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Benzodiazepine Sensitivity in Panic Disorder: Effects of Chronic Alprazolam Treatment

Deborah S. Cowley, M.D., Peter P. Roy-Byrne, M.D., Allen Radant, M.D., James C. Ritchie, M.P.H., David J. Greenblatt, M.D., Charles B. Nemeroff, M.D., Ph.D., and Daniel W. Hommer, M.D.

The aim of the current study was to determine the degree to which patients with panic disorder develop tolerance to subjective and physiological effects of benzodiazepine after chronic treatment with alprazolam. Response to acute administration of diazepam was assessed in 19 panic disorder patients receiving chronic treatment with alprazolam and 23 untreated panic disorder patients. At baseline in the laboratory, the two groups did not differ in peak saccadic eye movement velocity, saccade latency, short-term memory, plasma cortisol and growth hormone concentrations, heart rate, and self-rated levels of sedation and anxiety. Compared with untreated patients, alprazolam-treated patients displayed significantly less diazepam-induced change in peak saccadic velocity, saccade latency, growth hormone secretion, memory, and self-rated levels of sedation. There was no difference between groups in diazepam effects on plasma cortisol concentrations or self-rated anxiety. Within alprazolam-treated patients, diazepam-induced slowing of peak saccade velocity was significantly inversely correlated with illness severity, as measured by reported panic attacks per week and severity of phobic avoidance, but not with alprazolam dose, blood level, or duration of treatment. Because the alprazolam-treated group reported more panic attacks per week than the untreated panic patients, treated patients were divided into those who were asymptomatic versus those with continuing panic attacks. The subgroup of nine alprazolam-treated subjects who were asymptomatic also showed significantly less diazepam effects than the group of untreated panic disorder patients, suggesting that overall group differences were at least partially attributable to the development of tolerance to selected benzodiazepine effects with chronic alprazolam treatment.
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From the Department of Psychiatry and Behavioral Sciences (DSC, PPR-B), University of Washington and Harborview Medical Center; the Department of Psychiatry and Behavioral Sciences (AR), Veterans Affairs Medical Center, Seattle, WA; the National Institute on Alcohol Abuse and Alcoholism (DWH); the Departments of Psychiatry and Pharmacology (JCR), Duke University Medical Center, Durham NC; the Department of Psychiatry and Behavioral Sciences (CBN), Emory University School of Medicine; and the Division of Clinical Pharmacology (DJG), Tufts-New England Medical Center, Boston, MA.

Address correspondence to: Deborah S. Cowley, M.D., Department of Psychiatry and Behavioral Sciences ZA-99, Harborview Medical Center, Seattle, WA 98104.

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Several benzodiazepines, including alprazolam, diazepam, clonazepam, and lorazepam, have been shown to decrease anxiety levels, phobic avoidance, and the severity and frequency of panic attacks in patients with panic disorder (Ballenger et al. 1988; Dunner et al. 1986; Schweizer et al. 1988; Tesar et al. 1987). These patients thus represent a large group of individuals for whom chronic benzodiazepine treatment may be indicated. Patients with panic disorder also frequently require higher doses of benzodiazepines than do other anxious patients (Shader and Greenblatt 1993). Despite this, the behavioral and physiological effects of chronic benzodiazepine treatment in panic disorder patients have been little studied (Allen et al. 1991; Schweizer et al. 1993).

We have previously demonstrated that unmedicated patients with panic disorder, in comparison with

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nonpsychiatric control subjects, display decreased sensitivity to the behavioral and physiological effects of acutely administered diazepam, as assessed by diazepam effects on short-term memory, self-rated levels of sedation, and the peak velocity of saccadic eye movements (Roy-Byrne et al. 1990). The greatest patient-control differences were observed using peak saccadic eye movement velocity (SEV), a quantitative, reliable measure of benzodiazepine effects (Hommer et al. 1986; Roy-Byrne et al. 1993). The aim of the current study was to extend this finding by assessing the effects of chronic alprazolam treatment in panic disorder. Specifically, we wished to determine the degree to which chronically treated individuals displayed evidence of tolerance to these benzodiazepine effects. To do this, we utilized the same paradigm and compared the response to an acute benzodiazepine challenge in patients with untreated panic disorder versus those chronically treated with alprazolam.

The effects of chronic benzodiazepine administration have been examined in both preclinical and human studies. Behavioral studies in rodents suggest that animals chronically exposed to benzodiazepines display time-dependent decreases in benzodiazepine effects (File 1985; Nutt 1990). This decrease with time in the behavioral and physiological effects of the same blood and brain concentrations of a drug is termed *tolerance*. The neurochemical basis of tolerance remains unclear. Although some studies have demonstrated time-dependent receptor downregulation (Crawley et al. 1982; Miller et al. 1988a,b; Rosenberg and Chiu 1981), others have found no changes (Braestrup et al. 1979; Mohler et al. 1978a) or increases (DiStefano et al. 1979) in benzodiazepine receptor binding. Receptor binding changes, when observed, vary in time course and regional specificity within the central nervous system (CNS) with different benzodiazepines (Miller et al. 1989). Tolerance also develops at varying rates for different benzodiazepine effects. In animals, tolerance occurs rapidly to sedative and psychomotor effects, less rapidly to anticonvulsant actions, slowly to anxiolytic effects, and perhaps not at all to locomotor stimulation induced by low doses (File 1985; Nutt 1990). Strain differences in the extent of tolerance to different benzodiazepine effects (File 1983) suggest a possible genetic contribution to the development of tolerance.

The same phenomenon of differential tolerance to varying benzodiazepine effects is seen in humans. As in animals, sedative and psychomotor effects subside first (Smith and Kroboth 1987; Van Laar et al. 1992), with tolerance to impairment of driving ability observed after the first few weeks of treatment (Van Laar et al. 1992) or, in one study, after only a few doses of nitrazepam (Laurell and Tornros 1986). Petursson et al. (1983) reported almost complete suppression of the growth hormone response to diazepam in chronic benzodiazepine users (25). However, benzodiazepine effects on critical flicker fusion threshold, EEG beta activity, memory, and anxiety persist despite chronic use (Lader and File 1987; Lucki et al. 1986). The lack of tolerance to anxiolytic actions of benzodiazepines seen in most anxious patients (Rickels et al. 1983), as well as the observation that anxious patients without a history of substance abuse rarely escalate benzodiazepine dosages or overuse these medications (Woods et al. 1987, 1988), form the basis for their successful clinical use.

Few studies have examined the effects of long-term alprazolam use in panic disorder. Schweizer et al. (1993), in a double-blind study of patients with panic disorder treated for 8 months with alprazolam, imipramine, or placebo, observed the persistent efficacy of alprazolam at a mean dose of 5.6 mg daily, without escalation of dosage over the course of the trial. Other investigations of chronically treated panic disorder patients have actually observed gradual reductions in alprazolam dose over time, without loss of clinical efficacy (Nagy et al. 1989). Allen et al. (1991), in an 8-week trial of alprazolam treatment of panic and agoraphobia, noted continuing impairments in word recall throughout the study. Other measures of alprazolam effects have not been assessed in chronically treated patients with panic disorder.

As a preliminary examination of this issue, we measured response to an acute diazepam challenge in untreated patients with panic disorder and those chronically treated with alprazolam. Diazepam challenge was used since diazepam can be given intravenously, rapidly crosses the blood-brain barrier, and unlike alprazolam, which also interacts with noradrenergic receptors (Sethy and Hodges 1982), acts only at the benzodiazepine binding site. Thus, it provides a more specific measure of benzodiazepine effects and thus, presumably, of GABA-benzodiazepine receptor function. Diazepam response was assessed using peak SEV, saccadic latency, short-term memory, self-rated levels of sedation and anxiety, heart rate, and plasma growth hormone and cortisol. The following hypotheses were tested:

1. Compared with benzodiazepine-naive, unmedicated patients with panic disorder, patients chronically treated with alprazolam display less effect of acutely administered diazepam on SEV and latency, growth hormone and cortisol secretion, and self-rated levels of sedation, but similar amnestic and anxiolytic effects.
2. The different measures of benzodiazepine effects show different degrees of "tolerance" or diminished diazepam-induced changes in chronically treated patients with panic disorder.
3. In patients with panic disorder chronically treated with alprazolam, the degree of response to diaze-

pam is correlated with both treatment (dose, duration, plasma levels) and severity of illness measures.

METHODS

Subjects

Subjects were 42 patients meeting DSM-III-R criteria for panic disorder who were recruited from the University of Washington Center for Anxiety and Depression. Diagnoses were made using the Structured Clinical Interview for DSM-III-R. Patients had no history of alcohol abuse or dependence, did not meet criteria for current major depression, and were medically healthy. Twenty-three patients (14 women, 9 men) were untreated. Of these, 19 had never received benzodiazepines. One had taken alprazolam for 6 months until 12 months prior to the infusion procedure. One had taken diazepam for 6 months 3 years previously. Two had been prescribed lorazepam several years ago, one regularly for 4 months, and the other on an occasional, as-needed basis. None of these 23 untreated patients had taken any psychotropic or CNS-active medication within the month prior to the study. Their weights ranged from 44 to 95 kg (mean 72 ± 13 kg). Nine wore glasses or contacts, although all subjects were able to see the targets clearly without glasses. Nine of the 23 were included in our earlier report of benzodiazepine sensitivity in patients with panic disorder versus normal controls (Roy-Byrne et al. 1990).

Nineteen patients (10 women, 9 men) had received chronic alprazolam treatment for panic disorder at doses of 1 to 4.75 mg daily (mean 2.5 ± 1.1 mg daily) for a period of 4 to 98 months (mean 50.3 ± 25.5 months). These patients were entering a study comparing the efficacy of carbamazepine versus placebo in attenuating symptoms of alprazolam (Xanax) withdrawal. Ten wore glasses or contacts. Alprazolam-treated subjects ranged in weight from 49 to 108 kg (mean 71 ± 14 kg). Informed consent was obtained for all subjects after the procedures were fully explained to them. The study was approved by the University of Washington Institutional Review Board.

Procedure

Subjects arrived at the laboratory at 8 A.M. and were familiarized with the equipment and procedures. Alprazolam-treated patients had been instructed to take their morning alprazolam dose at 7 A.M. An intravenous catheter was inserted in the antecubital fossae bilaterally. Subjects completed the Spielberger Anxiety (Spielberger 1975) and Beck Depression (Beck and Beamsdorfer 1974) scales and an analogue scale measuring recent stress (the Global Assessment of Recent Stress, Linn 1985–86). One of us (DSC or PPR-B) then administered the Hamilton Anxiety Scale (Hamilton 1983).

Untreated panic disorder (PD) patients received two single-blind infusions in randomized order, approximately 1 week apart, beginning between 9 and 9:30 A.M. On one day, subjects received four intravenous doses of diazepam at 15-minute intervals (25, 25, 50, and 100 μg/kg), yielding logarithmically increasing cumulative diazepam doses of 25, 50, 100, and 200 μg/kg. On the other day, subjects were given propylene glycol vehicle of equal volumes. Drug or placebo was injected over a 60-second interval. Alprazolam-treated (ALP) subjects underwent only a single day of testing, receiving the four doses of diazepam as described. These patients did not have a placebo infusion day because only one week was available for testing between enrollment in the alprazolam discontinuation study and the introduction of carbamazepine or placebo treatment. Of note, there were no significant placebo effects on any diazepam response measure in the untreated PD group. Previous reports in normals (Roy-Byrne et al. 1993) and patients (Roy-Byrne et al. 1990) have demonstrated low placebo day variability in the eye movement measures used in this study.

On each infusion day, eight sets of measurements were taken at 15-minute intervals: two before drug administration, one after each of the four drug doses, and two more after the last dose. At baseline, a blood sample was drawn for measurement of plasma alprazolam concentration. At each time point, SEV was measured one minute after conclusion of drug administration. Plasma was then drawn for plasma diazepam, cortisol, and growth hormone concentrations from the arm contralateral to that used for drug injection. Ten visual analogue ratings of sedative and anxiolytic effects were then completed and blood pressure and pulse taken with an automated machine (Dinamap). Memory function was evaluated at baseline and again after doses 2 and 4.

Measurement of Eye Movements

Saccadic eye movement velocity and latency were recorded using a noninvasive infrared oculographic device (EyeTrac Model 210, ASL Laboratories, Waltham, Mass.) that has an accuracy of ± 0.1 degrees and ± 2 msec. Sensors were mounted on a rigid forehead support and bite bar attached to a solid table. Subjects removed glasses or contact lenses in preparation for the procedure. The sensors were adjusted so that they were positioned about 1 cm from the eyes, at the level of the lower lid, and pointed up toward the pupil in midposition. The first 14 PD and 11 ALP subjects were studied using as targets five red lights on a black background, 57 cm from the sensors, and spaced 7.5 degrees apart. The remaining subjects were studied after the equip-

ment was changed and targets were five white squares on a computer terminal screen, 26 cm from the sensors, and spaced 7.5 degrees apart. Target size and the angle subtended between targets were identical for both sets of targets.

Data were acquired on-line using an IBM 386 computer and a custom interface. A series of 23 targets were presented at 2-second intervals in a fixed, pseudorandom order, producing a series of target steps of 7.5, 15, 22.5, and 30 degrees. Subjects were instructed to follow the lights and not to try to anticipate where the next target would appear. Eye position data were digitized at 1,000 Hz. Calibration was achieved by asking the subject to fixate targets at 0 and 15 degrees to the right and left of center before each separate set of measurements. Recalibration with each dose minimized error due to movement of the subject's head.

Eye position data were stored and displayed on the computer, and velocity was calculated and displayed for each saccade. The peak velocity, latency, and amplitude of each saccade were identified and recorded using an automated program. Data from both eyes were combined, and peak velocity of each saccade was displayed as a function of saccade amplitude, creating a curve known as the main sequence (Bahill et al. 1975). In this function, peak velocity of saccades increases with distance of saccade until velocity reaches an asymptote at about 20 degrees of amplitude. Peak velocity and amplitude data from each dose and baseline measurement were fitted to an exponential equation of the form peak velocity $= a - b^{-x/c}$, where a, b, and c are constants and x is the amplitude of the saccade (Bahill et al. 1975). This equation was used to calculate the peak velocity for an idealized 20-degree ($= x$) saccade. The advantage of this method of data reduction is that all saccades contribute to this calculated peak velocity. Analysis by this technique required that subjects make at least 50% of the eye movements expected based on the target motion. All subjects met this requirement except for one untreated patient at the highest diazepam dose.

Other Measures of Benzodiazepine Effects

Ten items were extracted from the Lader Mood Analogue Scale (Bond and Lader 1974), five representing sedation and five anxiety. An average score for each cluster was employed as the two measures of subjective effects. Memory function was measured by presenting a list of 12 categorically similar words, 6 of which were repeated (total, 18 words). Subjects had to identify repeated words immediately, during the presentation of the list. They then freely recalled as many of the 12 words as they could after a 60-second interval (during which the analogue scales were completed). Finally, they attempted to recognize the 12 words embedded in a list of 24 categorically similar words and to identify recognized words as having been repeated or not in the original list. Scores on these four tasks were summed (perfect score, 36), since all four are known to be benzodiazepine sensitive (Roy-Byrne et al. 1987). Scores on each task were corrected for intrusions or errors to control for "correct guessing," as previously described (Roy-Byrne et al. 1987).

Cortisol was measured by a competitive protein-binding method with a sensitivity of 0.45 ng/dl. Cross-reactivity with endogenous steroids was less than 1%, except for 11-deoxycortisol (4.7%) and corticosterone (4.5%). Growth hormone was measured by an immunoradiometric assay (Nichols Institute, San Juan Capistrano) with sensitivity of 0.02 ng/ml. Cross-reactivity was less than 0.01% for other pituitary hormones. The intraassay and interassay coefficients of variation were 4.9% and 3.1%.

Alprazolam and diazepam plasma concentrations were measured by electron-capture gas-liquid chromatography (Greenblatt et al. 1990). Levels of the primary metabolite of diazepam, desmethyldiazepam, were also quantitated, but in all cases metabolite levels were much lower than those of the parent drug.

Clinical Measures

In order to determine which dimensions of illness severity or treatment factors might be associated with diazepam response in chronically treated patients (ALP group), several clinical measures were obtained at the time of initial evaluation in these patients. These data included the number of panic attacks per week, overall severity of phobias as measured by the Marks phobia scale (Marks and Mathews 1979), the dose and duration of alprazolam treatment, and overall lifetime duration of benzodiazepine treatment. Panic attack frequency was also assessed in the untreated group for the week preceding testing.

Data Analysis

For diazepam sensitivity testing, eight time points were available for analysis. As in our previously published report using the same paradigm (Roy-Byrne et al. 1990), data analysis was based on the first six time points (two baseline and four drug doses), because the aim was to measure acute effects of diazepam, rather than offset of pharmacodynamic effect corresponding to drug redistribution. Baseline scores did not differ significantly between groups (see Results). Analysis of group (PD versus ALP) × time effects was performed using a mixed-model analysis of variance (ANOVA), with a between-subjects factor (group) and a within-subjects factor (change with each of the four diazepam doses). Change scores were computed as the difference between the mean baseline score and the score at each

dose. Although no method of baseline correction is entirely satisfactory in the absence of random assignment, tests on simple pre-post difference or change scores are generally superior to analysis of covariance (ANCOVA) or percentage change for quasi-experimental designs in which subject groups represent samples from different populations (Overall and Ashby 1991). Analysis using change scores was performed on SEV, saccadic latency, sedation, anxiety, memory, cortisol, and growth hormone. Because growth hormone responses to challenge tests may be refractory when growth hormone concentrations are already elevated at baseline, patients with baseline plasma growth hormone concentrations above 3 ng/ml (5 PD, 4 ALP patients) were excluded (Siever et al. 1992).

The two groups differed significantly in age and panic attack frequency (see Results and Table 1). Since we wished to determine the effect of these differences on our ANOVA results and since we did not wish to use covariance analysis, which assumes that the covariate interacts with the dependent variable similarly in both groups, we performed median splits for the entire 42 subjects for both panic attack frequency and age. "High" versus "low" age and "high" versus "low" panic frequency were then used as additional, between-subjects, independent variables in the ANOVAs, both in comparing outcome measures at baseline and in assessing group differences over time with diazepam challenge. In general, these analyses yielded no significant panic frequency, age, or interaction effects and did not change the significance of the observed group differences (but see analysis of baseline saccade latency in Results). Thus, the analyses are presented in the Results section collapsed over age and panic attack frequency.

In addition, for SEV data, using the log-linear pharmacodynamic model (Dingemanse et al. 1988), diazepam day data for each subject were used to construct dose and concentration response curves by the following method: baseline SEV scores were averaged, and then the SEV value recorded with each successive dose was used to calculate the percent decrease in SEV occurring at each dose or plasma diazepam concentration. These values were plotted against log dose or log concentration, and the linear portion of the plot was fitted by least-squares regression analysis. The equation of the line was used to calculate the effective dose (ED30) or concentration (EC30) required to reduce SEV by 30%. A reduction of 30% in SEV was chosen to approximate the physiological limitations of the SEV system based on pilot data in normal subjects and our data, which has shown that individuals unable to make movements at the fourth dose show SEV reductions close to 30% on the previous (third) dose. ED30 and EC30 were compared between groups using t-tests. For the one subject unable to make 50% of expected eye movements at the highest diazepam dose (see above), ED30 and EC30 were calculated using an estimated fourth dose value derived by extrapolating from the line formed by the first three dose results. Of note, for technical reasons diazepam levels were not available at all time points, and thus EC30s could not be calculated for one untreated and two alprazolam-treated patients.

Demographic and clinical variables were compared in PD and ALP groups by using t-tests. Within the ALP group, Pearsons correlations were performed of ED30 and EC30 versus alprazolam plasma level, alprazolam dose, duration of alprazolam treatment, duration of total lifetime benzodiazepine treatment, panic attacks per week, and severity of phobic avoidance at baseline.

RESULTS

Demographic and clinical data for PD and ALP groups are shown in Table 1. The two groups did not differ in gender; distribution in scores on the Hamilton Anxiety, Beck Depression, or Spielberger State Anxiety scales; or in their global assessment of recent stress. Alprazolam-treated subjects were older (t = 3.7, df = 37, $p < .001$) and, despite their alprazolam treatment, reported significantly more panic attacks per week (t = 2.4; df = 40; $p < .02$).

At baseline, ALP and PD groups did not differ in SEV (ALP 483.8 ± 53.4 versus PD 478.6 ± 87.1 degrees per second; t = 0.23, df = 39, p = .82), cortisol (ALP 14.2 ± 7.1 versus PD 15.5 ± 7.1 ng/dl; t = 0.60, df = 39, p = .55), growth hormone (ALP 2.6 ± 4.6 versus PD 1.9 ± 2.4 ng/ml; t = 1.03, df = 38; p = .31), memory (ALP 22.9 ± 4.7 versus PD 25.1 ± 4.2 words recalled; t = 1.6, df = 39, p = .13), heart rate (ALP 73.4 ± 9.6 versus PD 68.1 ± 10.8 beats per minute; t = 1.6; df = 37, p = .12), or self-rated levels of sedation (ALP 37.2 ± 16.9 versus PD 37.6 ± 18.6; t = 0.07, df = 40, p = .94) or anxiety (ALP 42.6 ± 18.0 versus PD 40.0 ± 16.3; t = 0.49, df = 40, p = .63). Alprazolam-treated subjects did

Table 1. Demographic and Clinical Characteristics of Untreated and Alprazolam-Treated (ALP) Patients with Panic Disorder (PD)

	PD (n = 23)	ALP (n = 19)
Age (years)	29.5 ± 6.2[a]	36.9 ± 5.4[a]
Gender	14 F, 9 M	10 F, 9 M
Hamilton Anxiety score	20.7 ± 9.5	18.7 ± 10.2
Beck Depression score	15.0 ± 12.2	11.2 ± 7.3
Spielberger (state)	44.5 ± 11.8	48.4 ± 12.4
Global stress	41.7 ± 14.4	47.9 ± 24.2
Panic attacks/week	0.6 ± 0.3[b]	2.3 ± 3.2[b]

Figures represent mean ± standard deviation.
[a] $p < .001$.
[b] $p < .02$.

display a trend toward increased latency at baseline (ALP 229.2 ± msec versus PD 212.8 ± 30.7 msec; t = 1.9, df = 39; p = .07). However, saccadic latency increases with age (Abel et al. 1983), and when "high" versus "low" age was used as an additional between-subjects independent variable, differences in saccadic latency at baseline were no longer significant (F = 1.6; df = 1,37; p = .22).

Both groups displayed significant dose-dependent increases in plasma diazepam levels with the four diazepam doses (F = 182, df = 3,96; $p < .0001$). Plasma diazepam levels at each of the four doses (in ng/ml) were 96.1 ± 40.7, 152.8 ± 67.2, 311.6 ± 143.2, and 590.5 ± 199.4 for the untreated group and 106.8 ± 34.2, 187.3 ± 59.8, 340.3 ± 116.4, and 639.8 ± 203.2 for the alprazolam-treated group. There were no significant differences between the two groups in blood levels achieved at each dose. In the treated group, alprazolam levels on the morning of eye movement testing ranged from 6.2 to 50.6 ng/ml (mean 30.7 ± SD 13.9 ng/ml).

Analysis of variance revealed significant differences between groups, with ALP subjects displaying blunted diazepam responses for most outcome measures. Compared with the untreated PD patients, ALP patients showed less diazepam-induced SEV slowing (F = 6.1; df = 1,39; $p < .02$; see Figure 1), increase in saccadic latency (F = 7.5; df = 1,39; $p < .01$; see Figure 1), self-rated levels of sedation (F = 9.7; df = 1,40; $p < .005$; see Figure 2), and memory impairment (F = 9.5; df = 1,39; $p < .005$; see Figure 2). Dose-dependent (dose × group) interactions were seen for SEV (F = 7.8; df = 3,117; $p < .0001$), latency (F = 14.2; df = 3,117; $p < .0001$), sedation (F = 4.0; df = 3,120; $p < .01$), heart rate (F = 3.7; df = 4,148; $p < .01$; see Figure 3), and growth hormone (F = 3.6; df = 5,145; $p < .005$; see Figure 3). Both groups displayed significant diazepam-induced decreases (time effects) in self-rated levels of anxiety (F = 10.8; df = 3,120; $p < .002$) and in cortisol levels (F = 69.7; df = 5,145; $p < .001$). However, there were no significant differences between groups for diazepam effects on these measures. Of note, scores of self-rated levels of anxiety decreased by about 25% in both groups with diazepam.

Group differences in response to benzodiazepine challenge testing could be attributable to differences in

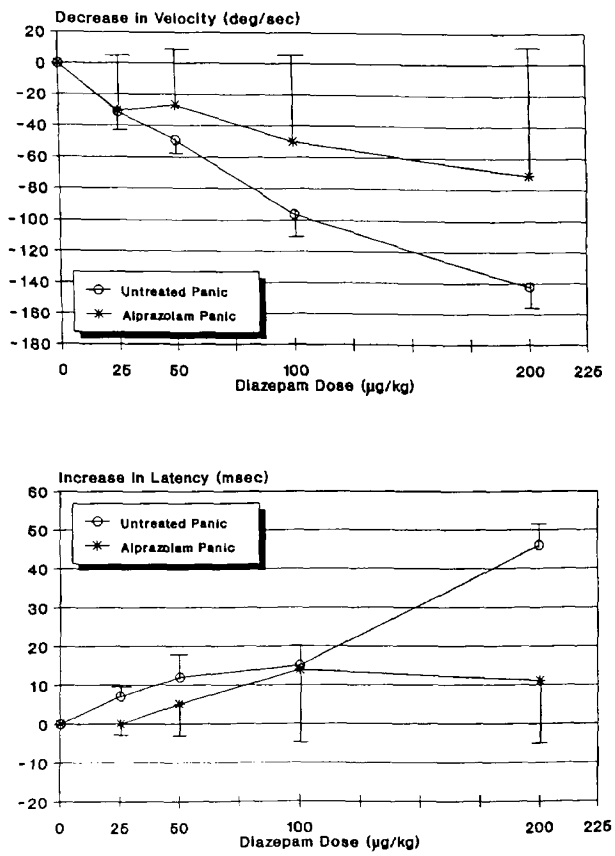

Figure 1. Decrease in saccadic velocity (degrees/sec) (*top*) and increase in saccadic latency (msec) (*bottom*) from baseline with each diazepam dose. Error bars represent the standard error of the mean.

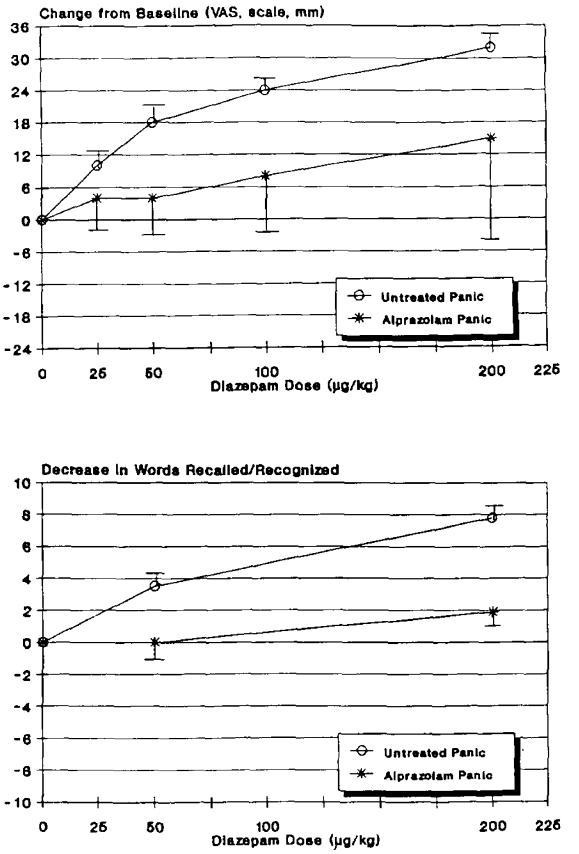

Figure 2. Change from baseline in self-rated sedation (*top*) (mm on a 100-mm visual analogue scale) and short-term memory (*bottom*) (words recalled or recognized, of a total of 36) with diazepam. Error bars represent the standard error of the mean.

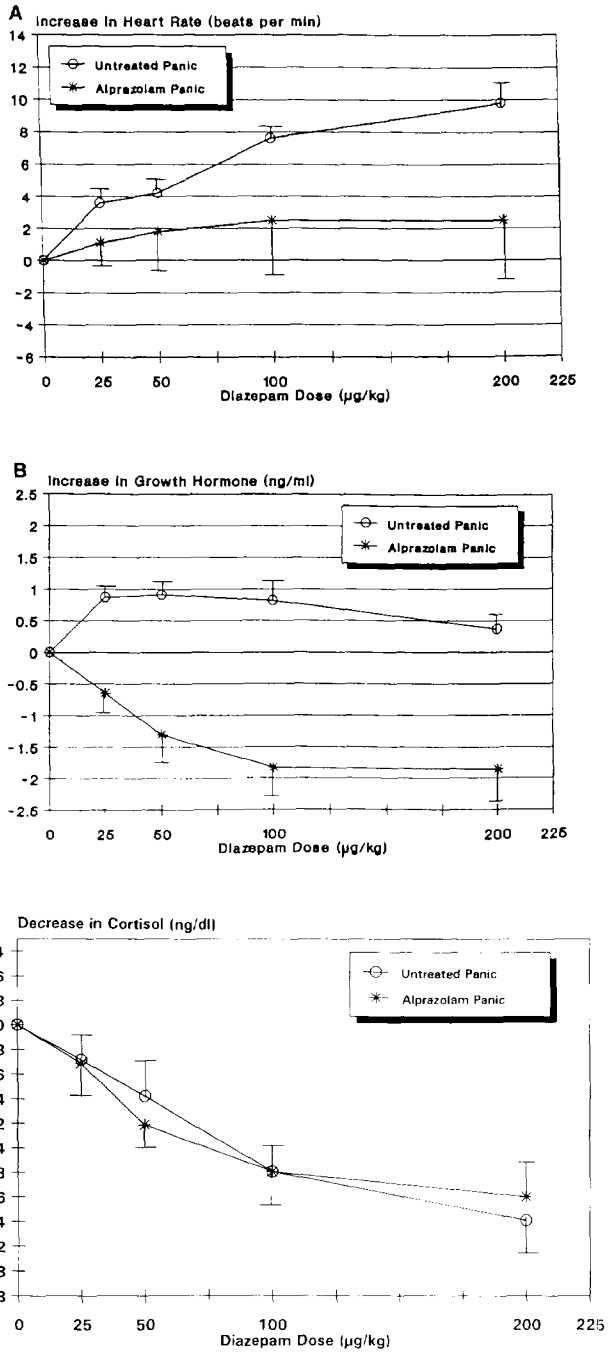

Figure 3. Change from baseline in heart rate (beats per minute) (**A**), plasma growth hormone concentration (ng/ml) (**B**), and plasma cortisol (ng/dl) (**C**) with each diazepam dose. Error bars represent the standard error of the mean.

sedation or arousal. Indeed, within the current sample, there was a significant correlation between SEV slowing and sedation at dose 4 in the alprazolam-treated group ($r = -0.46$, $p = .05$). There were no other significant correlations between sedation and SEV slowing at lower doses or between sedation and diazepam-induced change in the other response measures, however.

Both ED30s and EC30s were significantly higher in the ALP than in the PD group (Figure 4). The variability in ED30 and EC30 was also greater in the ALP patients ($F_{max} = 11.4$ for ED30, 4.2 for EC30; F test for homogeneity of variances), with one subgroup of patients having values similar to those of untreated PD patients but another subgroup showing markedly elevated values.

For the ALP group, correlations between ED30 and EC30 versus clinical variables revealed that less diazepam effects, as indicated by elevated ED30 and EC30s for SEV slowing, were associated with more frequent panic attacks (versus ED30: $r = 0.52$, $p = .02$; vs. EC30: $r = 0.53$, $p = .03$) and more severe phobic avoidance (vs. ED30: $r = 0.53$, $p = .03$; vs. EC30: $r = 0.67$, $p = 0.006$). Response to diazepam was not related to alprazolam blood level, daily alprazolam dose, duration of treatment with either alprazolam or all benzodiazepines, or to total exposure (dose × duration) to alprazolam.

The observed differences in benzodiazepine sensitivity between treated and untreated panic disorder patients could be due to greater severity of illness, as reflected by the higher mean frequency of panic attacks, in the alprazolam-treated group or to tolerance to benzodiazepine effects induced by chronic exposure to alprazolam. To examine this issue further, we divided alprazolam-treated patients into those with no panic attacks and with a Hamilton Anxiety score less than 20 (asymptomatic patients; $n = 9$) and those with ongoing panic attacks (range 2–9 attacks per week) or a Hamilton Anxiety score greater than 20 (symptomatic patients; $n = 10$; 9 with ongoing panic attacks, 1 with no attacks but a Hamilton Anxiety score of 33). Asymptomatic alprazolam-treated patients differed significantly in diazepam response from untreated PD patients. Compared with patients with untreated panic disorder (PD group), asymptomatic alprazolam-treated subjects displayed significantly less dose-dependent (group × dose) diazepam effects on SEV ($F = 4.0$; df = 3,90; $p < .01$), saccadic latency ($F = 5.6$; df = 3,90; $p < .002$), and human growth hormone ($F = 3.2$; df = 5,135; $p < .01$). There were significant group, but not group × dose, differences in diazepam effects on sedation ($F = 5.5$; df = 1,31; $p < .05$), heart rate ($F = 9.2$; df = 1,29; $p < .005$), and memory ($F = 9.2$; df = 1,30; $p < .005$), but no differences in anxiety or cortisol levels. Asymptomatic alprazolam-treated subjects also had significantly higher ED30s ($F = 14.8$; df = 1,30; $p < .001$) and EC30s ($F = 11.0$; df = 1,38; $p < .005$) than PD patients.

Symptomatic alprazolam-treated patients tended to have only nonsignificantly higher ED30s ($F = 2.9$; df = 1,16; $p = .11$) and EC30s ($F = 2.8$; df = 1,15; $p =$

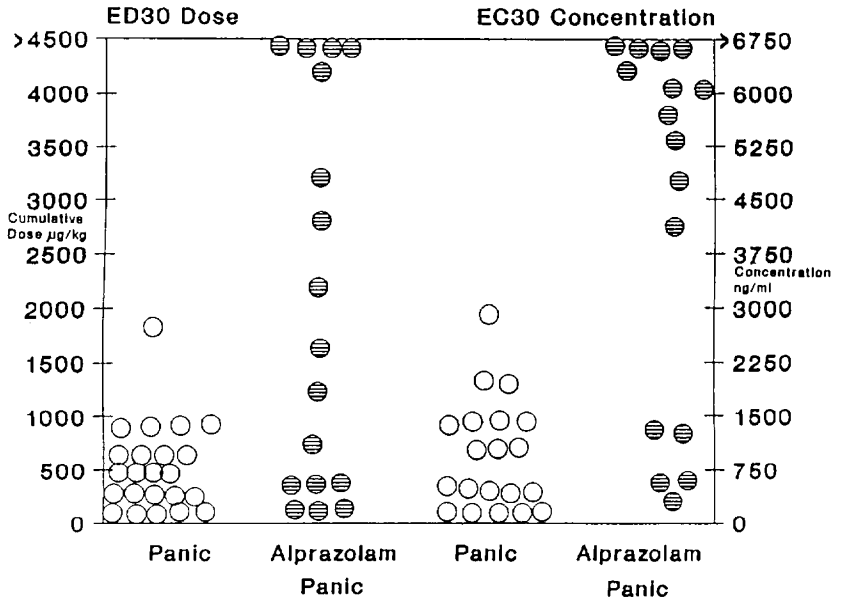

Figure 4. ED30 and EC30 (cumulative mcg/kg) for patients with untreated panic disorder (Panic) and alprazolam-treated patients with panic disorder (Alprazolam Panic).

.12) than the asymptomatic alprazolam-treated subjects. Although there were no significant differences between symptomatic and asymptomatic alprazolam-treated patients on any measure of diazepam effects, power analysis indicted that the sample sizes were too small to detect possible differences in SEV. The effect size after dose 3 was 0.80, suggesting that a sample size of 24 per group would be necessary for a power of 80%. For all other variables, effect sizes were 0.50 or less.

DISCUSSION

The current study is one of very few to examine the behavioral and physiological effects of long-term alprazolam treatment in patients with panic disorder using a benzodiazepine challenge paradigm and a wide variety of response measures to assess benzodiazepine effects. Peak SEV, in particular, provides a quantitative, reliable measure of benzodiazepine effects (Ball et al. 1991; Hommer et al. 1986; Roy-Byrne et al. 1993), which is both unaffected by motivation (Leigh and Zee 1991) and not subject to the diurnal or minute-to-minute variability of neuroendocrine variables. Areas involved in the generation and control of saccadic eye movements, which include the pontine reticular formation, substantia nigra, caudate nucleus, superior colliculus, prefrontal eye fields, and cerebellum (Leigh and Zee 1991), are rich in GABA-benzodiazepine receptors (Mohler et al. 1978b; Young and Kuhar 1979). Reductions in peak saccade velocity follow direct injection of muscimol, a GABA-agonist, into the superior colliculus (Hikosaka and Wurtz 1985). Benzodiazepine effects on peak SEV can be reversed in humans by the benzodiazepine receptor antagonist RO15-1788 (flumazenil; Ball et al. 1991; Hommer et al. 1986) and thus are likely to be mediated by the GABA-benzodiazepine receptor complex.

Consistent with past investigations of benzodiazepine effects (Ball et al. 1991; Hommer et al. 1986; Roy-Byrne et al. 1988, 1990, 1993), untreated patients with panic disorder demonstrated significant slowing of peak SEV; increased saccade latency; impairment of short-term memory; increases in self-rated sedation, heart rate, and growth hormone; and decreases in anxiety and plasma cortisol with diazepam. Alprazolam-treated patients in this study displayed reduced physiological and behavioral effects of acutely administered diazepam compared with untreated patients with panic disorder, as reflected by less diazepam-induced SEV slowing, increases in saccadic latency, memory impairment, self-rated levels of sedation, and increases in heart rate and growth hormone. The two groups did not differ in plasma diazepam levels, which in turn provide an accurate reflection of diazepam concentrations in the CNS (Arendt et al. 1983). Thus, the observed group differences cannot be attributed to pharmacokinetic factors.

The reduced responsiveness to diazepam challenge observed in our alprazolam-treated group is consistent with the phenomenon of tolerance. Furthermore, as would be expected based on past findings of differential tolerance to benzodiazepine effects, the two groups differed on some but not all diazepam response measures. Consistent with past observations of benzodiazepine-treated individuals (Laurell and Tornros 1986; Petursson et al. 1983; Smith and Kroboth 1987; Van Laar et al. 1992), our alprazolam-treated subjects displayed

reduced diazepam effects on sedative, psychomotor (eye movement), and growth hormone measures. Alprazolam-treated subjects did not differ from untreated patients in cortisol response or anxiolytic effects, consistent with Schweizer et al.'s (1993) report of sustained anxiolytic efficacy of alprazolam over time. Furthermore, the effect size in our sample was 0.06 for self-rated levels of anxiety and 0.22 for cortisol measures, suggesting that even with a larger sample size, we would be unlikely to observe group differences in diazepam effects on these measures.

Our finding of decreased benzodiazepine effects on short-term memory is at odds with the results of other studies. For example, Lucki et al. (1986) and Lucki and Rickels (1986) have shown continued short-term memory impairment after acute administration of benzodiazepines to chronically treated anxious patients. Recent investigations of the chronic cognitive and psychomotor effects of alprazolam in healthy volunteers (Allen et al. 1991) have demonstrated tolerance to decreased reaction speed, but not to memory effects, after 10 days of treatment with 0.5 to 0.75 mg t.i.d. The same group has found continuing impairments in word recall in patients with panic and agoraphobia who had been treated with alprazolam for 8 weeks (Allen et al. 1991). The present study differs from prior investigations in the particular memory test used, as well as in the chronicity of alprazolam use, with a mean of over 4 years of treatment.

The major limitation of the current study is the between-subjects, cross-sectional design, in which we compared two separate groups of subjects, rather than assessing diazepam response before and after treatment in the same subjects. This design limits the interpretation of some of the results. For example, the fact that the two groups did not differ in response measures at baseline before diazepam challenge might by itself suggest the presence of tolerance in the alprazolam-treated group. However, no such conclusion can be drawn since the baseline values for this group before alprazolam treatment, and thus the change in baseline values after chronic treatment, are unknown.

In addition, the alprazolam-treated group was clearly a selected sample, in that they wished to discontinue alprazolam, presumably because it was ineffective. They were older and more severely ill than the untreated group, as reflected by a significantly higher panic attack frequency. They may have been a particularly treatment-resistant group or one more prone to the development of tolerance than other patients maintained on chronic alprazolam treatment. Thus, these results cannot necessarily be generalized to all patients with panic disorder treated long-term with alprazolam.

There was no significant correlation between alprazolam dose, blood level, treatment duration, or overall exposure to alprazolam (dose *X* duration) and ED30 or EC30 levels. This is consistent with prior findings that behavioral tolerance to diazepam in humans is unrelated to blood, erythrocyte, or salivary levels of diazepam or desmethyldiazepam (Linnoila et al. 1983). In animal studies of tolerance (File 1985), the development of tolerance is unrelated to benzodiazepine dose, dosing interval, or half-life. However, the treated subjects in this study were trying to discontinue alprazolam, and thus to minimize their dose. The fact that many were quite symptomatic suggests that they may have benefited from higher alprazolam doses and were acutely undertreated. If they were more adequately treated with higher doses, and thus higher blood levels, then both their doses and blood levels may have been more likely to correlate with ED30 and EC30 values, and thus with their degree of tolerance.

The potentially unstable clinical condition of these patients, who were symptomatic and attempting to minimize their dose of alprazolam, may have had profound effects on our findings. For example, one possible explanation of our results is that the alprazolam-treated group was more severely ill and that patients who are more severely ill are less responsive to benzodiazepine challenge whether tested before or after treatment. This possibility is supported by the fact that the extent of diazepam-induced SEV slowing was inversely correlated with measures of illness severity within the alprazolam-treated group and that symptomatic alprazolam-treated patients showed a trend for higher ED30s and EC30s than panic-free alprazolam-treated subjects. However, this is unlikely to be the sole explanation for our findings. First, the alprazolam-treated subjects displayed considerable heterogeneity, with a subgroup having ED30s and EC30s markedly higher than those of any subjects in the untreated group. Second, and most important, even asymptomatic and panic-free patients on chronic alprazolam, who were *less* symptomatic than the untreated patients, were significantly less responsive to diazepam challenge, as reflected by a wide range of outcome measures.

Clearly, it is not possible, with the current sample, quantitatively to separate the relative contributions of illness severity and long-term treatment to the observed reduced responsiveness to acute benzodiazepine challenge in our alprazolam-treated group. The most likely possibility appears to be that both chronic benzodiazepine exposure *and* severity of panic symptomatology contribute to reduced responsiveness to diazepam. Perhaps the most robust finding of the present study is that of *differential* tolerance, with some outcome measures affected more than others by diazepam challenge after long-term benzodiazepine treatment.

Further investigation of the effects of chronic alprazolam treatment on acute benzodiazepine response would clearly be improved by studying the same group

of subjects before and then following treatment, optimizing doses to ensure adequate treatment, and including sufficient subjects to address more definitively the issue of the effect of symptom severity on benzodiazepine response after treatment. Nevertheless, our current findings provide interesting data suggesting that chronic treatment with alprazolam, as with other benzodiazepines, reduces responsivity to acute benzodiazepine challenge, that this is probably attributable both to the development of tolerance and to the severity of panic symptoms, and that the extent to which chronic treatment alters benzodiazepine response depends on the particular benzodiazepine effect measured. The use of multiple physiological and behavioral measures, and in particular the use of SEV, a quantitative, reliable measure of benzodiazepine effects, holds promise in further assessing the effects of chronic benzodiazepine treatment, comparing the development of tolerance to different benzodiazepines and benzodiazepine effects, and determining the relationship of initial sensitivity, psychiatric diagnosis, and other clinical characteristics to the degree and rapidity of development of tolerance.

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