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Effects of Diazepam and Two Beta-Carbolines on Epileptic Activity and on EEG and Behavior in Rats With Absence Seizures

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COENEN, A. M. L. AND E. L. J. M. VAN LUIJTELAAR. *Effects of diazepam and two beta-carbolines on epileptic activity and on EEG and behavior in rats with absence seizures.* PHARMACOL BIOCHEM BEHAV 32(1) 27–35, 1989.—In the present series of experiments, effects of a full benzodiazepine receptor agonist (diazepam) are described and compared with those of a partial benzodiazepine receptor agonist (ZK 91296) and an inverse partial benzodiazepine receptor agonist (FG 7142), both compounds of the beta-carboline family. In a rat model for generalized absence epilepsy, the anticonvulsant, the hypnotic and the myorelaxant properties were investigated, as well as effects on on-going behavior and effects on the electroencephalogram (EEG). While diazepam showed all behavioral and electrophysiological changes characteristic for the benzodiazepines, the partial agonist ZK 91296 reduced seizure activity without inducing any signs of sedation, sleepiness, myorelaxation and changes in behavior or EEG spectral content. The partial inverse agonist FG 7142 aggrevated epileptic activity, with slightly enhanced immobile behavior, suggesting some anxiogenic properties. The results not only demonstrate that the multiple effects of the benzodiazepines could be separated by these compounds, but also that the anticonvulsant activity is not related to changes in spectral content of the EEG. Because of its selective activity, ZK 91296 appears to be more suitable than diazepam in reducing seizure activity. Finally, FG 7142 seems a genuine partial inverse agonist which has some, but not all, of the inverse effects of a full agonist.

Diazepam Beta-carbolines Anticonvulsant activity Behavior Sleep EEG spectral analysis

A new class of interesting compounds are the beta-carbolines. These agents, chemically distinct from the benzodiazepines, appeared to be ligands of the benzodiazepine receptor (3). Three main subclasses of these compounds can be distinguished: agonists, imitating the benzodiazepines; antagonists, blocking the effects of the benzodiazepines; and inverse agonists, inducing opposite effects as the benzodiazepines. Moreover, it was established that various beta-carbolines did not show the whole spectrum of benzodiazepine effects or, for the inverse agonists, not all of the opposite effects. These partial or partial inverse agonists are of special interest, since they can, in principle, separate the multiple effects of the benzodiazepines (the anticonvulsant, the sedative, the hypnotic, the anxiolytic, and the myorelaxant properties). This is important with respect to the specificity of an aimed action (e.g., anticonvulsant activity without sedation and lowering of muscle tone).

The beta-carboline ZK 91296 is a partial benzodiazepine receptor agonist (30). It was found that ZK 91296 has a more specific anticonvulsive effect than the classical benzodiazepine diazepam (17). Using a rat model for absence epilepsy, Jensen *et al.* (17) claimed that ZK 91296 did not induce sedation and, furthermore, caused no changes in the background EEG. This fits into the profile of a partial agonist and is of interest viewed against the specificity of its anticonvulsant action. In the study of Jensen *et al.* (17), however, no quantitative data either of the sedation or of the spectral content of the EEG were given.

Based on biochemical data, the beta-carboline FG 7142 has been characterized as a partial inverse benzodiazepine receptor agonist (16). Behaviorally, the characterization of FG 7142 is not complete, although the (pro) convulsant properties are already documented (26). The effects of FG 7142 on sleep and wakefulness, on muscle tension, on anxiety and arousal level are less clear. Indications exist that this beta-carboline increases the arousal level and that it improves memory in mice (19). In rats, File and Pellow (11) and in humans, Dorow *et al.* (9) reported that FG 7142 has anxiogenic properties. This means that the partial inverse agonist has at least some effects opposite to that of the full agonist. However, the effects on the spectral content of the EEG have not been studied so far. If FG 7142 is indeed a partial inverse agonist, then the spectral changes should be opposite or at least differ from those seen after the full agonist.

An almost similar rat model for absence epilepsy with which Marescaux *et al.* (26) and Jensen *et al.* (17) performed their experiments with the beta-carbolines, was discovered

by us a few years ago. The WAG/Rij rats are homozygous and show all the electrophysiological and behavioral manifestations of generalized absence epilepsy (22). Several characteristics of this model are already known, such as the ontogenetic (5), this circadian (24) and the pharmacological (29) ones. It was concluded that this model, similar to that of Marescaux *et al.* (25), is a valid one for investigating epilepsy-modulating properties of psychoactive drugs.

The purpose of the present study was to investigate whether a partial agonist (ZK 91296) and a partial inverse agonist (FG 7142) do indeed have bidirectional effects on the amount of epilepsy of the WAG/Rij rats. Furthermore, the specificity of the epilepsy-modulating action was of interest. Therefore, the effects of both compounds on on-going behavior, on sleep and wakefulness, on spectral content of the EEG and on muscle tone were also topics to be studied. In order to indicate the sensitivity of our methods applied for ZK 91296 and FG 7142, the effects were compared with those of diazepam, the classical benzodiazepine, whose effects are well-known.

METHOD

Male and female rats of the WAG/Rij strain (10) were used. The age of the animals ranged between 13 and 19 months and their weights between 190 and 380 g. Rats were singly housed in cages (30×25×25 cm) and were maintained on a 12-12 hour LD cycle with lights on at 22.00 hours. They had ad lib access to standard food and water. A tripolar cortical electrode set (Plastic Products Company, MS 333 2A) was permanently implanted into all rats. During the experiment, EEG activity, measured between 1 and 70 Hz, could be registered on a polygraph (Elema-Schönander). More experimental details are given in van Luijtelaar and Coenen (22), and Coenen and van Luijtelaar (5).

After a complete recovery from the implantation and after habituation of at least one week to all experimental procedures, experiments started. At 9.00 hr of the experimental day, animals were connected to the recording cables. Baseline EEG measures took place from 12.00 till 13.00 hr. Then, animals were intraperitoneally injected with drug or solvent and again recorded for 3 (diazepam) or 4 (beta-carbolines) hours. During the whole time, rats stayed in their home cages. From a half hour after the injection and during the following half hour, specimens were closely observed through a window. Behavioral categories observed were: 'voluntary' behavior (walking, rearing, sniffing, digging), 'automatic' behavior (grooming, eating, drinking), and 'immobile' behavior (sitting, lying and standing still) (4,37). During the observation period, EEG and all behavioral codes were, aside from paper registration, also registered on magnetic tape. This was done to allow off-line frequency analysis of the EEG during a particular behavioral category.

Epileptic activity was identified by visual inspection of the EEG paper. Number of spike-wave complexes, the mean duration of spike-wave complexes and the total duration of seizure activity were determined for each hour. A spike-wave complex was identified as such if its duration was at least 1 sec, if it included a train of sharp spikes and slow waves (7.5–9 Hz), and if the spikes pointed upward and had an amplitude of at least twice the background amplitude of the EEG. Effects on behavior were obtained by measuring on paper the total duration in seconds of each behavioral category (voluntary, automatic and immobile), in the half-hour observation period. Effects on sleep were established by measuring in the observation period, the number of sleep periods, the mean length of these periods and the total duration of sleep. Sleep was defined as such when the behavioral category immobile behavior was accompanied by large EEG waves. Effects on EEG-spectral content were established by a method based on frequency analysis adapted from Coenen (4) and van Luijtelaar and Coenen (23). During periods of passive wakefulness (defined as the behavioral category immobile behavior, together with a low voltage, fast frequency EEG), the EEG was led through a hardware band pass filter set, covering the range of 2 till 30 Hz. Each 5 sec a spectrogram was calculated, containing a value for the delta-band (2–4 Hz), the theta-band (6–10 Hz), the spindle-band (11–14 Hz) and the beta-band (15–30 Hz). For each single animal a mean spectrogram for a period between 75 and 100 sec was constructed. This mean spectrogram was normalised (mean 0, variance = 1) to compensate for different amplification values.

At the end of the recording session, rats were picked up by hand and uncoupled from the recording cables. In doing this, the investigator tried to judge on a three-point scale (value 0, −1 and 1) the muscle tone of the animal. The value 0 stands for no clear changes in muscular tone, −1 for a clear reduction and 1 for a clear increase.

All statistics with respect to epileptic activity, behavioral data, hypnotic activity and EEG frequency analyses, were done with an ANOVA, with dose as factor (four or five levels). If a significant dose effect was found, differences among groups were subsequently tested with Duncan's multiple range test, wherein alpha was chosen to be 0.05.

RESULTS

Experiment 1: Effects of Diazepam (Valium)

Commercial diazepam in solution (Valium 10, Roche) was diluted further with a 3% Tween-80 in 0.9% NaCl solution, and was given to five groups of rats: group 0 (0 mg/kg) (n=6), group 1 (0.2 mg/kg) (n=6), group 2 (1 mg/kg) (n=8), group 3 (2.5 mg/kg) (n=7) and group 4 (5 mg/kg) (n=6). Diazepam and solvent were applied in a volume 1 ml per 400 g body weight. In contrast to all forthcoming experiments, recording time following injection was 3 hours.

Epileptic Activity (Fig. 1)

Before injection, there were no differences on any of the three dependent variables: number of spike-wave complexes, mean duration of these complexes and total duration in seconds.

During the first postinjection hour, a clear dose-effect in the number of spike-wave complexes was found, $F(4,28)=8.15$, $p<0.001$. Groups 0 and 1 had significantly more complexes than groups 2, 3, and 4. In the second postinjection hour, exactly the same effects were found, $F(4,28)=6.78$, $p<0.001$, whereas in the third hour a dose-effect was still detected, $F(4,28)=4.62$, $p<0.01$. During this hour, group 4 had fewer complexes than groups 1 and 0, and groups 3 and 2 less than group 1. During the first hour a dose-effect was found on the mean duration of spike-wave complexes, $F(4,28)=4.61$, $p<0.01$. Group 2 complexes were shorter than those of group 0. In the second and third hour, these effects were no longer present. During the entire three hour postinjectin period, effects were seen on the total duration of spike-wave complexes. In the first hour, $F(4,28)=5.98$, $p<0.01$, groups 4, 3, and 2 had a lower score than groups 1 and 0. In the second hour, $F(4,28)=5.31$,

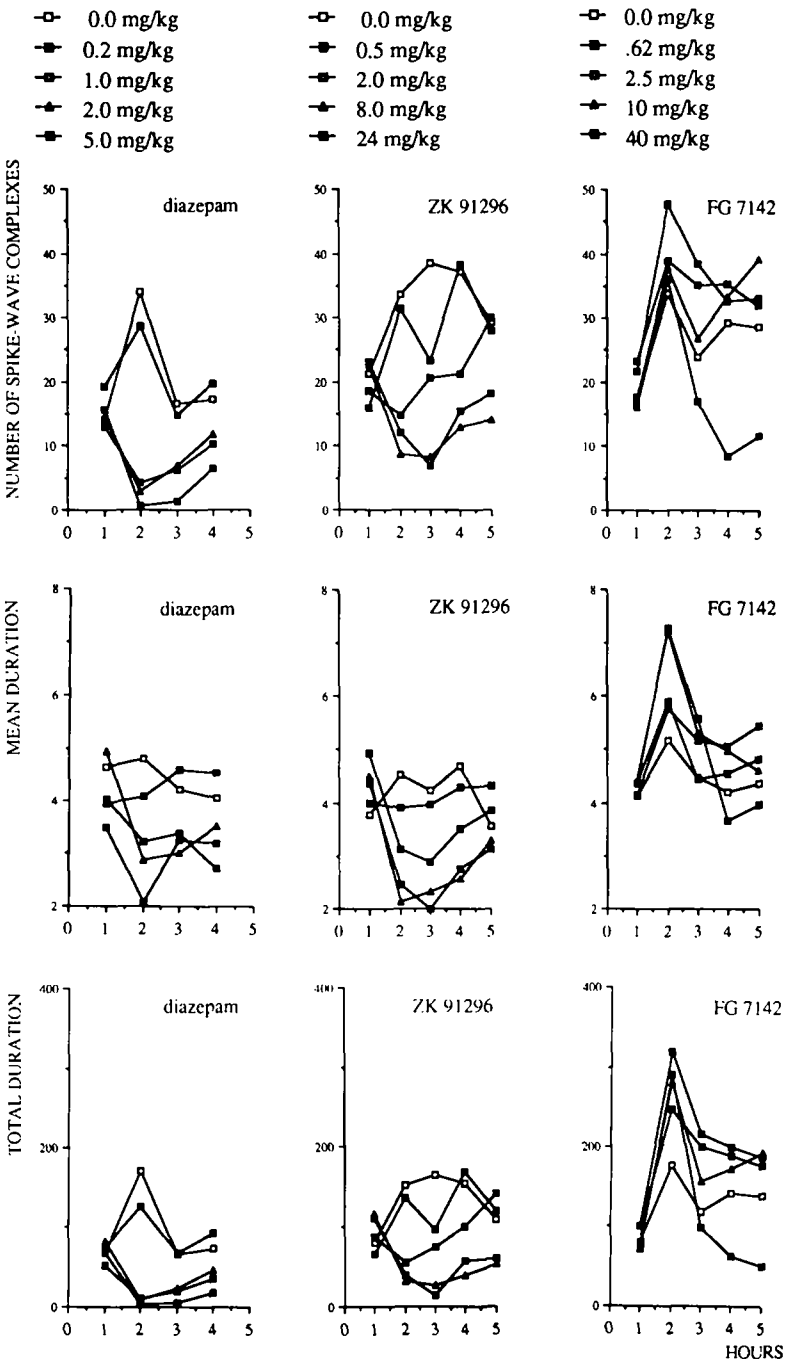

FIG. 1. Effects of diazepam (left vertical series of graphs), ZK 91296 (middle series) and FG 7142 (right series) on number of spike-wave complexes (upper series of graphs), on mean duration of spike-wave complexes (middle series) and on total duration of spike-wave complexes (lower series). All data are given per hour whereby 1 is the baseline hour and 2, 3, 4 and 5 are the 4 postinjection hours respectively. Each compound is given in 5 doses. For statistics see text.

$p<0.01$: groups 4, 3 and 2 had again fewer seconds with epileptic activity than groups 1 and 0. In the third hour, $F(4,28)=5.18$, $p<0.01$. Here, group 4 had less epileptic activity than groups 1 and 0, whereas groups 3 and 2 had less epileptic activity than groups 1 and 0, whereas groups 3 and 2 had less than group 1.

Behavior (Fig. 2)

No effects of diazepam were found on exploratory behavior; this in contrast to automatic behavior, $F(4,28)=2.74$, $p<0.05$, where group 4 showed less of this behavior than groups 1 and 0. A clear dose-response relationship was detected on immobile behavior, $F(4,28)=5.95$, $p<0.01$: group 4

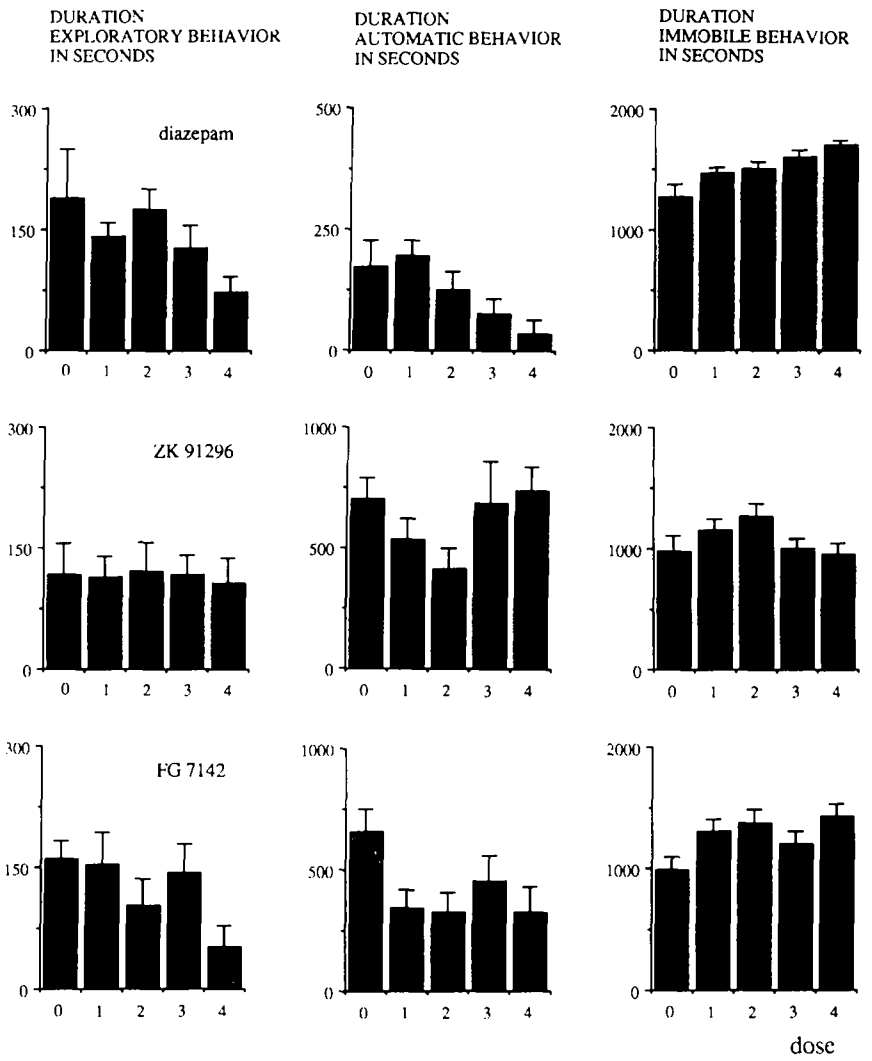

FIG. 2. Effects of diazepam (upper horizontal series of graphs), ZK 91296 (middle series) and FG 7142 (lower series) on exploratory behavior (left vertical series of graphs), on automatic behavior (middle series) and on immobile behavior (right series). Data are given in means and SEM's. All compounds are given in 5 doses (0, 1, 2, 3, and 4 respectively, whereby 0 is the zero-dose). Statistics are given in the Results section.

was more immobile than groups 2, 1 and 0 and groups 3, 2, and 1 were more immobile than group 0.

Sleep (Fig. 3)

There were no differences in the number of sleep periods, but there were differences in the total duration of sleep, $F(4,28)=4.82$, $p<0.01$. Group 0 had a shorter duration than groups 2, 3 and 4; and group 1 had shorter durations than group 4. In the mean length of the sleep period, $F(4,28)=3.13$, $p<0.05$, group 4 had a longer mean length than group 0.

EEG Spectral Analysis (Fig. 4)

As mentioned before, spectral analysis was done on the background EEG accompanying passive wakefulness. Aberrant EEG activity was excluded in this analysis.

No differences were found in the delta-band, but in the theta-band, $F(4,28)=9.06$, $p<0.0001$, the spindle-band, $F(4,28)=4.30$, $p<0.01$, and the beta-band, $F(4,28)=4.73$, $p<0.01$, effects of different doses of diazepam were found. In the theta-band, groups 0 and 1 scored higher than groups 2, 3 and 4; in the spindle-band, group 4 scored higher than groups 2, 1 and 0, whereas group 3 had more delta than group 1; in the beta-band groups 3 and 2 had more beta than groups 1 and 0.

Muscle Tone

As a rough impression obtained by picking up the rats, it was thought that diazepam gave rise to a considerable decrease of muscle tone. In particular, when higher doses were used, rats hung still and weak in the researcher's hand, suggesting firm muscular relaxation (value −1).

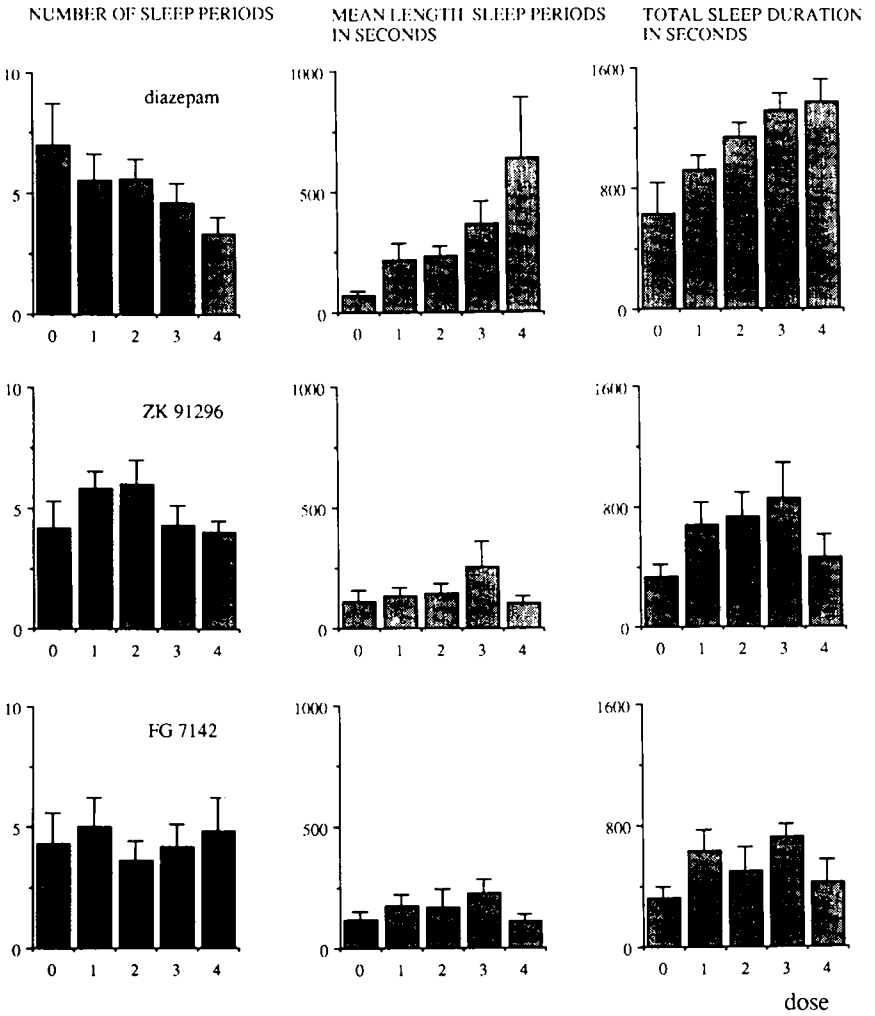

FIG. 3. Effect of diazepam (upper series of graphs), ZK 91296 (middle series) and FG 7142 (lower series) on number of sleep periods (left vertical series of graphs), on mean length of sleep periods (middle series) and on total duration (right series). All compounds are given in 5 doses (0, 1, 2, 3, and 4, in increasing order of dose, where by 0 is the zero-dose). See the Results section for statistics; all data are given in means and SEM's.

Experiment 2: Effects of the Beta-Carboline ZK 91296

ZK 91296 was suspended in 2.5% Tween-80 in 0.9% NaCl and then further diluted with distilled water. It was intraperitoneally given in a volume of 1 ml per 400 g body weight. Doses were 0 mg/kg (group 0), 0.5 mg/kg (group 1), 2 mg/kg (group 2), 8 mg/kg (group 3) and 24 mg/kg (group 4). All n's were 6.

Epileptic activity (Fig. 1). During the baseline hour, no differences in all three parameters could be detected, whereas in the first three postinjection hours several differences were found. All differences had disappeared in the fourth hour. In the number of spike-wave complexes, differences were found during the first hour, $F(4,25)=4.41$, $p<0.05$; during the second hour, $F(4,25)=5.78$, $p<0.05$ (in both cases groups 2, 3 and 4 had less complexes than groups 0 and 1); and during the third hour, $F(4,25)=3.45$, $p<0.05$, in which groups 3 and 4 had less complexes than groups 0 and 1.

For the mean duration, differences were discovered during the first hour, $F(4,25)=3.67$, $p<0.05$ (group 3 had a shorter mean duration than groups 0 and 1; and group 4 shorter than group 1); during the second hour, $F(4,25)=5.27$, $p<0.01$ (groups 3 and 4 scored shorter than groups 0 and 1); and during the third hour, $F(4,25)=2.75$, $p<0.05$ (groups 3 and 4 had a shorter mean duration than group 0).

For the total duration, differences were found in the first hour, $F(4,25)=4.89$, $p<0.01$ (groups 2, 3 and 4 had a shorter duration than groups 0 and 1); in the second hour, $F(4,25)=5.27$, $p<0.01$) (groups 2, 3 and 4 had a shorter duration than group 0); and in the third hour, $F(4,25)=3.90$, $p<0.05$ (groups 3 and 4 showed a shorter duration than groups 0 and 1).

Behavior (Fig. 2). No single significant difference for the three behavioral categories was found.

Sleep (Fig. 3). No differences could be detected in the number of sleep periods, the mean duration of these periods or in the total sleep duration, over the whole recording time.

EEG spectral analysis (Fig. 4). In neither the theta-, the

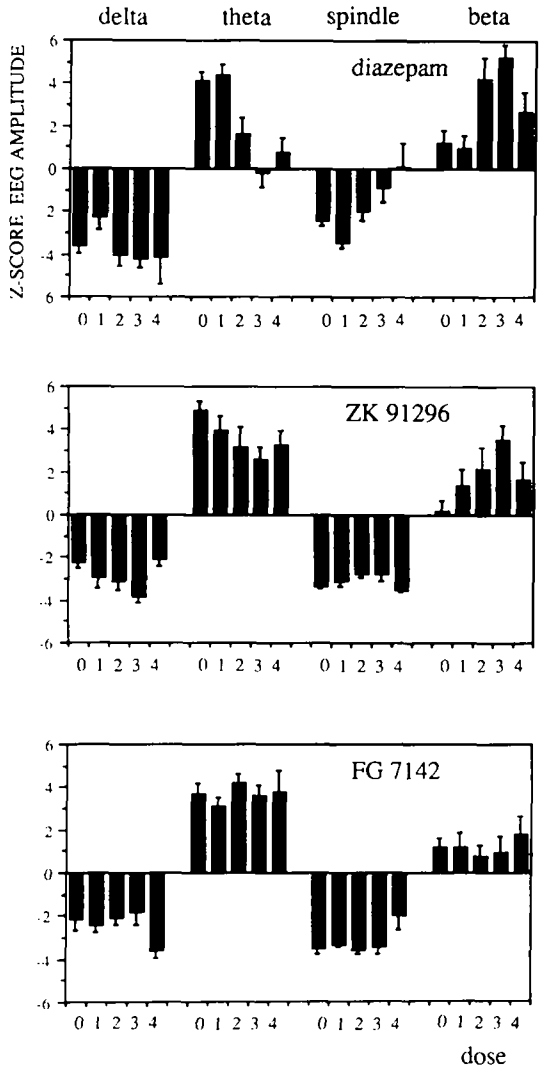

FIG. 4. Effects of diazepam (upper series of graphs), ZK 91296 (middle series) and FG 7142 (lower series) on EEG amplitude expressed in the Z-score (means and SEM's). Data are given for the delta-band (2–4 Hz) (left vertical series of graphs), the theta-band (6–10 Hz) (left middle series), the spindle-band (11–14 Hz) (right middle series) and the beta-band (15–30 Hz) (right series). Compounds are given in 5 doses (0, 1, 2, 3, and 4, in increasing order whereby 0 is the zero-dose). Statistics can be found in the Results section.

spindle-, nor the beta-band, could a significant finding be found as a result of treatment with ZK 91296. Only in the delta-band, $F(4,25)=3.79$, $p<0.05$, group 4 had less delta than groups 0, 2 and 3.

Muscle tone. Investigators were not able to feel any difference between the ZK 91296 rats (all doses) with their undrugged controls (value 0).

Experiment 3: Effects of the Beta-Carboline FG 7142

FG 7142 was suspended in 5% Tween-80. The suspension was further diluted with distilled water and homogenised. It was intraperitoneally given in doses of 0 mg/kg (group 0, n=9), 0.625 mg/kg (group 1, n=9), 2.5 mg/kg (group 2, n=9), 10 mg/kg (group 3, n=8) and 40 mg/kg (group 4, n=7).

Epileptic activity (Fig. 1). During the baseline hour there were no differences between the five groups. In the first postinjection hour, only a marginal significant effect was noticed on the mean duration of the spike-wave complexes, $F(4,37)=2.30$, $p<0.10$. The Duncan multiple range test showed that the mean duration of the complexes after 10 mg/kg and 40 mg/kg was longer than after zero-dose injection. The number of seizures of these groups were not significantly different despite the large increase (200–300%) compared to the previous hour. In a dose of 10 and 40 mg/kg, FG 7142 was able to induce strange effects on the morphology of the spike-wave complexes. Frequency of spike-wave activity, which is commonly 7–9 Hz, was slowly reduced till 3 per sec and the amplitude of the spikes increased. Such a period of an EEG convulsion, however, was not accompanied by a behavioral convulsion. It was followed by an almost flat and silent EEG. It was striking that in each animal such a convulsion occurred only once and always 10 to 20 minutes after the injection. These EEG changes were also described by Marescaux *et al.* (26).

Behavior (Fig. 2). No effects were found on automatic behavior. On exploratory behavior a significant drug effect was found, $F(4,37)=2.86$, $p<0.05$. Rats from group 4 spent less time exploring. Also on immobility, a significant effect was found, $F(4,37)=2.61$, $p<0.05$. Groups 4 and 2 showed longer immobile behavior than group 0.

Sleep (Fig. 3). On the number of sleep periods, the mean duration and on the total sleep duration, no significant differences could be detected.

EEG spectral analysis (Fig. 4). After FG 7142, a significant difference was found in the spindle-band, $F(4,39)=4.04$, $p<0.01$, in which group 4 had in the first postinjection hour more spindles than all other groups. Furthermore, a marginal significant point was found in the delta-band, $F(4,39)=2.49$, $p<0.06$. Here, group 4 showed also in the first hour following injection fewer delta waves than groups 0, 2 and 3.

Muscle tone. No indication for an effect of FG 7142 on muscle tone was obtained (value 0).

In all three experients, a post hoc test (t-test for correlated pairs of means) was performed to learn whether the zero-dose of each compound increased the number of spike-wave complexes in the first postinjection hour compared to the baseline. For diazepam, the results were, $t(5)=8.3$, $p<0.01$; for ZK 91296, $t(5)=8.6$, $p<0.01$; and for FG 7142, $t(8)=21.8$, $p<0.0001$.

DISCUSSION

First of all, an unexpected but consistent effect emerged in the experiments: the zero-dose of each compound, containing the solvent only (Tween-80 in saline), increased the number of spike-wave complexes. In a follow-up study, this finding was further explored. It turned out that of the two possible causes (stress induced by the injection or proconvulsive effects of the solvent) the latter appeared to be true. Tween-80 has proconvulsant properties (Peeters *et al.*, submitted). However, neither effects of Tween-80 on the mean duration of the spike-wave complexes were found, nor on the spectral content of the background EEG, nor on the behavior of the animals. The effects of the anticonvulsive agents, as reported in this paper, must be considered against the proconvulsive action of the control injection with Tween-80. It can be reasoned that differences among the various doses of a compound are facilitated by the increase caused by the vehicle. It seems much easier to find a signifi-

cant decrease with a compound if the score of the control group is increased. This may imply that the decrease found after antiepileptic agents is in fact an overestimation. Considering, however, that the anticonvulsive properties of diazepam and ZK 91296 are documented in a similar rat model (17,25), there is little doubt about the genuine anticonvulsant effects of these drugs.

With the full agonist diazepam, the well-known effects of the benzodiazepines could be established. A strong anticonvulsant activity was found on the number as well as on the total duration of spike-wave complexes and to a lesser degree on the mean duration of the spike-wave complexes. The sensitivity of benzodiazepines to suppress seizures and convulsions is notorious (17,25), except the effects on the mean duration. The decrease of the mean duration suggests that diazepam not only reduces the number of occurrences, but that it also has an effect on the mechanisms responsible for the termination of a spike-wave complex. It is of interest to know that other antiepileptic drugs, such as ethosuximide, valproate and trimethadione, only have an effect on the number of seizures, not on the mean duration (29).

Also, effects on behavior were found such as more immobility as the dose of diazepam increased. Viewed against the literature in which sedative effects of diazepam are well-known, the enhanced immobile behavior was interpreted accordingly. Hypnotic effects were detected as well, despite the relatively short observation period. Sleep time as well as mean duration of sleep bouts was enhanced after diazepam administration. Also, others have shown that diazepam enhances the time asleep, although light and deep slow wave sleep as well as REM sleep are differentially affected both in rats (31) and in rabbits (33).

Diazepam appeared to have a strong influence on the spectral content of the EEG. In particular, it favours the higher frequencies (the spindle- and beta-range) and reduces the theta-frequencies. Glatt *et al.* (13) noticed also in rats a reduction in the theta-range after 1.0 mg/kg of diazepam and an increase in the frequencies higher than 22 Hz. Depoortere and Granger (7) found an increase in the 12–14 Hz band after flunitrazepam in rats (0.1–10 mg/kg). Also, in man, spectral changes after benzodiazepine administration are found. Kubicki *et al.* (20) found increases in the number of spindles after various benzodiazepines. Borbély *et al.* (2) found a reduction of all low frequencies (0.25–10.0 Hz) during various sleep stages together with higher activity in the 11–14 Hz range and in the high frequency range (17–25 Hz). Our lack of effects in the delta band may be due to the fact that spectrograms were taken during passive wakefulness with a complete desynchronized EEG, while Borbély *et al.* (2) registered during sleep.

Finally, it was clear that diazepam reduced muscle tone, although only subjective estimations were made. So, in short, all classical benzodiazepine effects (anticonvulsive, sedative, hypnotic, shifts in EEG spectral content and muscle relaxation) could be established.

The effects of ZK 91296 are in sharp contrast to this; only a strong effect on the epileptic activity was found, confirming Jensen *et al.* (17) and Petersen *et al.* (30). ZK 91296 decreased seizure activity on all three parameters: total number, mean duration and, evidently, the total duration of spike-wave activity. In other models ZK 91296 was also effective: in gerbils with reflex epilepsy, both major and minor seizures (21) are reduced, whereas in the photosensitive baboon the paroxysmal EEG responses to stroboscopic stimulation were firmly decreased (27). In our hands, ZK 91296 was less potent than diazepam. This lower potency of ZK 91296 may agree with biochemical data which show that ZK 91296 needs higher benzodiazepine receptor occupancy than diazepam in order to obtain the same effects (30).

ZK 91296 shows no effects on behavior in the home cage: neither sedation nor sleep was induced. Others have also found no effects on behavior with the same drug in tests which are sensitive for the full benzodiazepine agonist (30), and in human volunteers no benzodiazepine-like effects were found (8).

We also found no changes in the EEG power spectra of the rats characteristic for diazepam after ZK 91296. Only some decrease in delta activity was found. The lack of diazepam-like effects of ZK 91296 is particularly interesting since it has been speculated that the changed frequency-amplitude spectrogram of the benzodiazepines may be related to anticonvulsive and anxiolytic properties of the drugs [e.g., (7)]. Our study, however, clearly shows that the changes in the background EEG in the theta, spindle and beta-range, are not related to the anticonvulsive effects. Whether the changes in the background EEG can be attributed to other benzodiazepine effects, e.g., the anxiolytic effects, is not completely clear yet, but worthwhile to investigate. Obviously, ZK 91296 selectively exhibits only some of the multiple effects of the full benzodiazepine receptor agonists. In this study, it only had an anticonvulsive effect and caused no sedation, hypnosis, or changes in the EEG and muscular relaxation. This lack of 'side-effects' of ZK 91296, combined with its anticonvulsive properties, seems to make further testing of this compound worthwhile in clinical trials, especially if less tolerance develops than in the case with diazepam. In first studies in healthy human volunteers, ZK 91296 failed, as expected, to induce typical benzodiazepine effects such as sedation and sleepiness (8). The specificity of ZK 91296 as a putative anticonvulsive drug needs further attention.

Almost equally specific as ZK 91296 is in suppressing epileptic activity is FG 7142 in provoking this activity. At the highest doses (10 and 40 mg), it is able to enhance the duration of the spike-wave complexes. This effect occurred despite the already higher number of spike-wave complexes induced by Tween-80. If another solvent had been used, which would not influence spike-wave activity, a more pronounced and statistically significant effect could be expected. On the other hand, it cannot be excluded that Tween-80 in combination with FG 7142 has stronger effects than FG 7142 alone. However, Stutzmann *et al.* (36) also used FG 7142 in combination with another solvent and they found no seizures after vehicle injection and spike-wave complexes after 30 mg/kg FG 7142. So it seems that the proconvulsant effect of FG 7142 in rats is solidly based. Jensen and Petersen (16) and Rossier *et al.* (32) also reported that FG 7142 in mice was proconvulsant, however, only in combination with pentylenetetrazol or picrotoxin, while Jensen *et al.* (18) showed that FG 7142 facilitated audiogenic seizures in susceptible mice. Moreover, next to the increase in the number of spike-wave complexes, we and others (34,36), found that in rats partial inverse agonists, including FG 7142, have the ability to induce an increase in seizure duration.

FG 7142 produced an increase in immobile behavior, without having an effect on sleep. Although a reduced locomotor activity or an increased immobility is difficult to interpret in terms of anxiogenic effects, it may not be completely unreasonable to presume that this enhanced motionlessness

is related to anxiogenic factors. Rats may show freezing behavior in response to fearful situations. It is of interest that Belzung *et al.* (1) found a decrease in spontaneous locomotor activity after beta-CCE, also an inverse benzodiazepine agonist, when administered in mice. Also in men (9), in cats (28) and in rats (6), anxiogenic effects of FG 7142 were shown. Sleep parameters were not changed under the influence of FG 7142. This should not necessarily be the case if FG 7142 is a true partial inverse benzodiazepine agonist (35), while an increase in wakefulness may be predicted for the full inverse agonist. FG 7142 showed, in a dose of 40 mg/kg, an increase in the spindle-frequency. This result is surprising considering that diazepam, the full agonist, also enhances the energy in this frequency band. However, it is possible that this finding must be considered as an artifact. In evaluating the raw EEG's by visual inspection, it appeared that it was difficult to find unchanged background EEG's. The 'spiky' character of the EEG is striking. However, these spikes are too small to meet the criteria for epileptic activity. Moreover, only a change was obtained for the spindle-frequency and not for the remaining freqencies, as was the case with diazepam. So it can be concluded that a partial agonist, and an inverse agonist lack the theta-reducing and beta-enhancing effects of the full agonists.

A final comment is devoted to the methodology of EEG spectral analysis. Various procedures exist for establishing effects of psychoactive drugs on the EEG (15). In almost all procedures (12,14), first a baseline frequency spectrum is made during a constant behavioral state. Next, EEG spectra are collected under drug conditions, which are subsequently subtracted from the predrug spectrogram. Reported dose-response relationships are then based on difference scores. The reason for this laborious procedure is that large interindividual differences are present in EEG characteristics. We evaded the predrug spectrogram and were, after a transformation of the mean spectrogram of each subject, still able to find the often reported diazepam effects on the spectrogram, showing that the present procedure is valid and sensitive for detecting effects of psychoactive drugs.

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