CHRONIC EFFECTS OF DIAZEPAM ON THE SPECTRAL CONTENT OF THE RAT EEG

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SUMMARY

We determined the effect of chronic continuous diazepam treatment on the spectral content of the EEG in rats. Diazepam was administered for 21 days using subcutaneously implanted silastic tubes, resulting in constant blood concentrations.
Diazepam caused a decrease in the power of the low frequency bands (1-8 Hz) and an increase in the power of the high frequency bands (21-40 Hz). These changes persisted during 21 days of treatment and were no longer detected in a post drug control measurement on the 9th day after removing the tubes.
No tolerance developed to the effect of diazepam on the spectral content of the EEG. This indicates that in 21 days there is no decline in the effect of interaction of diazepam with the $GABA_A$ - benzodiazepine receptor complexes responsible for the power spectrum changes.

KEY WORDS Benzodiazepine, Diazepam, Tolerance, Rats, EEG, Spectral Analysis.

INTRODUCTION

It is known that tolerance develops to the behavioural effects of benzodiazepines: e.g. in rats to the sedating effects, to the motor impairing effects and to the anticonvulsive activity [4,5]. A reduction in the course of time of the spectral changes in the EEG (i.e. a decrease in the power of the low frequencies and an increase in the power of the high frequencies [1,2,11]) is reported in multiple dose regime studies [12,13,17]. However, it is clinical knowledge that the EEG changes induced by benzodiazepines persist during chronic treatment (W. van Emde Boas, personal communication). Tolerance development might depend on the dose regime [4,9,19]. Repeated dosages of a benzodiazepine in rats result in major fluctuations in the concentrations of the drug [7] due to the short half life time in these animals (± 1 hour for diazepam, [6]). In humans, repeated dosages result in more constant blood concentrations due to the long half life time (± 40

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hours, [10]). We investigated the effect of diazepam on the EEG of rats having constant blood concentrations during 21 days. Silastic tubes containing diazepam were subcutaneously implanted, allowing a continuous release [7,8,16,20]. During the treatment we repeatedly determined the power spectrum of the EEG.

MATERIALS AND METHODS

Sixteen male WAG/Rij rats were used, age 10 months and weighing 350 ± 16 grams (mean ± SD) at the start of the experiment. Animals were maintained on a 12-12 hour light-dark cycle: lights off at 9 am. Rats were single housed in standard cages with ad libitum access to standard food and water.

Three electrodes were implanted under complete Hypnorm anaesthesia (Plastic Products Company, MS 333/2A). The coordinates related to bregma were: A 2.0, L 2.0; A -3.7, L 9.0. A ground electrode was placed above the cerebellum. Animals were allowed to recover one week. EEG signals were measured between 1 Hz and 100 Hz and recorded digitally with a sample frequency of 200 Hz. Recordings took place from 11 am until 1 pm. Two baselines were recorded.

Per animal we used 8 silastic tubes of 8 cm (Dow Corning, 0.062 inch inner diameter; 0.095 inch outer diameter), each containing 100 mg of solid diazepam. Controls received empty tubes. Implantation and removal were carried out under ether anaesthesia. Experimental recording started 24 hours after tube implantation. A post-drug recording was taken 9 days after tube removal.

Total benzodiazepine activity in the blood was determined with a receptor binding assay. Blood samples of 100 µL were taken from the tail venes and hemolyzed in water. The samples were extracted in pentane/dichloormethane. Evaporated extract fractions were incubated during 90 min at 0° C with rat-brain membrane preparations and 3 nM [methyl-^{3}H]diazepam, followed by rapid filtration. Specific benzodiazepine activity was expressed relative to the activity on day 2. Statistical analysis was performed by linear regression followed by a F-test of the slope.

The spectral content of the EEG was determined by Fast Fourier Transformation for 10 periods of 3.2 sec of EEG during passive wakefulness. This state was defined as observed immobile behaviour together with low voltage and fast frequency EEG [1,2]. A mean spectrogram was constructed for each animal per recording day and expressed in standardized scores (z-scores). We determined the mean power in the delta-band (1-4 Hz), the theta-band (4-8 Hz), the alpha-band (8-12 Hz) and the beta-band (12-40 Hz) [18]. Statistical analysis was performed by ANOVA for repeated measurements.

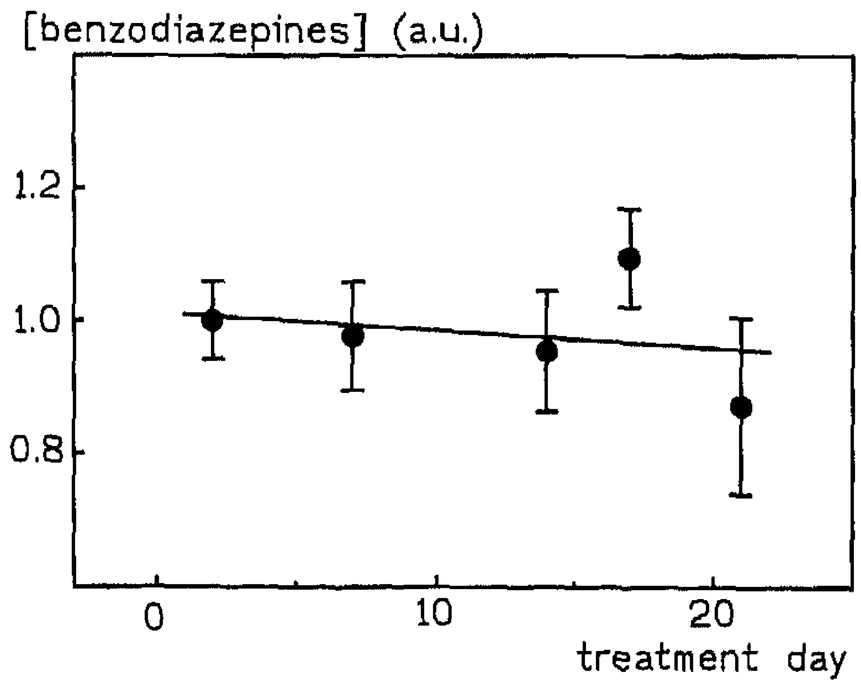

Fig. 1
Relative blood concentrations during 21 days of diazepam treatment with subcutaneously implanted silastic tubes. Concentration values are relative the activity on day 2. Mean's and S.E.M.'s on five treatment days (2, 4, 9, 18 and 21) are given (n=8 animals with 8 tubes of 8 cm each). The slope obtained by linear regression is not significantly different from zero (F-test; p=0.6).

RESULTS

The diazepam output from the implanted tubes was 17.6 ± 1.6 mg per animal per day (mean ± SD, n=8 animals with 8 tubes). The total benzodiazepine concentrations in the blood were constant during the 21 days (fig 1). A blood sample taken on day 2 analyzed by HPLC showed that the absolute concentration of diazepam was 0.7 μM (200 ng/ml) and of its main metabolite desmethyldiazepam was 1.2 μM (336 ng/ml).

The effects of these constant blood concentrations on the frequency bands of the EEG power spectra are presented in Fig. 2. During 21 days of treatment we found a decrease in the mean power of the low frequency bands (delta: $F_{1,15}=27.92$, $p<0.0001$ and theta: $F_{1,15}=17.96$, $p=0.0008$) and an increase in the high frequency band (beta: $F_{1,15}=47.15$, $p<0.0001$). No drug-day interaction was found. No differences were found on the baseline days nor on the 9th day after removal of the tubes between the control group and the experimental group.

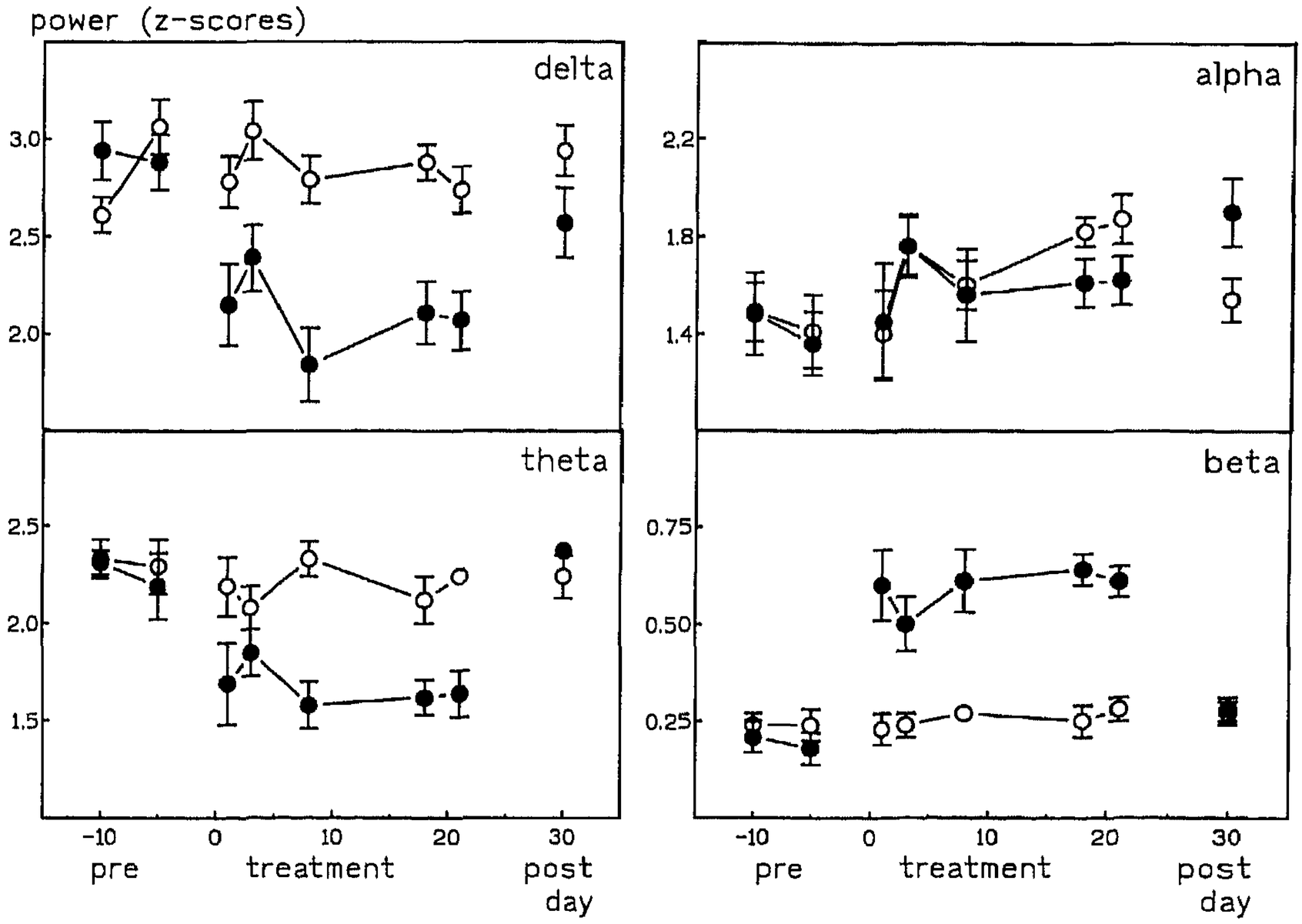

Fig. 2.
Effects of diazepam treatment during 21 days on the EEG frequency bands. Data points are given for controls (open circles, n=8) and for diazepam treated rats (filled circles, n=8).
Mean power is expressed in z-scores (mean's and S.E.M.'s) on two baseline recording days (-10 and -5), five treatment days (1, 3, 8, 18 and 21) and one post-treatment day (30).
Data are given for the delta-band (1-4 Hz), theta-band (4-8 Hz), alpha-band (8-12 Hz) and beta-band (12-40 Hz). On all treatment days diazepam induces a decrease in the delta- and the theta-band and an increase in the beta-band (ANOVA $p \leq 0.0008$). No drug-day interaction was found.

DISCUSSION

Blood concentrations remained constant during 21 days of implantation of silastic tubes with diazepam. This observation confirms earlier reports using this method [7].

The spectral content of the EEG was determined of EEG during passive wakefulness, a state known to be sensitive to drug effects [1]. During the entire treatment a decrease in the power of the low frequency bands and an increase in the power of the high frequency band was found. These changes are characteristic of single dose treatment with benzodiazepines [1,2,11]. During the treatment period no tolerance developed to the changes in the power spectrum. This observation confirms clinical knowledge that the EEG changes induced by benzodiazepines persist during chronic treatment. Humans, using benzodiazepines chronically, are likely to have fairly constant blood concentrations [10]. Tolerance development might be dependent on the dose regime, i.e. on changes in the concentration of the drug [4,9,19]. Sala et al. found that full tolerance developed to the increase in the beta band within 4 weeks using a single oral dose of 40 mg/kg chlordiazepoxide per day in rats [17]. We suggest that constant vs. fluctuating blood concentrations account for different results.

The changes in frequency bands of the EEG induced by diazepam reflect the interaction of the compound with the $GABA_A$ - benzodiazepine receptor complex. This was shown in a single dose study by Mandema et al. [11]. They found a perfect correlation between the benzodiazepine concentration producing half of the maximum EEG effect and the benzodiazepine affinity to the receptor site on the $GABA_A$ complex. This was determined in whole brain homogenates using tritiated flumazenil as the ligand [11]. Different $GABA_A$ - benzodiazepine receptor subtypes exist in different brain areas, presumably serving different physiological functions [3,14]. In order to clarify the mechanisms underlying tolerance development, it is important to know whether interactions of a benzodiazepine with its effector system remain intact during tolerance development. Our data indicate that in 21 days there is no decline of the effect of interaction of benzodiazepines with those receptor complexes that are responsible for the power spectrum changes. Three weeks of continuous treatment of rats with diazepam by the method described here did not alter the total benzodiazepine binding [16] but rather decreased the coupling between the GABA site and the benzodiazepine site [8]. It was found that the γ_2 subunit mRNA levels in the cortex were decreased [20]. The presence of the γ_2 subunit in the $GABA_A$ receptor causes the typical benzodiazepine effects in vitro [15]. Could it be that the effects of benzodiazepine treatment on the spectral content of the EEG are independent of the γ_2 subunit? Indeed, most of

the subunits investigated did not change during chronic treatment [20]. In favour of a difference in receptors involved in behavioural and EEG changes, benzodiazepines disrupt the regular relationship between EEG and behaviour [2]. The molecular basis underlying this pharmacological dissociation might manifest itself in chronic studies.

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