Psychopharmacology (1999) 146:180–184 

ORIGINAL INVESTIGATION

**John Potokar · Nick Coupland · Sue Wilson**
**Ann Rich · David Nutt**

# Assessment of $GABA_A$ benzodiazepine receptor (GBzR) sensitivity in patients on benzodiazepines

Received: 10 December 1998 / Final version: 27 April 1999

**Abstract** *Objectives*: To measure $GABA_A$ benzodiazepine receptor sensitivity in patients taking benzodiazepines and compare with matched controls. *Methods*: Seven patients who were on prescribed benzodiazepines for an anxiety disorder or insomnia were recruited from general practice and an adult mental health service outpatient clinic. They were matched with seven volunteers. All subjects received an intravenous injection of midazolam 50 μg/kg in 10 ml normal saline over 10 min. Objective responses to midazolam were assessed using saccadic eye movement velocity slowing and subjective assessments using visual analogue scales. Measurements were recorded for 120 min and plasma midazolam concentrations obtained at 15-min intervals post-infusion to 120 min. Ratios of pharmacodynamic/pharmacokinetic effects were obtained for each individual to estimate $GABA_A$ benzodiazepine receptor sensitivity. *Results*: Patients had an attenuated response to midazolam on both subjective and objective measures. $GABA_A$ benzodiazepine receptor sensitivity was significantly reduced in the patient group. *Conclusions*: Chronic treatment with benzodiazepines was associated with reduced effects of midazolam. Saccadic eye movement velocity was especially sensitive as a measure of attenuated response.

**Key words** Benzodiazepine · $GABA_A$ receptor · Tolerance · Saccadic eye movement

J. Potokar (✉) · S. Wilson · A. Rich · D. Nutt
Psychopharmacology Unit, University of Bristol,
School of Medical Sciences, University Walk,
Bristol BS8 2BA, UK
Fax: +44-117-9277057

N. Coupland
Department of Psychiatry, University of Alberta,
1E7.28 MacKenzie Centre, 8440-112 Street, Edmonton, Alberta,
Canada T6G 2B7

## Introduction

Chronic use of benzodiazepines may result in the development of tolerance, dependence and characteristic withdrawal symptoms on cessation. Tolerance is the phenomenon whereby increasing amounts of a drug have to be taken to produce the original effect. The understanding of this process is critical, not only to improve treatments for anxiety but also to further our understanding of other (more lethal) drugs of addiction. The latter include alcohol, the drug most frequently taken for anxiolysis and which, like benzodiazepines also has effects on the $GABA_A$ receptor. One theory of the mechanism of tolerance is that chronic exposure to benzodiazepines results in reduced sensitivity of the GBzR. Clinical research has used various techniques to explore both the effects of chronic exposure to benzodiazepines and GBzR sensitivity, including endocrine and biochemical responses, psychometric, performance and cognitive tasks, and electroencephalography. However, there are several limitations with these approaches, including variability of responses across gender, time and between individuals which restrict their usefulness.

We have been using the technique of saccadic eye movement (SEM) analysis to study GBzR sensitivity. SEMs are fast, conjugate gaze changes which enable the subject to center a target of interest onto the fovea. They have the advantages of sensitivity to low doses of benzodiazepines (Roy-Byrne et al. 1993) and once initiated they are beyond voluntary control. Additionally, SEM parameters reliably reflect plasma benzodiazepine levels (van Stevenick et al. 1991).

The purpose of this study was to assess GBzR sensitivity in patients on chronic benzodiazepines, compared with matched controls, by measuring slowing of saccadic eye movement peak velocity (SEMV) in response to an IV infusion of midazolam. This benzodiazepine agonist was chosen because it is available in IV formulation and has a short terminal half-life, which allows effects to be determined over a range of concentrations in a short experiment. Our hypotheses were that pa-

tients on chronic benzodiazepines would have less slowing of their eye movements in response to this challenge and less subjective sedation, reflecting reduced GBzR sensitivity.

## Materials and methods

Seven patients on chronic benzodiazepine treatment were recruited from general practice and an adult mental health services outpatient clinic. These were matched for age and sex with seven volunteers. All subjects gave written informed consent for the study which was approved by the local ethics committee. Patients were taking 1–20 mg diazepam equivalents per day, for an anxiety disorder or insomnia. At the screening visit, a medical and psychiatric history was taken by an experienced Psychiatrist and diagnoses made according to DSM1V criteria. Patients underwent a physical examination and ECG. Routine bloods (urea and electrolytes, full blood count and liver fuction tests) were done and a urine sample obtained for a toxicology screen. Exclusion criteria for patients and volunteers were significant physical illness, females not using adequate contraception and excessive alcohol intake (>28 units per week for males; >21 units per week for females). None of the healthy volunteers had a current or past history of an axis 1 disorder. Subjects were told of the nature of the study and that an attempt was being made to understand the effect of long-term benzodiazepine treatment on brain receptors. They were told that midazolam might make them feel sleepy, heavy eyed and might reduce co-ordination. They were asked to follow a normal diet and had their usual breakfast on the day of the study. They were not allowed to consume caffeine-containing drinks during the test period. Subjects were also told that they should not drive until the following day, and that they could withdraw from the study at any time.

### Procedure

Subjects attended the testing room at 0900 hours. They were rested in a semi-supine position on a comfortable couch and IV cannulae were inserted (one in each arm) for midazolam administration and blood sampling.

At baseline the following parameters were recorded: anxiety using the Spielberger trait and state anxiety inventories (STAI-T/STAI-S). Subjective sedation using a visual analogue scale (VAS), was also assessed. This was a 100-mm scale in intervals of 10 mm. Zero represented “not at all”, and 100 represented “the worst ever”. SEMs and VAS were recorded at 15-min intervals for 120 min and blood was drawn at these times for midazolam assay.

Eye movements in response to a light moving across a screen were recorded, analysed and stored using the Cardiff system (CSGAAS) – see below. Baseline saccadic eye movement ratings were recorded at t=–30 min, t=–20 min and t=–10 min.

At t=0 min, the subject received midazolam 50 μg/kg made up to 10 ml with normal saline, which was infused via a syringe driver over 10 min. This regime was chosen after analysis of preliminary data showed that volunteers felt drowsy at this dose but the majority were still able to perform saccadic eye movements.

### Measurement of eye movements

A silver/silver chloride disposable EEG electrode (Medicotest, Denmark) together with a small amount of electrode gel was placed 1 cm laterally to the outer canthus of each eye and on the glabella, after scarification with abrasive cream (Skinpure, Nihon Kohden). Electrode impedances were measured and confirmed to be less than 5 kohm. The electrodes were connected to a DC amplifier with a gain of ×1000; output from the amplifier was then sampled 256 times per second via an analogue to digital converter. The resulting digital information was then analysed by an IBM compatible PC. Since vertical eyeball movement significantly alters EOG amplitude in a non-linear way (Barry and Melville-Jones 1965), only lateral saccades were studied. Forty-eight saccade trials were recorded at each time point, at target displacements of 10–40°. Peak velocity for each saccade was plotted against the angle of displacement, to produce a main sequence curve. The saccade peak velocity value for each time point was produced by interpolation into the main sequence curve at an angle of 35°. For a fuller description of the methodology used see Wilson et al. (1993**)**.

### Midazolam assay

Blood samples were placed in lithium heparin tubes, immediately stored in ice and centrifuged within 30 min. Plasma was then stored at –20°C until analysed. Midazolam was measured in plasma by a gas liquid chromatographic (GLC) method with nitrogen/phosphorus end-point detection utilising a three-stage extraction process together with an internal standard to monitor recovery.

Standards (0–200 ng/ml) were extracted from drug-free plasma obtained from normal healthy volunteers in the same manner.

The inter and intra-assay coefficients of variation (CV) were both within 10% and the assay limit of detection (defined as 3 times baseline noise) was 0.5 ng on column. The analytical (actual) recovery was 70%. A number of psychoactive compounds were tested for interference in the assay; none of these were found to cause problems.

### Data analysis

Baseline variables were compared between groups using unpaired *t*-tests or Mann-Whitney tests, where appropriate. Repeated measures mixed ANOVA was used to examine group effects, time effects and group×time interaction. Heterogeneity of covariance was tested with the Mauchly sphericity test and degrees of freedom modified using the Greenhouse-Geisser adjustment, where appropriate. For significant effects, unpaired *t*-tests were used at the first time point (t=15) when midazolam effect was greatest. Area under the curve was used to estimate both total pharmacodynamic effect (reduction in SEMV) and total pharmacokinetic effect (concentration of midazolam) from t=0 to t=120 min.

## Results

Demographic data and baseline variables are given in Table 1. The patients had increased lifetime anxiety as measured by the STAI, but although there was a trend towards higher state anxiety prior to the study, this did not reach statistical significance.

### Demographic and baseline clinical variables

Clinical details are presented in Table 2. A variety of benzodiazepines were being prescribed both for anxiolysis and as hypnotics.

**Table 1** Demographic data and baseline variables

| | Benzodiazepine group | Controls | |
|---|---|---|---|
| Age | 44.0 (12.4) SD | 36.1 (14.2) SD | NS |
| Sex | 4F:3M | 4F:3M | NS |
| STAI | 53.0 (10.8) | 34.0 (5.2) | *P*=0.01 |
| SSAI | 43.9 (18.5) | 29.1 (3.1) | NS |
| VAS anxiety | 24.4 (23.2) | 5.2 (4.5) | NS |

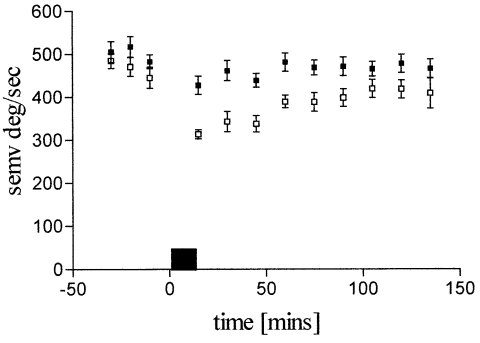


**Fig. 1** Mean SEM velocity versus time in both groups. ■ Patients (*n*=7), □ controls (*n*=7). *Black bar* represents midazolam infusion

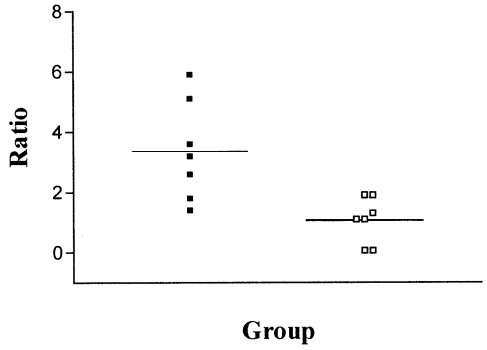


**Fig. 2** Ratio of SEMV response to plasma midazolam concentration in patients (□) and controls (■). *Black line* represents median

**Table 2** Clinical details

| Subject number | Diagnosis | Benzodiazepine |
|---|---|---|
| 1 | Primary insomnia | 20 mg temazepam |
| 2 | OCD | 6 mg clonazepam |
| 3 | Social phobia | 15 mg diazepam |
| 4 | Primary insomnia | 20 mg nitrazepam |
| 5 | GAD | 9 mg diazepam |
| 6 | GAD | 4 mg clonazepam |
| 7 | Primary insomnia | 10 mg temazepam |

### Plasma midazolam

Plasma midazolam concentrations were higher in the patients. Repeated measures mixed ANOVA showed a group effect ($F$=5.66; $df$=10,1; $P$=0.039), a time effect ($F$=16.03; $df$=17.7, 1.77; $P$=0.0001 and a group-time interaction ($F$=6.9; $df$=17.7, 1.77; $P$=0.007).

### Effects of midazolam on saccadic eye movement parameters

#### *SEMV*

There was no significant difference in baseline velocities (controls 488.6, SD 46.7; patients 516.2, SD 79.4, NS). Midazolam produced a reduction in velocity that peaked in both groups at t=15. At t=105 min, velocities in both groups were similar to baseline, and to each other (Fig. 1).

Repeated measures ANOVA (mixed) showed a main effect of group ($F$=8.2, $df$=12,1; $P$=0.014), time ($F$=27, $df$=40.6, 10, $P$=0.000) and group by time ($F$=3.9, $df$=10, 3.4, $P$=0.013).

At t=15 min, there were very significant differences in SEMV between the groups (t=–3.3, $df$=12, $P$=0.006 (2-tailed); 95% CI=–171.7 to –35.1).

In order to get a measure of both pharmacodynamic and pharmacokinetic effect we adapted the method recently used by Roy-Byrne and colleagues to assess GBzR sensitivity in patients with panic disorder and OCD (Roy-Byrne et al. 1996). Area under the curve was calculated for both SEMVs and plasma midazolam concentration from t=0 min to t=120 min. To obtain a measure of SEMV effect, we subtracted the values for AUC from that obtained by extrapolating mean baseline SEMVs to t=120, for each individual. Ratios of AUC for SEMV and plasma midazolam concentration were then compared to give a measure of receptor sensitivity (Fig. 2).

The ratios of pharmacodynamic/pharmacokinetic effect were significantly different between the two groups ($t$=3.36, $df$=12, $P$=0.006 (2-tailed unpaired $t$-test). The 95% confidence interval for difference between means was 0.81–3.8.

#### *Sedation*

There was an increase in sedation in both groups following midazolam, although there was a wide variation in response. There was no group effect, but there was a time effect ($F$=5.88, $df$=3.4,41; $P$=0.05) and a group by time effect ($F$=2.8, $df$=3.4,41; $P$=0.05), with the patients experiencing less sedation at 15 and 30 min.

AUC for the sedation time plots was also examined as a pharmacodynamic variable. There was no difference in PD/PK ratios using sedation as the pharmacodynamic variable ($t$=1.82, $df$=12; $P$=0.09).

## Discussion

This pilot study extends our earlier work which showed a reduction in SEMV in response to midazolam in volunteers (Ball et al. 1991). The current study demonstrates that patients on chronic benzodiazepines have a significantly attenuated saccadic eye movement velocity response to intravenous midazolam compared with matched controls, despite higher plasma midazolam concentrations. Furthermore, there were no baseline differences between patients and controls in terms of both SEMV and sedation ratings, suggesting that patients had become tolerant at least to some effects of their medica-

tion. There are several possible explanations for these findings.

Firstly, it is possible that the patient group have reduced GBzR sensitivity as a trait phenomenon. There is some evidence for this. Our group has shown that the benzodiazepine antagonist flumazenil induces panic attacks in patients with panic disorder, but not in controls, suggesting that this disorder is associated with a shift in GBzR sensitivity in the inverse agonist direction (Nutt et al. 1990). Roy-Byrnes group found reduced SEMV response to IV diazepam in unmedicated patients with panic disorder (Roy-Byrne et al. 1990). The same group also found that chronic alprazolam treatment was associated with significantly attenuated SEMV responses to IV diazepam compared with untreated patients (Cowley et al. 1995) which argues against trait differences in GBzR function or number being the sole explanation for the attenuated SEMV responses in subjects taking benzodiazepines chronically.

An alternative explanation is that chronic benzodiazepine use results in a decrease in number of GBzR in the brain. Some groups have found this in animal work (Chiu and Rosenberg, 1978; Sher et al. 1983; Miller et al. 1988), but others have not (Gallagher et al. 1984; Stephens and Schneider 1985; Nutt and Costello 1988). The question of whether tolerance involves altered interaction between the GABA and benzodiazepine site has also been studied, again with conflicting results. Gallagher found a decreased effect of GABA agonists on enhancing benzodiazepine binding in chronically treated animals (Gallagher et al. 1984) whereas Stephens and colleagues have not found this effect (Stephens and Schneider 1985; Stephens et al. 1988).

In animal models, chronic treatment with benzodiazepines increases the activity of inverse agonists (Little et al. 1988**;** Nutt and Costello 1988), although the mechanism for this is not known. Advances in molecular biology have demonstrated the complex heterogeneity of the GBzR and an explanation for tolerance may be that it results from changes in subunit isoforms possibly in specific brain regions. There is some evidence for this but again the results are mixed, with some groups finding a reduction in the expression of $\alpha_1$ and $\gamma_2$ isoforms (Heninger et al. 1990; Kang and Miller 1991) and others finding no changes in $\alpha_1$ mRNA, but increases in $\alpha_3$ and decreases in $\alpha_5$ mRNA (O' Donovan et al. 1992).

The differences in plasma midazolam concentrations between individuals emphasise the importance of including pharmacokinetic measurements when assessing receptor sensitivity. The increased concentrations in the patient group may reflect altered protein binding, or slower metabolism. Interestingly, Roy-Byrne's group also found increased plasma benzodiazepine levels in a study comparing slowing of SEMVs in patients with panic disorder versus controls (Roy-Byrne et al. 1990).

Caveats to the design include both diagnostic grouping and the use of different benzodiazepines in the patient group. In practice, there is considerable co-morbidity in patients with anxiety disorders and many report sleep problems including insomnia. Clearly, it would have been ideal if all patients had been taking the same benzodiazepine and future studies should control for this variable. The cross sectional nature of the study means that preceding trait anxiety rather than chronic exposure to benzodiazepines may explain the attenuated response in the patient group. A longitudinal study would help clarify this issue.

Finally, the SEMV response to IV midazolam appears to be less variable than subjective sedation. Preliminary data on repeat administration also suggest that the method is reliable (Potokar et al. 1998). Recruiting patients for studies of this nature is often a slow, laborious process and it is therefore essential to use paradigms which are reliable and sensitive if type 1 error is to be avoided. Future studies would benefit from a larger number of patients as well as less diagnostic diversity. This may help clarify whether GBzR sensitivity is a state or trait phenomenon, whether drug usage can alter this sensitivity and perhaps most importantly whether tolerance is reversible.

## References


Ball D, Glue P, Wilson S, Nutt DJ (1991) The pharmacology of saccadic eye movements (1): effects of the benzodiazepine receptor ligands midazolam and flumazenil. Psychopharmacology 105:361–367

Barry W, Melville-Jones G (1965) Influence of eyelid movement upon electro-oculographic recording of vertical eye movements. Aerospace Med 36:855–858

Chiu TH, Rosenberg HC (1978) Reduced diazepam binding following chronic benzodiazepine treatment. Life Sci 23: 1153–1158

Cowley DS, Roy-Byrne PP, Radant A, Ritchie JC, Greenblatt DJ, Nemeroff CB et al. (1995) Benzodiazepine sensitivity in panic disorder: effects of chronic alprazolam treatment. Neuropsychopharmacology 12:147–157

Gallager D, Lakoski JM, Gonsalves SF, Rauch SL (1984) Chronic benzodiazepine treatment decreases postsynaptic GABA sensitivity. Nature (Lond) 290:74–77

Heninger C, Saito N, Tallman JF, Garret KM, Vitek MP, Duman RS et al. (1990) Effects of continuous diazepam administration on GABA A subunit mRNA in rat brain. J Mol Neurosci 2:101–107

Kang I, Miller LG (1991) Decreased GABA A receptor subunit concentrations following chronic lorazepam treatment. Br J Pharmacol 103:1285–1287

Little HJ, Gale R, Sellars N, Nutt DJ, Taylor SC (1988) Chronic benzodiazepine treatment increases the effects of the inverse agonist FG 7142. Neuropharmacology 27:383–389

Miller LG, Greenblatt DJ, Roy RB, Summer WR, Shader RI (1988) Chronic benzodiazepine administration. 11: Discontinuation syndrome is associated with upregulation of gamma-aminobutyric acid A receptor complex binding and function. J Pharmacol Exp Ther 246:177–182

Nutt DJ, Costello M (1988) Rapid induction of lorazepam dependence and its reversal with flumazenil. Life Sci 43:1045–1053

Nutt DJ, Glue P, Lawson CW, Wilson SJ (1990) Flumazenil provocation of panic attacks: evidence for altered benzodiazepine receptor sensitivity in panic disorder. Arch Gen Psychiatry 47:917–925

O'Donovan MC, Buckland PR, Spurlock G, McGuffin P (1992) Bi-directional changes in the levels of messenger RNAs encoding gamma-amino butyric acid A receptor alpha subunits after flurazepam treatment. Eur J Pharmacol 226:335–341

Potokar JP, Nash J, Sandford J, Rich A, Nutt DJ (1998) GABA A benzodiazepine (GBzR) receptor sensitivity in man: reliability and relationship with alcohol consumption. J Psychopharmacol 12:A13

Roy-Byrne PP, Cowley DS, Greenblatt DJ, Shader RI, Hommer D (1990) Reduced benzodiazepine sensitivity in panic disorder. Arch Gen Psychiatry 47:534–538

Roy-Byrne PP, Cowley DS, Radant A, Hommer D, Greenblatt DJ (1993) Benzodiazepine pharmacodynamics: utility of eye movement measures. Psychopharmacology 110:85–91

Roy-Byrne P. Wingerson DK, Radant A, Greenblatt DJ, Cowley DS (1996) Reduced benzodiazepine sensitivity in patients with panic disorder: comparison with patients with obsessive compulsive disorder and normal subjects. Am J Psychiatry 153:1444–1449

Sher PK, Study RE, Mazzetta J, Barker JL, Nelson PG (1983) Depression of benzodiazepine binding and diazepam potentiation of GABA-mediated inhibition after chronic exposure of spinal cord cultures to diazepam. Brain Res 268:171–176

Stephens DN, Schneider HH (1985) Tolerance to the benzodiazepine diazepam in an animal model of anxiety. Psychopharmacology 87:322–327

Stephens DN, Schneider HH, Weidmann R, Zimmermann L (1988) Decreased sensitivity to benzodiazepine receptor agonists and increased sensitivity to inverse agonists following chronic treatments: evidence for separate mechanisms. Adv Biochem Psychopharmacol 45:337–354

Van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF (1991) A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 50:172–180

Wilson SJ, Glue P, Ball D, Nutt DJ (1993) Saccadic eye movement parameters in normal subjects. Electroenceph Clin Neurophysiol 86:69–74