Electroencephalographic effects and serum concentrations after intranasal and intravenous administration of diazepam to healthy volunteers

Karsten Lindhardt,[1] Sveinbjörn Gizurarson,[2,3] Sigurjón B. Stefánsson,[4] David R. Òlafsson[2] & Erik Bechgaard[1]

[1]*The Royal Danish School of Pharmacy, Department of Pharmaceutics, Universitetsparken 2, 2100 Copenhagen, Denmark,* [2]*Lyfjathroun hf, Hafnarbudir, Geirsgata 9, 101 Reykjavik,* [3]*Department of Pharmaceutics, University of Iceland, 107 Reykjavik and* [4]*Landspítalinn, University Hospital of Iceland, Department of Neurology, 121 Reykjavik, Iceland*

Aims To evaluate the electroencephalographic (EEG) effects, blood concentrations, vehicle irritation and dose-effect relationships for diazepam administered nasally.

Methods The study had a cross-over design with eight healthy volunteers (one drop out). It consisted of four legs with four different administrations: intranasal (i.n.) placebo, 4 mg diazepam i.n., 7 mg diazepam i.n. and 5 mg intravenous (i.v.) diazepam. Polyethylene glycol 300 (PEG300) was used as a vehicle in the nasal formulations to solubilize a clinically relevant dose of diazepam. Changes in N100, P200 and P300 brain event-related potentials (ERP) elicited by auditory stimulation and electroencephalographic β-activity were used to assess effects on neurological activity.

Results The mean [95% confidence intervals] differences between before and after drug administration values of P300-N100 amplitude differences were −0.9 [−6.5, 4.7], −6.4 [−10.1, −2,7], −8.6 [−11.4, −5.8] and −9.6 [−12.1, −7.1] for placebo, 4 mg i.n., 7 mg i.n. and 5 mg i.v. diazepam, respectively, indicating statistically significant drug induced effects. The bioavailabilities of 4 and 7 mg i.n. formulations, were found to be similar, 45% [32, 58] and 42% [22, 62], respectively.

Conclusion The present study indicates that it is possible to deliver a clinically effective nasal dose of diazepam for the acute treatment of epilepsy, using PEG300 as a solubilizer.

Keywords: benzodiazepine, diazepam, EEG, electroencephalography, ERP, event-related potential, intranasal, nasal, PEG300, polyethylene glycol 300

Introduction

The present study was carried out to assess the intranasal administration of diazepam as a potential alternative to intravenous and rectal dosing in the treatment of acute epileptic seizures. A nasal spray is beneficial when a rapid onset of effect (within seconds or minutes) is required. Animal experiments have shown that the intranasal administration of diazepam may induce effects within 5 min. In rabbits, a peak serum concentration is obtained about 5 min after the administration [1]. Diazepam has poor water solubility, but polyethylene glycol 300 (PEG300), a vehicle causing relatively little local irritation, has been found to solubilize an expected clinically relevant dose (4–10 mg) of diazepam in the limited volume necessary for nasal administration [2].

In an earlier clinical study a nasal dose of 2 mg diazepam was administered by use of PEG300 as the solubilizing vehicle [2]. Within 30 min the nasal bioavailability was found to be about 37%. The neurological measurements in this study were rather crude, and comprised parameters such as memory tests and the ability to catch a ruler. The quantification of drug effects on attention and vigilance was based on questionnaires. The results of this study showed only minor drug effects, probably because the dose was too low. Therefore, it was decided to administer higher nasal doses of diazepam (4 and 7 mg).

Correspondence: Dr Sveinbjörn Gizurarson, University of Iceland, Department of Pharmaceutics, Hofsvallagata 53, 107 Reykjavik, Iceland. Tel.: 00354 5112020; E-mail: sg@lyf.is

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The electroencephalographic (EEG) effects of benzodiazepines are well known. Changes after drug administration have been observed in event-related potentials (ERP) and beta-activity [3–9]. The brain generates electrical waves of various wavelengths creating a spectrum, which may be divided into several frequency bands. The most important bands are found in the frequency range 8–12 and 12–35 Hz, named the alpha and beta-activity, respectively. An increase of beta-activity has been found to be a more sensitive measure of benzodiazepine effect than a decrease in alpha-activity [10].

Exposing study subjects to target tones (e.g. 2000 Hz auditory stimuli) among neutral tones (e.g. 1000 Hz auditory stimuli) generates ERPs. The neutral tones occur five times more frequently than the target. A positive wave appears 300 ms (P300) after the target tone. This wave is generated from the sensory discrimination of the target tone among the neutral tones. The P300 potential has been found to be particularly useful in measuring the intracerebral effects of benzodiazepines [8]. After both neutral and target tones a negative wave appears after 100 ms (N100), but only limited changes appear after benzodiazepine administration [3]. As well as being related to the sedative and cognitive effects of benzodiazepines [8], changes in EEG, particularly the increase in beta-activity, has been found to correlate with anticonvulsant effect [10].

Unrug *et al.* [3] found that the decrease in the P300 potential after a 10 mg oral dose of diazepam was most pronounced at the vertex electrode. The peak of the P300 potential is usually identified as the most positive point in the waveform range between 200 and 400 ms and a change in the latency of this peak may also be useful in evaluating changes in P300 caused by diazepam [8].

Fink *et al.* [9] found a linear correlation between the increase in EEG and beta-activity and blood concentrations of diazepam after oral administration to healthy volunteers. More recent studies of the pharmacological effects of benzodiazepines in man have been of nonblinded design, and only 3 out of 18 were controlled, emphasizing the need for additional well designed studies in this field [11].

The aims of the present study were (1) to provide information on the pharmacodynamic response to nasally administered diazepam formulated in a polyethylene glycol 300 vehicle (2) to evaluate and optimize EEG methods for measuring the neurological effects of diazepam, and (3) to define any relationship between the effect of diazepam on the EEG and serum concentrations of the drug.

Methods

Three male and five female healthy Caucasians, weighing 84 ± 17 kg, and between 20 and 40 years of age were studied (one of whom dropped out). They were asked not to drink alcohol during the entire study period and none was taking regular medication. Subjects received both written and oral information before giving their written consent. The National Icelandic Ethics Committee and the Icelandic Health Department approved the study.

The study had a double-blind, randomized, cross-over design. Eight subjects received on separate occasions (1) placebo intranasal (i.n.) administration of PEG300, (2) 4 mg diazepam i.n. solubilized in PEG300 (4 mg i.n.), (3) 7 mg diazepam i.n. solubilized in PEG 300 (7 mg i.n.), and (4) 5 mg diazepam intravenously (i.v.) administered in a commercially available formulation (Stesolid Novum®). The latter and PEG300 were obtained from Dumex-Alpharma A/S (Copenhagen, Denmark) and Union Carbide (Charleston, U.S.A.), respectively. In order to improve the spray properties of the viscous formulation, it was necessary to modify a unit-dose device from Pfeiffer (Radolfzell, Germany). Each device was filled to spray 75 μl in each nostril (two devices per nasal round).

Blood sampling

Venous blood samples were taken at −10, −2, 3, 5, 8, 11, 15, 20, 30, 45 and 60 min after drug administration. A commercially available enzyme-immunoassay (EIA) kit from STC Technologies, Inc. (Bethlehem, U.S.A.) was used for the analysis of diazepam in serum. The measurements were performed on a HTS7000 microplate reader from Perkin-Elmer (Wellelsly, U.S.A.) with u.v. detection at 450 nm. Samples were centrifuged at 3200 *g* for 10 min and serum was transferred to 1 ml cryotubes from NUNC (Copenhagen, Denmark) and stored at −80° C until analysis. Standards of 1, 2, 5 and 20 ng ml^{-1} ($n = 9$) were analysed on separate days and a mean coefficient of variation was found to be 10% (range 7–13). The lowest level of detection was about 0.1 ng ml^{-1}. All samples had concentrations higher than 1 ng ml^{-1}.

ERP and β-activity

The hardware and software systems used for the EEG recordings and analyses were from Neuroscan® (Sterling, U.S.A.). Nineteen silver scalp electrodes (F1, F2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2) were placed on the head according to the international 10–20 system [12]. Electrodes placed on the left and right mastoid process (electrodes A1 and A2) were connected together and used as a reference. Two electrodes, one above the other below the left eye, were used to monitor eye movements. The impedance of the electrodes was tested before the recording started.

The subjects sat in a chair during the recording and with their eyes shut. They were asked to listen to auditory stimuli delivered through earplugs to both ears. The neutral

stimulus was a 1000 Hz tone occurring five times more frequently than the 2000 Hz target tone. A preliminary test was performed to ensure the subjects were able to hear and separate the stimuli. The interstimulus-interval was 2 s, tone duration was 100 ms, and rise and fall time was 5 ms. The subjects were asked to tap with their finger and count when a target tone appeared. If subjects fell asleep, as happened a few times, especially in the i.v. group, they were gently awoken by touching their hands.

The EEG was sampled at 200 Hz after low pass filtering at 40 Hz and high pass filtering at 0.3 Hz. Each auditory stimulus triggered a sampling of EEG that started 100 ms before the stimulus and had a total duration of 1280 ms (256 sampling points). The EEG epoch triggered by each auditory stimulus was stored for further analysis.

The EEG was recorded before and after the subjects had received medication. Each recording session lasted about 15 min during which 400–500 stimuli were delivered. The recordings were analysed by averaging the EEG epochs to the neutral and the target stimuli, respectively. Epochs were rejected when amplitudes exceeded ± 100 μV either in the lead across the eye or at the Fz, Cz or Pz electrodes. The peak of the P300 potential was defined by the highest potential between 200 and 500 ms and was located in the average ERP for each individual before and after treatment. The peak of the N100 potential was defined by the lowest potential between 50 and 200 ms. The Cz electrode resulted in the most sensitive effect measurements and was therefore chosen for the ERP calculations. To assess changes in beta activity, the mean amplitude and relative power spectrum were obtained after Fourier transformation of each epoch. The mean amplitude of the β-activity within different frequency bands between 16 and 35 Hz was calculated.

Questionnaires

The questionnaires were answered as soon as possible after each measurement. Subjects were asked to score a prefabricated list of various possible irritant effects from each formulation.

Data analysis

The area under the serum concentration-time curve from 0 to 60 min (AUC(0,60 min)) was calculated using the trapezoidal method. AUC from 0 to 2 min for intravenous administrations were determined by extrapolation to zero by using logarithmic regression analysis on the initial two concentrations. A two-factor ANOVA was used to compare differences between pharmacodynamic measurements obtained by subtracting values after drug administration from pre-dose values. P300, P300-N100 differences and β-activities were tested. A two-sample *t*-test (one-sided) was used in the statistical analysis to compare the various data sets.

Results

Mean serum concentration-time profiles of seven subjects are shown in Figure 1. The mean bioavailability, C_{max} and t_{max}, [95% confidence interval], for the 4 and 7 mg i.n. diazepam formulations were found to be; 45% [32, 58] and 42% [22, 62], 99 ng ml^{-1} [83, 115] and 179 ng ml^{-1} [126, 232], 18 min [11, 25] and 42 min [25, 59], respectively. t_{max} was significantly ($P<0.05$) higher after 7 mg i.n. administration than after 4 mg. The slower absorption from the 7 mg dose was substantiated by differences in the $AUC_{i.n.}/AUC_{i.v.}$ ratio of the drug between the two formulations at the early time points (Table 1).

A two-factor ANOVA was used to compare P300, P300-N100 amplitude differences and β-activity effects

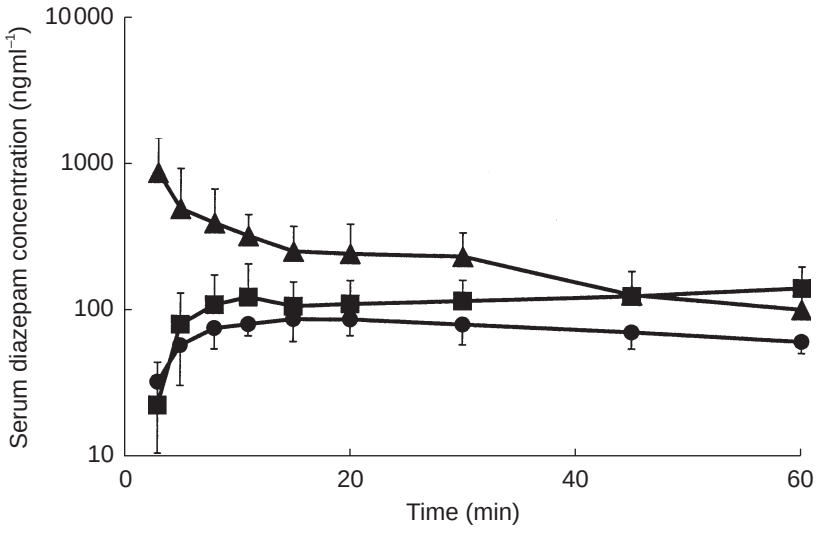

Figure 1 Mean (±s.d.) serum concentration-time profiles after intranasal (i.n.) administration of 4 (●) and 7 mg (■) diazepam and 5 mg (▲) diazepam intravenous (i.v.), respectively, to seven healthy subjects.

Table 1 Mean $AUC_{i.n.}/AUC_{i.v.}$ ratios (expressed as percentage) at various times after the administration of 4 and 7 mg diazepam to seven healthy subjects.

	$AUC_{i.n.}/AUC_{i.v.}$ (%) Time (min)								
I.n.dose	*3*	*5*	*8*	*11*	*15*	*20*	*30*	*45*	*60*
4 mg	12[a]	14	17	21	25	29	34	40	45
7 mg	7[a]	8	12	15	19	22	27	34	42
P value[b]	0.07	0.05	0.09	0.14	0.17	0.12	0.09	0.19	0.63

[a]One AUC value was left out because it was thought to be an outlier, being more than three times the standard deviation above all the other values.
[b]*P* values from the comparison between the two doses.

Table 2 Mean (±s.d., $n=7$) in P300 potential (μV) at the vertex electrode between before and after administration.

Formulation	*Difference between before and after drug administration*	*95% confidence[b] intervals*
Placebo	−0.8±2.2	[−3.0, 1.4]
4 mg i.n.	−2.8±3.1	[−5.9, 0.3]
7 mg i.n.	−5.0±2.4★,[a]	[−6.7, −3.2]
5 mg i.v.	−5.6±2.8★★	[−8.4, −2.7]

[a]$P<0.05$(★), $P<0.01$(★★), $P<0.001$(★★★) *vs* placebo.
[b]No significant differences were found between formulations or subjects.

Table 3 Mean (±s.d., $n=7$) changes in P300-N100 potential differences (μV) at the vertex electrode between before and after drug administration.

Formulation	*Difference between before and after drug administration*	*95% confidence[b] intervals*
Placebo	−0.9±7.5	[−6.5, 4.7]
4 mg i.n.	−6.4±5.0★	[−10.1, −2.7]
7 mg i.n.	−8.6±3.8★★	[−11.4, −5.8]
5 mg i.v.	−9.6±3.4★★★	[−12.1, −7.1]

[a]$P<0.05$(★), $P<0.01$(★★), $P<0.001$(★★★) *vs* placebo.
[b]Significant differences were found between formulations, but not between subjects.

before and after treatment (Tables 2–4). No significant difference was found between subjects. However for the P300-N100 amplitude differences a significant drug effect ($P<0.05$) was found. A significant reduction in P300 amplitude was observed, using a two-sample *t*-test, compared with placebo, after 7 mg i.n. ($P<0.05$) and the 5 mg i.v. administrations ($P<0.01$), but not after the 4 mg i.n. administration (Table 2). Significant decreases compared with placebo was also found in the P300-N100 amplitude differences ($P<0.05$) ($P<0.01$) and ($P<0.001$) for the 4 mg i.n., 7 mg i.n. and 5 mg i.v. formulations, respectively (Table 3). The corresponding significance values for the beta-activity were ($P<0.05$) ($P<0.05$) and ($P<0.05$), respectively (Table 4).

Table 4 Mean (±s.d., $n=7$) EEG amplitude (nV) values of differences in the β-frequency range (16–35 Hz) at the vertex electrode between before and after drug administration.

Formulation	*Difference between before and after administration*	*95% confidence[b] intervals*
Placebo	64±163	[−57, 184]
4 mg i.n.	189±232★	[17, 360]
7 mg i.n.	139±68★	[89, 189]
5 mg i.v.	222±120★	[133, 342]

[a]$P<0.05$(★), $P<0.01$(★★), $P<0.001$(★★★) *vs* placebo.
[b]No significant differences were found between formulations or subjects.

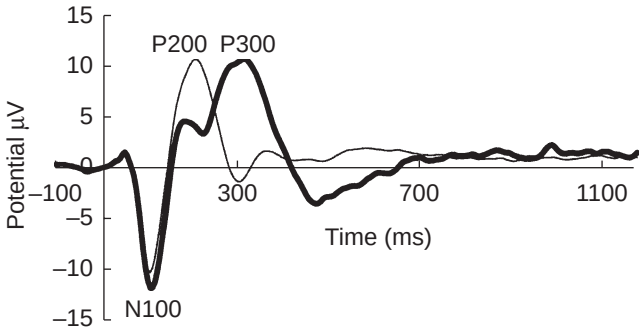

Figure 2 Mean values from seven healthy subjects of the ERPs at the vertex electrode elicited by frequent nontarget events (1000 Hz tones, thin line) and rare target events (2000 Hz tones, thick line), respectively. Note the P300 evoked by the rare tones.

The differences [95% confidence intervals] between the before and after values for the P300-N100 amplitude differences were −0.9 [−6.5, 4.7], −6.4 [−10.1, −2,7], −8.6 [−11.4, −5.8] and −9.6 [−12.1, −7.1] for placebo, 4 mg i.n., 7 mg i.n. and 5 mg i.v. diazepam, respectively. The overall means of the ERPs (all subjects) elicited, respectively, by neutral and target stimuli before treatment are shown in Figure 2 and those elicited by the target stimuli after treatment in Figure 3.

Mean differences [95% confidence intervals] on the P300-N100 measured between the placebo and drug treatment were −3.3 [−0.4, −6.1], −4.8 [−2.4, −7.2] and −5.8 [−3.9, −7.8] for the 4 and 7 mg i.n. formulations and the 5 mg i.v. formulation, respectively, all of which were statistically significant effect of diazepam was found for all formulations. The mean difference [95% confidence intervals] between 4 mg i.*n* and 7 mg i.n., 5 mg i.v., respectively, were −1.5 [−3.3, 0.3] and −2.6 [−4.1, −1.0], indicating statistical difference between 4 mg i.n. and 5 mg i.v. The mean difference [95% confidence] between 7 mg i.n. and 5 mg i.v. was −1.1 [−3.1, 1.0], indicating no statistical difference in the neurological effect between these two diazepam formulations.

No shift in latency of the ERP components was found after diazepam administration, and therefore, the ERP data are based on changes in ERP amplitudes obtained at the vertex electrode.

By averaging the ERP epochs evoked by target stimuli within each consecutive 2 min period (approximately 10 epochs of rare stimuli), it was possible to determine how the P300-N100 difference changed with time for different formulations of the drug. In Figure 4, this is shown as change in the ratio relative to placebo of the P300-N100 difference in drug treatment.

The questionnaires revealed that the adverse effects following drug treatment were limited. Bitter taste after nasal administration, was the most frequently reported adverse event.

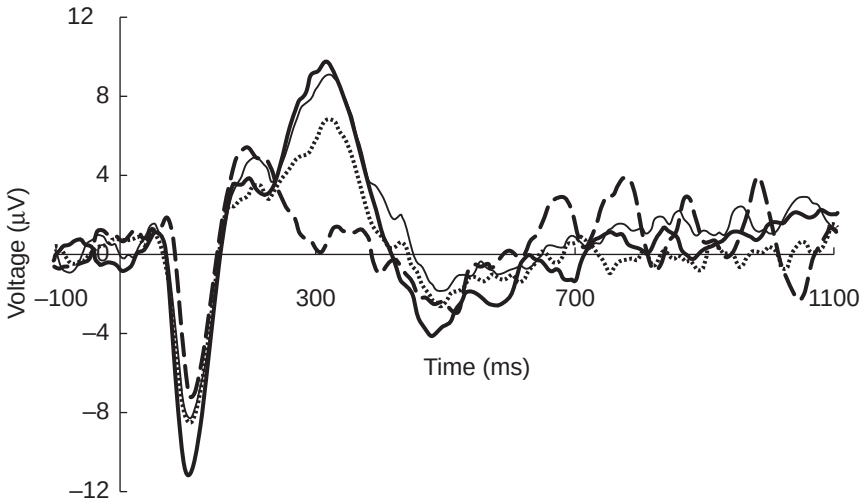

Figure 3 Mean values from seven subjects of the ERPs at the vertex electrode after hearing a rare target event (2000 Hz tone) after administration of placebo (thick line), 4 mg diazepam (thin line), 7 mg diazepam (dotted line) intranasally or 5 mg diazepam (dashed line) intravenously. Note the negative peak at 100 ms (N100) and the positive peak at 300 ms (P300).

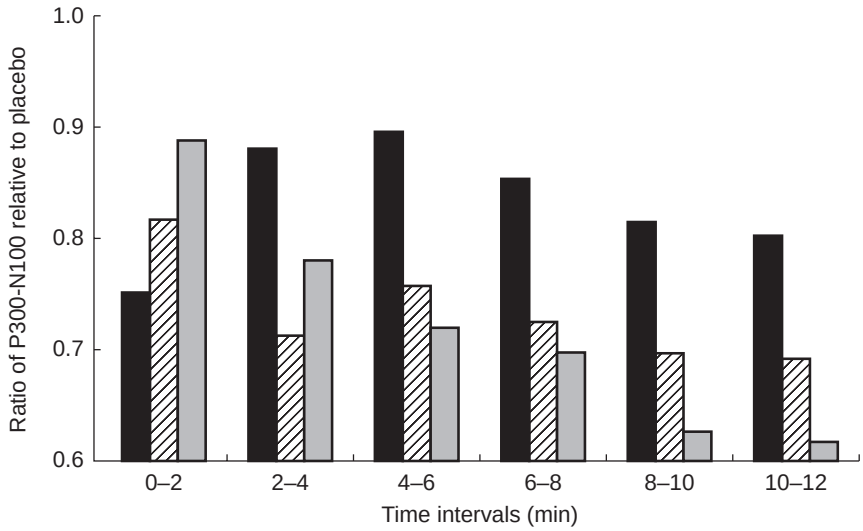

Figure 4 Mean values from seven subjects of N100-P300 potential differences (obtained by averaging ERPs within each consecutive 2 min period) after administration of placebo intranasally, 4 mg diazepam intranasally (■), 7 mg diazepam intranasally (▨) or 5 mg diazepam intravenously (▩). Values (range 0.6–1.0) are illustrated as ratios of the P300-N100 difference for drug relative to placebo.

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