A benzodiazepine receptor (GBzR) may be especially important for several reasons. Acutely, alcohol enhances GABA neurotransmission, at least at those receptor subtypes which contain the long form of the γ-2 subunit (Wafford et al., 1991). Clinically, drugs which have actions on the GBzR have been used to treat withdrawal and currently benzodiazepines are the treatment of choice, although high doses need to be given, probably because of cross-tolerance between alcohol and benzodiazepine agonists.

Inverse agonists are ligands that bind to the GBzR and have opposite effects to full agonists, i.e. they are negative modulators at the receptor, decrease the effects of GABA and consequently are anxiogenic, procognitive and convulsant (Pole et al., 1982). Recently it has been shown in experimental animals that chronic alcohol administration can alter the activity of ligands at the GBzR, such that partial inverse agonists (e.g. FG 7142 and RO15-4513), behave as full inverse agonists (Buck and Harris, 1990). These effects are similar to those which occur following chronic treatment with benzodiazepines (Little et al., 1987) and have been conceptualized as a shift in the receptor spectrum that reduces GABA-A inhibition, in the inverse agonist direction (see Fig. 1). The benzodiazepine antagonist flumazenil has been shown to reset this shift after both chronic alcohol (Buck et al., 1991) and benzodiazepine (Nutt and Costello, 1988) administration. This suggests that the effects of chronic treatments with these drugs (as measured by the increases in inverse agonist-provoked seizure rate) is reversed by giving flumazenil.

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A receptor shift theory has also been considered as an explanation for the anxiogenic effects of flumazenil in human panic disorder (Nutt et al., 1990) which clinically has some similarities with alcohol withdrawal (George et al., 1988). The provocation of panic attacks suggests inverse agonist-like effects of flumazenil in panic disorder, unlike the resetting that has been shown in animal models of GABAergic drug tolerance.

Although receptor shift could explain some of the symptoms of alcohol withdrawal, an alternative explanation is that during withdrawal, endogenous inverse agonists are acting unopposed at the GBzR. It has been suggested that these agents, which are anxiogenic and proconvulsant, may increase during chronic alcohol intake, in an attempt to negate the sedating effects of alcohol. When alcohol levels fall, their actions would be unopposed and so cause withdrawal symptoms (Sandler, 1982). There is some evidence for this in that increased levels of tribulin (a putative endogenous agonist) have been found in the urine of recently withdrawn alcoholics (Bhattacharya et al., 1982). Furthermore, flumazenil has been shown to reduce signs of alcohol withdrawal in both animal (File et al., 1989; Buck et al., 1991) and human (Gerra et al., 1991) studies.

These two theories (receptor shift versus endogenous inverse agonist) can be tested by giving flumazenil to alcoholics in withdrawal. If chronic alcohol intake results in receptor shift, flumazenil should be anxiogenic, at least initially, since it would be expected to act as a partial inverse agonist. If an endogenous anxiogenic substance is produced by alcoholics in withdrawal, flumazenil should be anxiolytic, since it would bind to the GBzR and prevent binding of endogenous inverse agonist. We therefore carried out a double-blind placebo-controlled study of the effects of intravenous flumazenil on ratings of withdrawal and anxiety in alcohol-dependent patients who were experiencing significant withdrawal. The main aims of the study were: (1) to study the effects of flumazenil in withdrawing alcoholics; (2) to see whether receptor shift or the presence of an endogenous inverse agonist is the more likely explanation for withdrawal effects caused by altered GBzR activity.

SUBJECTS AND METHODS

Subjects

Fifteen male subjects who fulfilled DSM-III-R criteria for alcohol dependency (American Psychiatric Association, 1987) and who had experienced mild/moderate withdrawal symptoms were recruited from local alcohol treatment clinics, or from general practitioners in the Bristol area. These were matched for age within 2 years with 15 healthy male volunteers. All subjects gave written informed consent to the study, which was approved by the local Ethics Committee. Exclusion criteria for alcoholics were: history of seizures (withdrawal-related or otherwise), delirium tremens, psychotic disorder, intercurrent infection, major medical illness, marked hypertension (defined as systolic blood pressure >180 or diastolic >120 mmHg), dependence on other drugs excluding nicotine and caffeine, use of benzodiazepines in the last 3 weeks and a positive urine drug screen. Exclusion criteria for volunteers were: history of drug or alcohol abuse, use of benzodiazepines in the last 3 weeks, history of seizures, significant medical illness, a first degree relative with any psychiatric disorder including alcohol or drug abuse. Prior to entering the study, all subjects were physically examined and blood was taken for full blood count, urea and electrolytes, and liver-function tests. Their urine was screened for the presence of benzodiazepines. Subjects were told of the nature of the study and that an attempt was being made to understand what contributions if any benzodiazepine receptors made to symptoms of alcohol withdrawal. They were told that flumazenil may temporarily increase or decrease levels of anxiety and that possible side effects of the active treatment included transient tinnitus and dizziness. They were also told that they could withdraw from the study at any time.
**Procedure**

Patients attended the testing room at 09:00. The time since their last consumption of alcohol was recorded and withdrawal symptoms/signs were assessed using CIWA (see below). Breath levels of alcohol were measured using a Lion alcometer. If alcohol was detected, the measurement was repeated after an hour. If alcohol was still detectable, patients were excluded from the study. Subjects were then rested in a semi-supine position, on a comfortable couch, and an intravenous cannula was inserted into an antecubital vein. Baseline ratings were carried out over 30 min, and then at time $t = 0$ min the first of the two infusions was administered, as a bolus over 1 min. Heart rate was continuously monitored by electrocardiography during the infusion, and ratings were carried out as detailed below. Blood pressure and heart rate were automatically recorded (using Dinamap, Critikon) at 2, 5, 15, 30 and 45 min after the infusion. The second infusion was started after ratings at time $t = 60$ (min), and subsequent measurements were as for the first infusion until $t = 120$ (min). After the study, patients were given a withdrawing course of chlordiazepoxide and follow-up arrangements were made.

**Ratings**

Prior to insertion of the cannula, a Clinical Institute Withdrawal Assessment from Alcohol (revised) (CIWA-Ar) scale was administered. This is a 10-item scale which effectively measures rapid changes in symptom level (Sullivan et al., 1991). Only those patients who scored $>10$ (i.e. those experiencing moderately severe withdrawal symptoms) were entered into the study.

At baseline the following parameters were measured. Anxiety and mood ratings using the Spielberger trait/state anxiety inventories (STAI/SSAI) (Spielberger et al., 1970) and the Beck depression inventory (BDI) (Beck et al., 1961) respectively. Subjective assessments of anxiety using visual analogue scales (VAS) were also done. This was a 100 mm scale in intervals of 10 mm. Zero represented 'not at all', and 100 represented 'the worst ever'. After each infusion, at times $t = 5, 15, 30$ and 45 min, VAS and anxiety rating scales were performed. Objective assessments were made of withdrawal symptoms, using a shortened 4-item version of the CIWA (sweating, agitation, tremor and flushing). At baseline and at 5 min after the start of each infusion, patients were also rated on a panic symptom inventory. This rated the presence of symptoms and their severity: $0 =$ not present, $1 =$ slight, $2 =$ moderate, $3 =$ severe and $4 =$ very severe. Two measures were derived. Firstly whether or not the subject met criteria for a panic attack. This required an increase in the anxiety or fear item of $\geq 2$, to a score of at least 3, together with an increase in score of $\geq 2$ in at least four DSM-III-R panic symptoms. Secondly the scores for DSM-III-R panic items were summed in two categories; somatic and psychological symptoms. The psychological symptoms were anxiety, fear, fear of going mad, fear of losing control and fear of dying.

**Drugs**

The dosage of flumazenil (2 mg) was the same as was used in our previous panic disorder study (Nutt et al., 1990) and was chosen on the basis that positron emission tomography studies show ~75% occupancy of brain benzodiazepine receptors at this dosage (Savic et al., 1991). The placebo infusion was an equal volume of normal saline.

**Statistics**

Data were analysed using the SAS package (SAS Institute, 1988).

The main statistical plan was analysis of variance (ANOVA) with repeated measures. The factors included in the ANOVA were diagnosis (alcohol withdrawal or control), drug (flumazenil or placebo), order (whether flumazenil was administered first or second). For the CIWA scores, controls were not rated. Due to baseline differences between alcoholics and controls on several analyses, analysis of covariance (ANCOVA) using the baseline values as covariates was used. The main interest was in significant diagnosis by drug interaction, which would indicate a different response in the alcohol-dependent group (as opposed to the control group) to flumazenil or placebo. Post-hoc 2-tailed paired or unpaired $t$-tests were then used to test for significant effects. Data are reported as means $\pm$ SD.
Table 1. Demography and baseline anxiety/depression ratings

<table>
<thead>
<tr>
<th></th>
<th>Alcoholics (n = 15)</th>
<th>Controls (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.7 (6.9)</td>
<td>34.9 (7.8)</td>
</tr>
<tr>
<td>SSAI</td>
<td>56.1 (11.7)</td>
<td>30.0 (6.5)</td>
</tr>
<tr>
<td>STAI</td>
<td>56.2 (10.5)</td>
<td>31.4 (8.2)</td>
</tr>
<tr>
<td>BDI</td>
<td>20.3 (7.3)</td>
<td>3.3 (4.1)</td>
</tr>
<tr>
<td>Daily alcohol intake (units)</td>
<td>29 (12)</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

SSAI = Spielberger state anxiety inventory; STAI = Spielberger trait anxiety inventory; BDI = Beck depression inventory. Values are given as means, with standard deviations in parentheses.

RESULTS

Demographic data for patients are given in Table 1. None of the patients screened positive for benzodiazepines. The patients with alcohol-dependence diagnosis had been drinking for a mean of 15 years (range 1–32) and had a mean of 2 detoxifications (range 0–10). The mean time from last alcohol consumption was 19 h (range 8–48). Despite chronic heavy alcohol consumption, liver-function tests were in the normal range (mean bilirubin = 11, mean alkaline phosphatase = 93, mean aspartate aminotransferase = 35).

Psychological effects

Baseline Beck depression inventory scores were significantly higher in patients than controls (20.3 ± 7.6 versus 3.3 ± 3.9; P < 0.001) as were Spielberger trait anxiety rating scales (56.2 ± 10.1 in patients and 31.4 ± 7.9 in controls; P < 0.001).

Spielberger state anxiety ratings at baseline were also significantly higher in the patients (52.6 ± 10.1) than in controls (30.0 ± 6.2) (P < 0.001).

The mean baseline visual analogue scores for anxiety were significantly higher in the patient group (39.7 ± 23.1), compared with controls (9.3 ± 7.5) (P < 0.001).

Since there were baseline differences in anxiety, ANCOVA was used with the baseline value as a covariate. There was a significant main effect of group, but no drug and no drug x group effects (Fig. 2a and b). Repeated measures analysis of variance for within-subject effects showed no significant effects of time; there were no time x group, time x drug or time x group x drug interactions (Table 2). The trend to a time x group interaction was due to a slight fall in anxiety in the alcoholics during both the placebo and flumazenil treatment periods.

Clinical Institute Withdrawal Assessment scores

The mean CIWA score at baseline was 15.6 ± 3.1. The score at the end of the study was 8.6 ± 6.1, which represented a significant reduction (P = 0.002). Analysis of the observer ratings of withdrawal showed a main effect of time, (F = 4.1, P = 0.002), but no significant effect of drug (F = 0.05, P = 0.8), and no time x drug interaction (F = 0.5, P = 0.8).

Panic inventories

No patients or controls met the criteria for a panic attack. Only one patient reported an increase in anxiety following the flumazenil infusion and this was mild. The ANCOVA was not significant.

Fig. 2. Visual analogue scores (anxiety) after (a) flumazenil and (b) placebo.
Table 2. Repeated measures analysis of covariance for visual analogue anxiety ratings

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>F</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>4</td>
<td>0.64</td>
<td>0.63</td>
</tr>
<tr>
<td>Time × group</td>
<td>4</td>
<td>2.34</td>
<td>0.06</td>
</tr>
<tr>
<td>Time × drug</td>
<td>4</td>
<td>0.81</td>
<td>0.52</td>
</tr>
<tr>
<td>Time × group × drug</td>
<td>4</td>
<td>1.02</td>
<td>0.39</td>
</tr>
</tbody>
</table>

The trend in the time × group effect was due to a slight fall in anxiety in the alcoholics during both the placebo and flumazenil periods.

for the somatic symptoms of panic ($F = 1.3$; df = 1,1 $P = 0.26$) or the psychological symptoms ($F = 1.0$; df = 1,1 $P = 0.32$).

Cardiovascular data

Systolic blood pressure. There was a significant main effect of diagnosis, with higher scores for the alcoholics compared with controls ($F = 28.8$, $P = 0.0001$). There was no effect of drug or drug × diagnosis interaction. For within-subject comparisons, there was no effect of time, or significant interactions.

Diastolic blood pressure. Again, there was a significant effect of diagnosis, with higher pressures in the alcoholic group ($F = 8.5$, $P = 0.005$). There was no significant drug effect or drug × diagnosis interaction. For within-subject comparisons, there was no effect of time, or significant interactions.

Heart rate. Alcoholics had significantly higher heart rates ($F = 20.7$, $P < 0.001$), but there were no drug effects, and no drug × diagnosis interactions. For within-subject comparisons, there was no effect of drug, or time × drug interactions, but there was a significant time effect ($F = 5.5$, $P < 0.0001$).

DISCUSSION

The main study findings were: (1) flumazenil was neither anxiogenic nor anxiolytic in withdrawing alcoholics; (2) flumazenil did not provoke panic attacks, as it does in panic disorder patients.

The fact that flumazenil was not anxiogenic argues against the GBzR shift theory of alcohol withdrawal. However, it may be that occupation of the GBzR could cause a change in the configuration of the receptor, leading to almost instantaneous resetting of receptor shift, as has been suggested by animal and in-vitro studies (Nutt and Costello, 1988; Buck and Harris, 1990). It has been suggested that the GBzR is in constant flux, oscillating between allosteric coupling that results in positive GABA modulation (agonism) and an alternative configuration which negatively modulates GABA (inverse agonism). Tolerance, as caused by chronic intake of alcohol or benzodiazepines, may lock receptors in the inverse agonist state whilst occupation by the antagonist may unlock the coupling and return the receptor to a neutral state. This possibility would not, however, be consistent with the panicogenic effects of flumazenil in panic disorder, nor would it be consistent with the anxiogenic effects of flumazenil in chronic users of benzodiazepines (Bernik et al., 1991).

Flumazenil was not anxiolytic, which suggests that an endogenous inverse agonist is not acting unopposed during withdrawal. However, the CIWA scores did significantly decrease during the course of the study, when one might expect withdrawal scores to be worsening. The half-life of flumazenil in the human brain is 12–20 min (Lassen et al., 1995) and thus one would expect a differential effect of flumazenil and placebo if flumazenil was preventing the effects of an inverse agonist (since the infusions were 60 min apart).

Since this was not observed, this theory is difficult to support on the basis of our study. However, our results conflict with a previous clinical study of flumazenil in alcohol withdrawal (Gerra et al., 1991), in which a significant reduction in withdrawal scores was found, with parallel increases in withdrawal scores in the comparison placebo group. The main difference in methodology between the studies was that we used a bolus over 1 min whereas a 48 h infusion was given in the earlier study. It may be that in humans prolonged occupation of the GBzR is needed to reduce significantly withdrawal symptoms, but this will only be determined by further work.

Pharmacokinetic factors may also be important. Flumazenil is metabolized to the free carboxylic acid and then the corresponding glucuronide via hepatic pathways. It may be that the lack of effect seen in the alcohol-dependent group was due to increased metabolic activity. It may also be that
flumazenil would have more of an effect on withdrawal if it was given before, rather than during, withdrawal as has been the case in some animal studies. The time course of changes in GABA function in relation to downstream effects on other neurotransmitter systems is also likely to be relevant. All patients will have had noradrenergic overactivity and this might obscure any changes (especially anxiolytic) caused by flumazenil (i.e. one set of receptors is being tested in patients who have changes in multiple transmitter systems). It should also be mentioned that animal studies often use seizure activity as the main measure of withdrawal. It may be that flumazenil does not prevent withdrawal per se, but rather it has intrinsic anticonvulsant activity through actions on the GBzR (see Nutt et al., 1992). A further methodological comment is that blindness was not complete, because some patients had noticeable mild dizziness after flumazenil.

The differences in response to flumazenil between the alcohol withdrawal subjects and the panic disorder patients previously studied (Nutt et al., 1990) is perhaps the most interesting finding. Panic attacks and alcohol withdrawal are clinically similar, and subjectively patients find it hard to differentiate the symptoms (George et al., 1988). Both states are characterized by increased noradrenergic function and people with panic disorder frequently use alcohol to damp down their anxiety. In addition, alcoholics often drink to relieve panic symptoms associated with alcohol withdrawal. Furthermore, benzodiazepines are effective in both panic disorder and alcohol withdrawal. The differential response to flumazenil in these two groups suggests that the GBzR is functioning differently in these two similar and often co-existent conditions.

Further work to measure tolerance is needed to clarify the effects of chronic alcohol intake on GBzR function. In the past, this has proved difficult, because measures used can be crucially influenced by psychological factors. We have been using the technique of saccadic eye movement analysis (Glue, 1991) to quantify receptor sensitivity using the benzodiazepine midazolam (Potokar et al., 1996). Hopefully this, together with other evolving techniques (including neuroimaging), will further define the effects of alcohol on the GBzR system and lead to better treatments for withdrawal.

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REFERENCES


