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ORIGINAL INVESTIGATION

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GABA-benzodiazepine receptor function in alcohol dependence: a combined ^{11}C-flumazenil PET and pharmacodynamic study

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Abstract *Rationale:* Gamma-aminobutyric acid (GABA)-benzodiazepine receptor function is hypothesised to be reduced in alcohol dependence. *Objectives:* We used positron emission tomography (PET) with [^{11}C]flumazenil, a non-selective tracer for brain GABA-benzodiazepine (GABA-BDZ) receptor binding, to determine in vivo the relationship between BDZ receptor occupancy by an agonist, midazolam, and its functional effects. *Methods:* Abstinent male alcohol dependent subjects underwent [^{11}C]flumazenil PET to measure occupancy of BDZ receptors by midazolam whilst recording its pharmacodynamic effects on behavioural and physiological measures. Rate constants describing the exchange of [^{11}C]flumazenil between the plasma and brain compartments were derived from time activity curves. *Results:* A 50% reduction in electroencephalography (EEG)-measured sleep time was seen in the alcohol dependent group despite the same degree of occupancy by midazolam as seen in the control group. The effects of midazolam on other measures of benzodiazepine receptor function, increasing EEG beta1 power and slowing of saccadic eye movements, were similar in the two groups. No differences in midazolam or flumazenil metabolism were found between the groups. *Conclusions:* In summary, our study suggests that alcohol dependence in man is associated with a reduced EEG sleep response to the benzodiazepine agonist, midazolam, which is not explained by reduced BDZ receptor occupancy, and is consistent with reduced sensitivity in this measure of GABA-BDZ receptor function in alcohol dependence. The lack of change in other functional measures may reflect a differential involvement of particular subtypes of the GABA-BDZ receptor.

Keywords PET · Flumazenil · Alcoholism · GABA-benzodiazepine receptor · EEG · Saccadic eye movements

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Introduction

Adaptive responses, such as tolerance, to exogenous pharmacological agents are a common experience in neuropsychopharmacology. However, understanding the nature of such changes is difficult as, until recently, it has not been possible to control for levels in drug occupation of brain receptors. To overcome this confound, we developed a positron emission tomography (PET) in vivo paradigm that directly measured the relationship between brain gamma-aminobutyric acid (GABA)-benzodiazepine (GABA-BDZ) receptor occupancy and pharmacodynamic effects (Malizia et al. 1996). Using the non-selective BDZ PET tracer, [^{11}C]flumazenil, it was found that increased occupancy of this receptor by the benzodiazepine agonist, midazolam, positively correlated with increases in electroencephalographic (EEG) beta activity.

The GABA-BDZ receptor is hypothesised to function abnormally in a number of different neuropsychiatric con-

ditions including anxiety, epilepsy and alcoholism (Nutt and Malizia 2001). In this study we used [^{11}C]flumazenil PET with concomitant functional measures to test the hypothesis that GABA-BDZ receptor function is reduced in alcohol dependence in man. Clinically, altered sensitivity of the $GABA_A$ receptor complex to anaesthetics was raised as a concern in patients who misuse alcohol (Bruce 1983) and there are a number of studies suggesting significant alterations of the function of this receptor in alcohol dependence.

Alcohol affects many neurotransmitter pathways, but augmenting GABA-ergic function is recognised as key in the anxiolytic, hypnotic, sedative and ataxic effects of alcohol (see Nutt 1999). Animal models show that acutely, alcohol increases Cl^- flux through the GABA-BDZ receptor (Suzdak et al. 1986) but chronic alcohol exposure results in decreased sensitivity to alcohol and other benzodiazepine agonists, i.e. tolerance (Buck and Harris 1990). Such neuro-adaptation is associated with functional changes in specific subunits of the GABA-BDZ receptor in particular regions of the brain (see Grobin et al. 1998). For instance, reduced levels of α1 subunit mRNA and peptide has been consistently reported in rat cortex (Montpied et al. 1991; Mhatre and Ticku 1992) with increased expression of α4 subunit mRNA (Devaud et al. 1995).

However, it is not yet clear what processes might explain the human experience of tolerance to alcohol and cross-tolerance to other drugs acting at the $GABA_A$ receptor. Human post-mortem studies using a variety of radioligands show increases, decreases or no changes in GABA-BDZ receptor levels (Tran et al. 1981; Freund and Ballinger 1988; Dodd et al. 1992; Korpi et al. 1992). One post-mortem study revealed increased expression of α1 subunit mRNA and peptide in the superior frontal cortex of alcoholics, but no such increase was seen either in the motor cortex or in the α3 subunit mRNA and peptide (Lewohl et al. 1997, 2001). By contrast, Mitsuyama et al. (1998) reported no significant increase in α1 subunit mRNA or peptide expression in the frontal cortex of alcohol dependent patients.

In vivo neuroimaging studies using [^{11}C]flumazenil PET or [^{123}I]iomazenil and single photon emission computed tomography (SPECT) to label the GABA-BDZ receptor have consistently shown reduced GABA-BDZ receptor binding, particularly in the frontal cortex in abstinent alcohol dependent subjects (Gilman et al. 1996; Abi-Dargham et al. 1998; Lingford-Hughes et al. 1998). There is also evidence from an [^{18}F]FDG [2-deoxy-2[^{18}F] fluoro-D-glucose] PET study that GABA-BDZ receptor function may be reduced in alcoholism since the inhibitory response to a challenge with the benzodiazepine agonist, lorazepam, was found to be blunted in the orbitofrontal cortex and cingulate gyrus in alcohol dependent subjects abstinent for 8–11 weeks (Volkow et al. 1997).

Conventional ways in which benzodiazepine receptor function has been evaluated in man include effects of agonists on EEG and saccadic eye movements. Benzodiazepines increase EEG beta power, produce sedation and reduce saccadic eye movements in a dose-dependent manner (Domino et al. 1989; Feshchenko et al. 1997; Hommer et al. 1986; Ball et al. 1991). However, the relationship between occupancy at brain benzodiazepine receptors and function had to be inferred from the plasma concentration.

The aim of this study was, therefore, to directly measure for the first time the function of GABA-BDZ receptor in humans to specifically test the hypothesis that alcohol dependence is associated with reduced benzodiazepine receptor sensitivity in man by using PET to simultaneously assess of the brain pharmacokinetics and pharmacodynamics of midazolam.

Materials and methods

Subjects

Abstinent male subjects who fulfilled DSM IV criteria for alcohol dependence were recruited from local alcohol treatment services. They had all been abstinent for at least 6 weeks. The average length of abstinence was 7.3 months (SE±1.5, range 2–18 months). This avoided early withdrawal/abstinence phase, when significant changes in blood flow are reported to occur which might confound comparisons (Volkow et al. 1994). The control group consisted of male subjects who had never fulfilled such criteria, i.e. were non-dependent alcohol drinkers and were recruited through colleagues and advertisements. Subjects with clinical evidence of hepatic, cognitive or neurological impairment or medical disorder were excluded. All subjects had no previous or current history of psychosis or anxiety disorders. One alcohol dependent and one non-dependent control subject each had a history of major depressive disorder; they were not depressed at the time of PET but were receiving treatment with paroxetine and clomipramine, respectively. The effect of depression or chronic serotonergic antidepressants therapy on benzodiazepine receptors is not clear (see Kugaya et al. 2003). All parameters measured for these two subjects were within the range of the other subjects and results of analyses performed with and without them were not different. The results described hereafter therefore include these two subjects. No other prescribed psychotropic drugs were being currently taken and benzodiazepines had not been taken for at least 6 weeks since detoxification in the alcohol dependent group. Recreational or occasional use of illicit drugs (e.g. cannabis), but not dependency (DSM IV defined) was admissible, although for most subjects, drug use was not current. Technical difficulties resulted in rejection of scan data from five subjects. This paper describes the 21 subjects (11 alcohol dependent and ten non-dependent) from whom [^{11}C]flumazenil PET images were analyzable.

All subjects were assessed for their severity of dependency (Severity of Dependency Questionnaire (SADQ), Stockwell et al. 1983). Anxiety and depression were assessed using the Beck Depression Inventory (BDI; Beck et al. 1961), and Spielberger State–Trait Anxiety Inventory (SSAI, STAI; Spielberger 1983).

Informed consent was obtained before participation in the study, which had been approved by the local Ethics Committees and from the UK Administration of Radioactive Substances Advisory Committee.

[^{11}C]Flumazenil PET

[^{11}C]Flumazenil was prepared with >95% radiochemical purity and with a specific radioactivity of ~24,000 MBq μmol^{-1} at the end of the synthesis by *N*-methylation of the corresponding *N*-desmethyl derivative with [^{11}C]iodomethane, as described previously by Pike et al. (1993).

[^{11}C]Flumazenil scans were performed using an ECAT-953B PET camera in 3D mode (CTI/Siemens, Knoxville, TN, USA) with performance characteristics as described previously by Spinks et al. (1992). The axial field of view was 10.6 cm. The final image resolution (using a Hanning 0.5 filter) was 8.0×8.0×4.3 mm [at full width half maximum (FWHM)].

Subjects were positioned in the scanner such that the transaxial images were parallel to the intercommissural line (AC-PC line) and the majority of the cerebellum was scanned. A bolus injection of [^{11}C]flumazenil (370 MBq, 10 mCi) in ~2 ml was administered through an intravenous cannula sited in the dominant antecubital fossa vein. Thirty minutes after the start of the scan they received an i.v. infusion of midazolam (50 μg kg^{-1}) over 5 min through an intravenous cannula sited in the non-dominant arm. We chose 50 μg kg^{-1} of midazolam since this dose produced the most robust effect in a previous study (Malizia et al. 1996).

Each subject had a radial arterial cannula in the non-dominant wrist to allow continuous counting of blood radioactivity concentration with a bismuth germanate (BGO) counter during the course of the experiment. Discrete samples were also taken at 4, 6, 8, 10, 20, 35, 50 and 65 min after injection to derive the metabolite-corrected plasma curve. Fractional concentrations of unchanged [^{11}C]flumazenil and of less lipophilic radioactive metabolites were determined by solid phase extraction of plasma followed by high-performance liquid chromatography (HPLC). Samples were also taken at 5, 20, 35, 50 and 60 min after the start of the midazolam infusion and frozen for later measurement of total plasma midazolam levels using HPLC. The area under the curve (AUC) was then used to derive a measure of the amount of midazolam available during the scan (ng ml^{-1}).

Twenty-six dynamic frames of data in 3D mode were acquired over 105 min (3×1, 19×3, 2 or 3×15 min; except for first three scans where only 2×15 min were acquired) and produced images containing 31 contiguous slices.

Image analysis

The methods used to analyze the resulting images described in Malizia et al. (1996) were slightly modified in this study.

The dynamic images were reconstructed with measured attenuation and dual energy window scatter corrections. Each reconstructed frame of [^{11}C]flumazenil uptake acquired was analysed on a Sun SPARC workstation (Sun Microsystems, Mountain View, CA, USA) using Analyze AVW version 3.1 (Mayo Foundation; Robb and Hanson 1991). Regions of interest (ROI) were drawn on an individual volume of distribution maps of the GABA-BDZ receptor as labelled by [^{11}C]flumazenil with reference to a stereotaxic atlas (Talairach and Tournoux 1988). The same ROI map was used on each dynamic image and moved to correct for any patient movement. The same ROI map was used for all the subjects. The ROIs were placed on the following regions: right/left (R/L) frontal, R/L orbitofrontal, anterior cingulate, and occipital cortices, thalamus and R/L cerebellum. Due to movement during the scan, the position of each ROI was checked on each frame manually, independently by two researchers (A.L.H., S.W.).

For each ROI, time activity curves (TACs) were analysed kinetically using a one-tissue compartment model with a fixed blood volume component [5%]. Estimates of K_1, the unidirectional clearance of [^{11}C]flumazenil from plasma to brain, and k_2, the rate constant for efflux from tissue to plasma, were derived from least squared fits to data from the first 30 min until the midazolam infusion, i.e. pre-displacement (see Fig. 1). It was anticipated that occupancy of BDZ receptors by midazolam would lead to increased efflux of [^{11}C]flumazenil from the brain compartment to plasma (rate constant, k_{2d}). To achieve an adequate fit for the remainder of the TAC, it was found necessary to include both a k_{2d} and also a further constant (k_{back}) to fit its tail. The rate constants K_1, k_2 and k_{back} were then fixed and the time activity curve after midazolam was fitted to determine k_{2d}. The displacement index was then defined as k_{2d}/k_2 and was taken as a measure of occupancy of midazolam at the benzodiazepine receptor.

Pharmacodynamic measures

EEG measurements

The EEG was measured continuously throughout the procedure, using silver–silver chloride electrodes placed on the scalp according to the International 10-20 system of electrode placement; recordings made from four channels, F4–C4, P4–O2, F3–C3 and P3–O1. Offline analysis was performed both visually and automatically for the whole period. Spectral analysis with fast Fourier transform was used to derive a measure of power for each 1-Hz band from 1 to 30 for epoch lengths of 77 s (Spike 2, Cambridge Electronic Design). After visual exclusion of epochs containing muscle and movement artefact, total power in each of the EEG bands (delta, 1–4 Hz, theta 4–8 Hz, alpha1 8–10 Hz, alpha2 10–13 Hz, beta1 13–18 Hz, beta2 18–30 Hz) was calculated for each epoch and plotted against time. Peak beta power and AUC were calculated for each subject, and was used as a prime EEG measure of

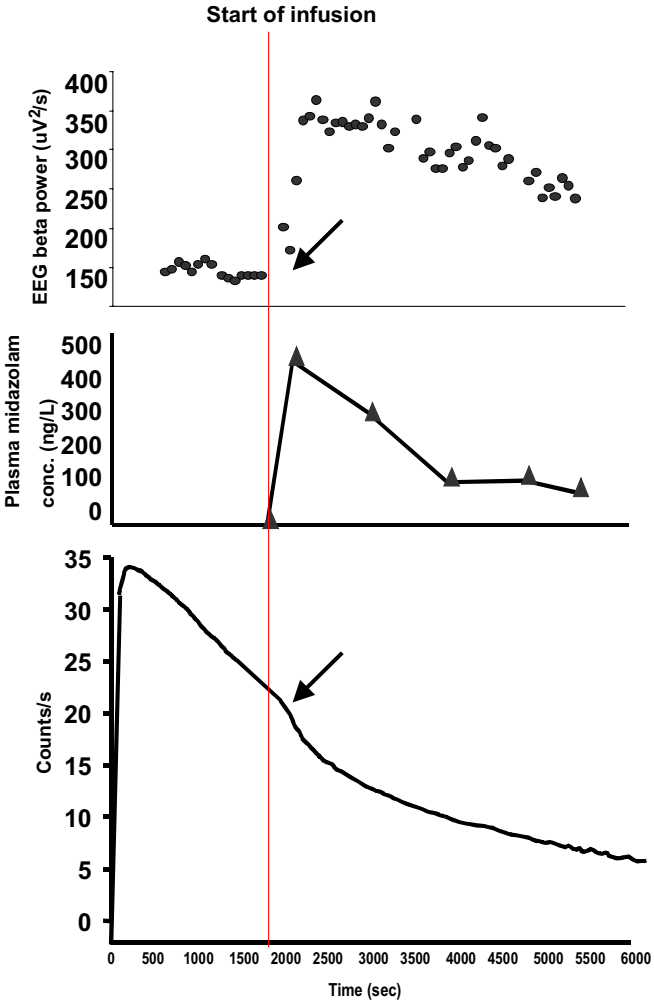

Fig. 1 *Bottom panel*: time activity head curve of [^{11}C]flumazenil. *Middle panel*: concentration of midazolam. *Top panel*: EEG beta power activity. The infusion of midazolam at 30 min resulted in an increased efflux of [^{11}C]flumazenil coincident with increased EEG beta power (marked with *arrows*). The *graphs* are from a typical study

midazolam's effect, as has been described previously (Malizia et al. 1996). The ratio of the peak beta1 value at F4–C4 after midazolam infusion to that at baseline was used as the pharmacodynamic index.

In addition, the EEG was analysed visually and any sleep was scored according to Rechtschaffen and Kales (1968) criteria. All periods of stage 2 or deeper and lasting longer than 10 s were summed over the whole 60-min period following midazolam infusion and referred to as 'total EEG sleep time'.

Saccadic eye movements

Saccadic eye movements to a target were measured as described by Ball et al. (1991) using electro-oculography. Only lateral saccades were studied. The subject was asked to fixate on a red light as it appeared on the screen mounted 67 cm on front of him. Data was then collected for 48 eye movements of 15–40° and a main sequence curve obtained, the measurement for peak velocity being the interpolation at 35°.

Anxiety and sedation

Anxiety and sedation were rated by the subjects at regular intervals (30 and 5 min prior to the injection of [^{11}C] flumazenil, and at 15, 25, 35, 50, 65, 80, 90 min afterwards) using a visual analogue scale (1–100).

Cardiac monitoring

Systolic and diastolic blood pressure and heart rate were recorded at the same time points as ratings of anxiety and sedation.

Statistical analysis

Two-tailed Student's *t*-test was used to determine differences in the means of other variables (midazolam levels, demographic details) or pharmacodynamic measures between the two groups. The relationship between clinical variables and pharmacokinetic or pharmacodynamic measures was examined using Pearson's correlation. A repeated measures analysis of variance (ANOVA; with pharmacokinetic data as the dependent variable and regions as the repeated measure) with Greenhouse–Geisser correction using SPSS software (SPSS Inc, Chicago, IL, version 12.0.1) was used to determine whether there were any differences between the groups in any of the pharmacokinetic data (K_1, k_2, k_{2d}, K_1/k_2, k_{2d}/k_2) (Sargent et al. 2000). Due to incorrect positioning in the scanner, cerebellar data from one alcohol dependent and one control subject was missing; these were replaced by the mean value of the remainder of the subjects in the group. ANOVA was used to compare the rate constants pre- and post-midazolam, k_2 and k_{2d}, with Bonferroni correction for multiple comparisons.

Results

Subject details

The alcohol dependent and non-dependent groups were well matched with regard to age (all results presented as mean±SE, 44.45±1.8 and 46.2±2.6, respectively). Predictably, the severity of alcohol dependency questionnaire score for the alcohol dependent subjects was significantly (p<0.001) higher (36.5±3.2) than that for the non-dependent control subjects (4.1±1.8). The average number of years of heavy drinking in the alcohol dependent group was 20.1±2.0 years. No significant (p<0.05) differences were found between the groups in their scores on the BDI,

Table 1 Demographic details of the subjects

	Control±SE (n=10)	Alcohol dependent±SE (n=11)
Age	46.2±2.6	44.45±1.8
SADQ (10, 10)	4.1±1.8	36.5±3.2
Years of heavy drinking	N/A	20.1±2.0
SSAI (8, 11)	27.4±2.4	32.5±2.8
STAI (10, 11)	32.7±1.8	40.4±4.6
BDI (10, 7)	3.1±0.9	6.3±1.5
Family history	2	2

Heavy drinking was defined as greater than 50 units week^{-1} (1 unit=8 g alcohol). Family history was 'positive' if there was a first-degree relative with alcoholism. The numbers after the name of the questionnaire denote the number of questionnaires that could be analysed for each group (control, alcohol dependent)
SADQ Severity of Alcohol Dependence Questionnaire, *SSAI* Spielberger's State Anxiety Questionnaire, *STAI* Spielberger's Trait Anxiety Questionnaire, *BDI* Beck's Depression Inventory

Spielberger SSAI or STAI inventory. Unfortunately due to missing data, not all the questionnaires could be analysed, as described in Table 1.

Midazolam levels

No difference was seen in the plasma concentration of midazolam between the two groups (p<0.05). Only samples from seven alcohol dependent and nine non-dependent subjects were available for analysis due to technical difficulties. In alcohol dependent subjects, the area under the curve for midazolam was 453±50 ng ml^{-1}, while in control subjects, 537±68 ng ml^{-1}.

Pharmacokinetic measures: [^{11}C]flumazenil image analysis

There was no difference in the AUC for levels of parent [^{11}C]flumazenil between the two groups. In the alcohol dependent group, the AUC was 1,153±61% [^{11}C]flumazenil in plasma min^{-1} and for control group, 1,122±51% [^{11}C]flumazenil in plasma min^{-1}.

In the nine brain regions studied, K_1 was higher in the alcohol dependent group than in controls. However, a repeated measures ANOVA revealed that there was no main effect of group ($F_{1,\ 19}$=2.267, p>0.05), but there was a significant main effect of region ($F_{3.771,\ 71.646}$=p>0.05) on K_1. No significant group×region interaction ($F_{3.771,\ 71.646}$= 0.620; p>0.05) was evident (see Table 2). In all brain regions the k_2 rate constant was higher in the alcohol dependent group compared with controls (see Table 2). A repeated measures ANOVA showed a significant main effect of group ($F_{1,\ 19}$=8.306, p>0.05) and of region ($F_{3.004,\ 57.076}$= 248.1, p>0.05) but no significant group×region interaction ($F_{3.004,\ 57.076}$=0.811; p>0.05). Post-hoc unpaired t-test revealed significant differences in k_2 between the control and alcohol dependent groups in thalamus, anterior cingulate, left orbitofrontal frontal and cerebellar regions (p>0.05).

The volume of distribution [V_D] was calculated [K_1/k_2] and repeated measures ANOVA revealed that there were no significant main effect of group ($F_{1,\ 19}$=0.775; p>0.05) but a significant main effect of region ($F_{3.346,\ 63.566}$=123.5; p>0.05) on V_D (see Table 2). There was no significant group×region interaction ($F_{3.346,\ 63.566}$=1.439; p>0.05). As expected though, in some brain regions, such as the orbitofrontal and anterior cingulate cortices, a reduction by 6–8% in GABA-BDZ receptor binding was seen in the alcohol dependent group compared with the control group.

In all brain regions studied, midazolam resulted in an increase in the rate of efflux of [^{11}C]flumazenil as evi-

Table 2 Rate constants (±SE) derived from time activity curves for individual regions of interest

Rate		R frontal	L frontal	Thalamus	R cereb	L cereb	Ant cing	Occipital ctx	R orbitofr	L orbitofr
K_1	N	0.295±0.008	0.294±0.009	0.339±0.008	0.350±0.011	0.357±0.010	0.323±0.009	0.400±0.014	0.293±0.012	0.286±0.001
	A	0.316±0.016	0.333±0.015	0.359±0.014	0.365±0.013	0.386±0.011	0.326±0.012	0.413±0.027	0.317±0.017	0.317±0.013
k_2	N	0.050±0.001	0.049±0.001	0.100±0.002	0.080±0.002	0.080±0.002	0.046±0.001	0.054±0.002	0.048±0.001	0.048±0.001
	A	0.053±0.001	0.054±0.001	0.109±0.002	0.084±0.003	0.088±0.002	0.050±0.001	0.062±0.004	0.056±0.004	0.057±0.004
k_{2d}	N	0.079±0.007	0.080±0.006	0.156±0.006	0.100±0.008	0.092±0.006	0.073±0.004	0.096±0.008	0.096±0.020	0.087±0.013
	A	0.092±0.008	0.091±0.008	0.200±0.024	0.112±0.013	0.140±0.018	0.086±0.011	0.096±0.007	0.115±0.014	0.130±0.018
K_1/k_2	N	5.97±0.20	6.00±0.21	3.39±0.10	4.38±0.15	4.47±0.15	7.03±0.25	7.40±0.25	6.08±0.27	6.05±0.27
	A	5.99±0.27	6.13±0.26	3.31±0.13	4.37±0.19	4.41±0.15	6.50±0.23	6.80±0.31	5.74±0.27	5.64±0.25
k_{2d}/k_2	N	1.6±0.1	1.6±0.1	1.6±0.1	1.2±0.1	1.2±0.1	1.6±0.1	1.9±0.4	1.8±0.3	1.8±0.2
	A	1.8±0.2	1.7±0.1	1.9±0.2	1.3±0.2	1.6±0.2	1.7±0.3	2.2±0.3	2.4±0.4	1.6±0.2

R and L cerebellum controls (N) n=9; alcohol dependent (AD) n=10 due to cerebellum positioned outside the field of view. Value±SE. Rate constants are expressed in ml plasma ml tissue^{-1} min^{-1}. Repeated-measures ANOVA revealed no significant region×group interaction for K_1, k_{2d}, K_1/k_2 or k_{2d}/k_2, but there was a significant interaction in k_2 ($F_{1,19}$=2.207; p>0.05)
K_1/k_2 equals volume of distribution and k_{2d}/k_2 equals the displacement index, taken as a measure of occupancy
N Non-dependent control subject, *A* alcohol dependent subject, *cereb* cerebellum, *cing* cingulate cortex, *ctx* cortex, *orbitofr* orbitofrontal cortex

denced by the higher k_{2d} compared to k_2. In the control group, k_{2d} was significantly higher ($p<0.0056$ with Bonferroni correction) than k_2 in all regions except right and left cerebellum; although a significant difference ($p<0.05$) was found between k_{2d} and k_2 in the right and left orbitofrontal cortex, this did not survive Bonferroni correction. In the alcohol dependent group, k_{2d} was significantly higher ($p<0.0056$ with Bonferroni correction) in all regions except for the right and left cerebellum. Concerning k_{2d}, a repeated measures ANOVA revealed no main effect of group ($F_{1,\ 19}=3.437$, $p>0.05$), a main effect of region ($F_{4.204,\ 79.881}=19.714$, $p<0.05$) but no significant group×region interaction ($F_{4.204,\ 79.881}=1.808$; $p>0.05$) (see Table 2).

Although a higher displacement index (k_{2d}/k_2) was found in the alcohol dependent group compared with the control group in all brain regions, repeated measures ANOVA showed no main effect of group ($F_{1,\ 19}=0.847$, $p>0.05$), a main effect of region ($F_{3.041,\ 57.771}=5.384$, $p<0.05$) but no significant group×region interaction ($F_{3.041,\ 57.771}=0.916$; $p>0.05$) (see Table 2).

Pharmacodynamic measures

Visual analogue scales

After the midazolam infusion, the majority of the subjects were unable to respond to the request for a rating about how sedated or anxious they were feeling. Even for those that responded, their reply did not necessarily reflect the actual experience since the ratings of sedation were often inaccurate. For instance, subjects sometimes rated themselves very low for sleepiness immediately after being woken by the investigator asking for a response. They were often disorientated and forgetful of previous instructions. In general, however, the sleepiness scores were higher in the non-dependent control group. Due to the low numbers of responses, formal statistical analysis was not undertaken.

Cardiovascular effects

No significant differences were seen in systolic or diastolic blood pressure or heart rate between the groups nor during the procedure (results not shown, see Table 3).

Saccadic eye movements

Subjects were too drowsy at the initial time points after midazolam infusion to complete the task so analysis of the saccadic eye movement data to reflect benzodiazepine receptor function was not possible (see Table 3).

Table 3 Pharmacodynamic measures (±SE) obtained during the PET scan pre- and post-midazolam infusion 30 min into the procedure

	Minutes from start midazolam infusion																	
	−35		−15		−5		5		20		35		60		70			
	A[a]	N	A	N	A	N	A	N	A	N	A	N	A	N	A	N	A	N
Saccadic eye movements																		
No. of subjects able to respond	8	8	10	10	9	10	1	0	3	1	5	3	4	5	7	6		
Peak velocity (degs^{-1})	497±81	462±74	473±95	429±53	473±88	435±68					314±114	280±31	424±74	317±36	420±101	337±31		
Error (deg)	−1.4±1.7	−0.7±1.7	−1.7±1.7	−1.2±1.4	−1.1±1.5	−1.1±1.6					−2.9±0.9	−2.4±0.9	−2.2±1.1	−1.5±1.2	−1.4±1.4	−1.5±1.6		
Peak deceleration (deg s^{-2})	−47±13	−36±12	−43±15	−36±11	−45±11	−36±14					−32±6	−24±5	−40±14	−27±8	−37±14	−24±6		
Visual analogue scales																		
No. of subjects able to respond	10	8	11	10	11	10	3	2	6	3	7	4	8	9	9	6		
Anxiety (mm) mean	18±16	17±23	16±14	14±17	16±14	14±21	(17)	(25)	12±14	(18)	9±8	15±13	14±11	21±13	10±10	8±8		
Sleepiness (mm) mean	20±23	30±25	18±31	42±16	18±18	46±32	(17)	(15)	32±29	(70)	29±24	65±19	45±30	56±32	29±19	46±31		

There were no differences in baseline saccadic eye movements. Since these were unobtainable for some time after the midazolam infusion for the majority of subjects, this measure could not be used as an index of midazolam occupancy/function. Visual analogue scales were not felt to represent changes seen objectively. In both groups of subjects, no change was seen in the diastolic blood pressure, but a transitory non-significant increase in heart rate was seen in both groups, accompanied by a reduction in systolic blood pressure in the alcohol dependent group only

[a] *A* Alcohol dependent, *N* non-alcohol dependent

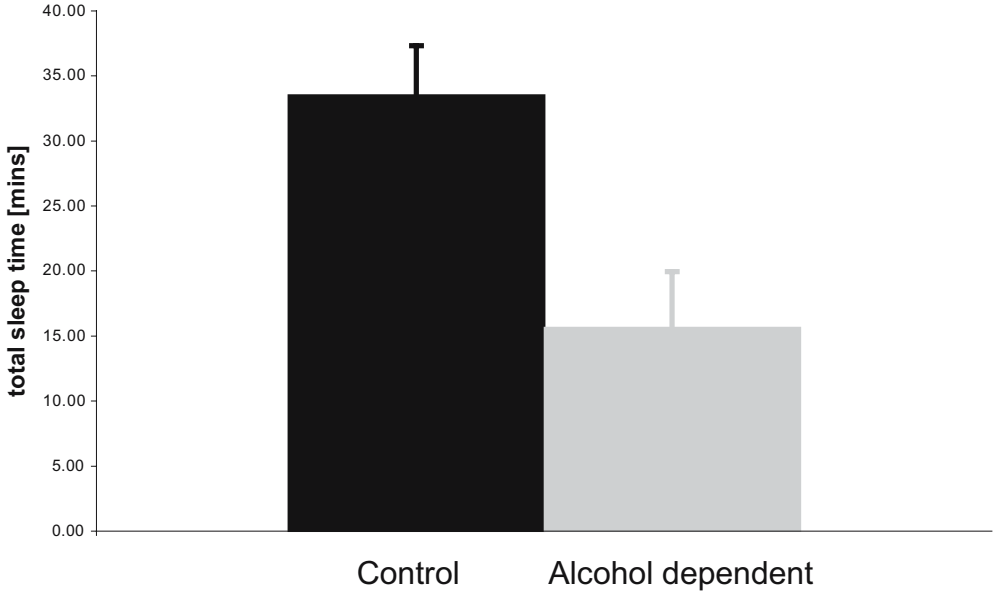

Fig. 2 Midazolam resulted in significantly ($p<0.05$) less time asleep in the alcohol dependent group compared to the non-alcohol dependent group. Sleep was sleep scored according to Rechtschaffen and Kales (1968) criteria. All periods of stage 2 or deeper and lasting longer than 10 s were summed over the whole 60-min period following midazolam infusion

EEG

Sleep. Visual analysis revealed the expected change in the EEG. About halfway through the 5-min infusion, subjects usually closed their eyes, and later fell asleep, with the appearance of low amplitude mixed frequencies and marked rhythmic beta activity at 13–18 Hz. After an interval that varied from subject to subject, they fell asleep with sleep spindles and K complexes characteristic of stage 2 sleep. Subjects spent the rest of the scanning period either asleep or alternating between sleep and waking. EEG beta power and more specifically, drug-related beta1 power does not change during stage 1 and 2 sleep.

The time taken to fall asleep after midazolam infusion (sleep onset latency) was not significantly different in the two groups. However, the total EEG sleep time was significantly lower in the alcohol dependent group [mean±SE (one recording from the alcohol dependent group was uninterpretable due to artefact) alcohol dependent group: 15.7±4.3 min; control group: 33.5±3.8 min; $p<0.05$; see Fig. 2]. From visual inspection of the data, it was apparent that increasing midazolam AUCs were associated with longer sleep times in the control subjects but this relationship was not evident in the alcohol dependent subjects. However, the limited number of subjects in whom both these data were available precluded formal statistical comparison.

There was a significant correlation between sleep onset latency and score on the STAI (all subjects $r=0.6$, $p<0.05$; control $r=0.5$, $p>0.05$; alcohol dependent $r=0.6$, $p>0.05$), but not between total EEG sleep time (all subjects $r=-0.2$, $p>0.05$; control $r=-0.2$, $p>0.05$; alcohol dependent $r=0.05$, $p>0.05$), suggesting those that were more anxious took longer to show signs of sleep but anxiety did not affect

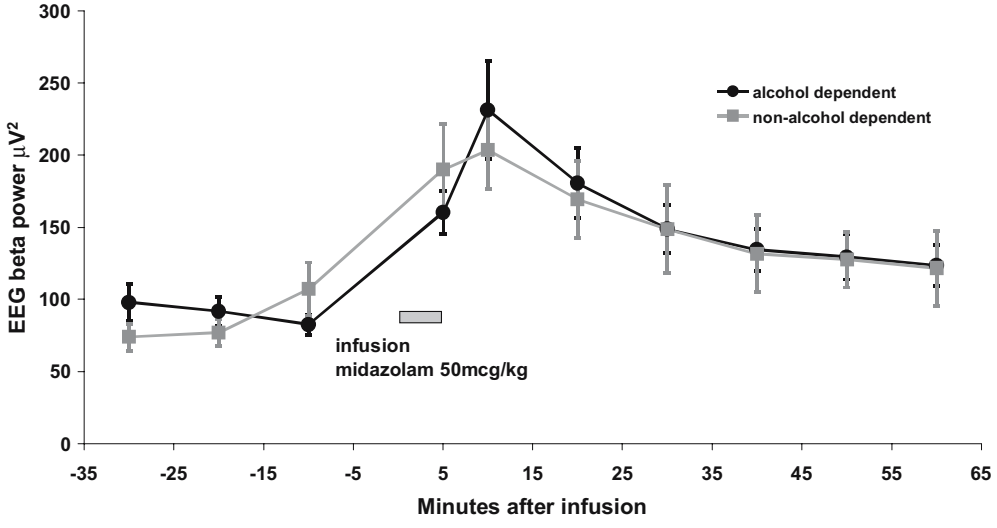

Fig. 3 Midazolam resulted in increased EEG beta power in alcohol dependent and non-dependent groups. There was no difference in the responses

total EEG sleep time. No other questionnaire measures were significantly related to sleep.

EEG betal power. Automatic analysis revealed that in all subjects there was an increase in beta1 power that began between 2 and 5 min into the midazolam infusion and remained increased for 30–60 min (see Fig. 3). One EEG recording in an alcohol dependent subject was uninterpretable due to artefact. There was no significant difference in beta1 power at baseline, and no significant difference in the change (peak/baseline) in EEG beta1 power after the midazolam infusion between the two groups, nor in power in the alpha and theta bands. The EEG beta1 power ratio in the alcohol dependent group was 2.79±0.85 and in the control group, 2.75±1.18. Areas under the curve were calculated for each patient and these also showed no difference between the two groups.

Relationship between pharmacokinetic and pharmacodynamic measures

The relationship between the brain pharmacokinetics of midazolam, i.e. displacement index as defined from the PET scan, and its functional effects was explored, i.e. EEG and sleep measures. There was no correlation between the displacement index and change in EEG beta1 power ratio or any indices of sleep. In addition, we looked at the relationship between plasma midazolam levels and absolute EEG beta1 power at the five time points during the scanning procedure. As expected in most individuals in both groups, there was a strong, significant correlation between EEG beta1 power and plasma midazolam levels supporting previous studies (e.g. Van Steveninck et al. 1993). There was no significant difference between the two groups in terms of these correlations.

Discussion

Using this [^{11}C]flumazenil PET paradigm to directly measure the pharmacokinetics and pharmacodynamics of midazolam at the brain benzodiazepine receptor, we have shown that alcohol dependence is associated with reductions in a pharmacodynamic response (EEG sleep time) in the absence of reduced brain receptor occupancy (indeed, slightly greater receptor occupancy in the alcohol dependent group was seen). This clearly demonstrates reduced sensitivity to this action of the benzodiazepine receptor agonist, midazolam, in alcohol dependence. We found no differences in another index of benzodiazepine function, EEG beta1 power.

This study was technically challenging to perform, requiring simultaneous acquisition of PET imaging and EEG data in one session before and after an infusion of midazolam. Associated movement artefact and loss of arterial input function resulted in the rejection of almost a fifth of the scans obtained (five of 26). Nevertheless, acquiring scan data in a single scan protocol has significant advantages over a two-scan protocol when the volunteers are difficult to recruit and may not tolerate two scans. Videbaek et al. (1993) used a two-scan SPECT [^{123}I]iomazenil protocol to measure the pharmacokinetics of midazolam and ran into difficulties. Subjects fell asleep with the midazolam infusion (6 mg h^{-1} compared to 3.5 mg bolus in our study), some not waking throughout the procedure and spending the night in intensive care as a precaution. Such an approach does not therefore lend itself to wider clinical experimental use.

The rate constants derived in our present study for the control population are similar to those found by Malizia et al. (1996). Alcohol dependence has been commonly reported to be associated with reduced blood flow and metabolism (Volkow et al. 1992; Adams et al. 1993; Gilman et al. 1996). However, we found no evidence of reduced blood flow since the rate constant K_1 was not lower in the alcohol dependent group compared to control group. Our patients were all abstinent for at least 6 weeks and this likely contributed to the blood flows in the two groups being similar. A study comparable to the present one using patients abstinent for 2 months reported no significant reduction in metabolism, whereas it has been reported in studies using patients abstinent up to about 1 month (Nicolas et al. 1993; Volkow et al. 1994).

Alcohol dependent patients had slightly fewer GABA-BDZ receptors in some brain regions compared to the control group. Lower benzodiazepine receptor levels would be consistent with other studies showing reduced levels of the GABA-BDZ receptor (Lingford-Hughes et al. 1998; Abi-Dargham et al. 1998). The protocol we used was optimal for determining the pharmacokinetics of midazolam at the benzodiazepine receptor rather than the volume of distribution (V_D) and so the full [^{11}C]flumazenil washout curve was not available for calculation of V_D as in routine neuroimaging protocols. This likely contributed to a probable underestimation and may have reduced our sensitivity to detect group differences (see Lassen et al. 1995).

Several important kinetic factors that could have contributed to the displacement index (k_2/k_2) were considered. Similar to other studies, we could find no evidence that the metabolism of [^{11}C]flumazenil or midazolam was altered in our alcohol dependent patients who were medically, neurologically and cognitively healthy (Abi-Dargham et al. 1998; Bauer et al. 1997). Although we did not measure its major active metabolite, α-hydroxymidazolam, relatively low concentrations of this metabolite have been reported when midazolam is given i.v. as in the present study (Mandema et al. 1992). Changes in blood flow secondary to midazolam will influence the measured rate (k_{2d}) of efflux of [^{11}C]flumazenil and midazolam has been shown to reduce blood flow in a dose-dependent manner. Veselis et al. (1997) reported that midazolam at doses two to three times higher than in the present study (7.5–9.7 mg compared with 3.5 mg/70 kg) reduced global blood flow by 12%. Therefore in the present study, a smaller reduction is likely and results in a reduced rate of efflux of [^{11}C]flumazenil. This would reduce the likelihood of detecting a difference between the rate constant pre- (k_2) and post-midazolam

(k_{2d}) due to benzodiazepine receptor occupancy by midazolam and of detecting a difference in the displacement index (k_{2d}/k_2) between the groups.

We found that time spent asleep, as determined by EEG, was significantly different between groups, with alcohol dependent subjects asleep for half the time of that seen in the control group, despite no reduction in occupancy of the benzodiazepine receptor by midazolam. In their study Bauer et al. (1997) found that the same amount of midazolam was required for sleep induction but did not report time asleep. Elsewhere, abstinent alcohol dependence has been reported to be associated with sleep disturbance (Currie et al. 2003). We did not collect detailed information about sleep patterns in our patients, and so cannot fully exclude the possibility that the alcohol dependent group may have had a greater level of 'pre-existing' sleep disturbance that contributed to the reduced sedative effects of midazolam seen. In addition, whilst we believe that the benzodiazepine receptor is critical in midazolam induced sleep, we are not able to exclude a contribution from other neurotransmitters involved in modulating sleep (see Brower 2001). However, our finding that alcohol dependence is associated with substantially less time asleep is consistent with pre-clinical models of alcohol dependence, where reduced anaesthetic effect of diazepam and pentobarbital, but not thiopental, have been reported (Newman et al. 1986; Curran et al. 1988; Doudet et al. 1995). In human alcohol dependence, tolerance to anaesthetic agents working through the $GABA_A$ receptor (thiopental, propofol) has been reported, including requiring higher doses and shorter time spent asleep, though not consistently (Loft et al. 1982, 1983; Swerdlow et al. 1990; Fassoulaki et al. 1993).

The reduced EEG sleep time seen was in contrast to finding no differences in the other selected benzodiazepine pharmacodynamic indices, saccadic eye movements and EEG beta1 power. We did not find that alcohol dependence was associated with any alteration in baseline EEG or saccadic eye movement parameters or in the effect of midazolam on EEG beta1 power or saccadic eye movements although the data available on the latter was limited due to sedation. Other studies have also reported limited changes in measures typically considered to reflect benzodiazepine receptor function such as no difference in the dose of midazolam required to sedate alcohol dependent (abstinent for 2–3 weeks) and control subjects, nor in EEG responses, including beta power (Ciraulo et al. 1997; Bauer et al. 1997). We chose EEG beta power since it is a well-recognised and validated index of benzodiazepine function (Domino et al. 1989; Feshchenko et al. 1997). Although we found a significant correlation between plasma midazolam levels at the five time points during the scanning procedure and absolute EEG beta1 power, we did not find a significant correlation of EEG beta1 power ratio (peak/baseline) with midazolam occupancy, i.e. displacement index derived from the PET image as Malizia et al. (1996) did using this PET protocol. We believe that we did not see a correlation between brain occupancy and EEG beta1 power ratio since we only used a single dose of midazolam and so had a narrower range of plasma concentrations and effects than in Malizia et al.'s (1996) study, which used three doses of midazolam. In addition, the displacement index gives a single value for each subject which represents the entire period of the central effects of midazolam. We suspect this did not correlate with EEG beta1 power ratio (peak/baseline) because the latter is predominantly determined by the initial rise in midazolam concentrations. Such a lack of attenuation of drug induced EEG beta power in alcohol dependence is consistent with a study showing no attenuation of beta power after chronic benzodiazepine administration (Lee and Lader 1988).

Given the profound differences in midazolam-induced sleep EEG activity, why is reduced sensitivity not seen with all benzodiazepine pharmacodynamic measures? The reason for this is unclear but probably reflects the fact that a number of different subtypes of the GABA-BDZ exist, each of which appears to have a particular association with a specific function (Mohler et al. 2002). For instance, the α1 containing subtype is involved in mediating the sleep promoting effects of benzodiazepines and alcohol (Blednov et al. 2003). We know from animal studies that chronic alcohol exposure differentially affects subunit expression in different brain regions (see Kumar et al. 2004) and our finding of reduced sleep time would therefore be consistent with reports from animal models that chronic alcohol exposure in rat brain leads to reduced levels of the α1 subunit (Montpied et al. 1991). In man, subunit changes have not been systematically investigated with post-mortem studies so far showing increased or no changes in α1 and α3 polypeptide and diazepam-sensitive (α1, α2, α3, α5) benzodiazepine receptor levels in various parts of the cortex (Tran et al. 1981; Dodd et al. 1992; Lewohl et al. 1997, 2001; Mitsuyama et al. 1998). It is not clear which subtype or subtypes are involved in saccadic eye movements or EEG beta-power although recent evidence suggests a role for the α2 subtype in EEG beta frequency (Edenberg et al. 2004). This study cannot inform the important question about which subtypes may be different in alcohol dependence in man since midazolam is an agonist at the α1, α2, α3 and α5 subtypes and [^{11}C]flumazenil is a non-selective ligand (Lingford-Hughes et al. 2002).

In addition, whilst it is clear that chronic alcohol exposure can alter the benzodiazepine receptor subunit profile, it is not apparent what happens in prolonged abstinence or indeed if any of the changes reported in man pre-dated their abuse of alcohol. Previous neuroimaging studies that showed reduced GABA-benzodiazepine receptor levels in alcohol dependence could not determine whether this reduction was secondary to alcohol consumption (Lingford-Hughes et al.1998; Abi-Dargham et al. 1998). There is evidence from neuroimaging and pharmacological challenge studies in people with a family history of alcoholism supporting the hypothesis that reduced GABA-benzodiazepine receptor function is involved in mediating vulnerability to alcoholism (Volkow et al. 1995; Cowley et al. 1994; Sarid-Segal et al. 2000), as well as more recent genetic studies (Loh et al. 1999; Edenberg et al. 2004). In

the current study, only two subjects in each group had a family history of alcoholism suggesting that the differences seen here are more likely to be as a result of their alcohol consumption.

In conclusion, the objective of this study was to simultaneously assess the pharmacokinetics and pharmacodynamics of midazolam at the benzodiazepine receptor in man to test the hypothesis that alcohol dependence is associated with reduced benzodiazepine receptor function. Whilst we achieved this, the experiment was challenging to conduct and the sensitivity of the paradigm may not have been great enough to detect differences between two groups of subjects. We acknowledge that the statistical power of the study is limited; however, power calculations revealed that for some parameters derived from this data, an inappropriately large number of subjects would have to be scanned. Nevertheless, we showed that alcohol dependence in man is associated with decreased midazolam induced time asleep, in the absence of reduced occupancy of the benzodiazepine receptor. We propose that this reduced sensitivity in alcohol dependence may reflect a difference in the subunit profile of their GABA-BDZ receptors, either as part of their vulnerability to or as a consequence of their alcohol dependence.

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