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Midazolam Suppresses Spike-and-Wave Rhythm Accompanying Three Different Models of Epileptic Seizures

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Summary

The action of a water-soluble benzodiazepine midazolam (0.1 and 1 mg/kg i.p.) was tested against three models of spike-and-wave rhythm in rats: rhythmic metrazol activity (a model of human absence seizures), minimal metrazol seizures, and epileptic afterdischarges induced by low-frequency cortical stimulation (probably models of human myoclonic seizures). Midazolam was able to reduce spike-and-wave activity in all three models, but there were quantitative differences: the lower dose was effective only against rhythmic metrazol activity, but its action against two other models was negligible, whereas the higher dose of midazolam resulted in significant effects in all three models. These quantitative differences are not sufficient to prove our hypothesis that the spike-and-wave rhythm represents different phenomena in various models. A spread of epileptic activity into brain structures other than the thalamocortical system determines the type of epileptic seizures.

Key words

Experimental epilepsy • EEG • Cerebral cortex • Rat • Midazolam

Introduction

Human electroencephalographic spike-and-wave rhythm accompanies not only absences, but also other types of seizures (Engel 1989, Gastaut and Tassinari 1975). On the other hand, spike-and-wave rhythm serves as a main tool for identification of models of absence seizures in experimental animals (Gloor 1979, Gloor and Fariello 1988, Gloor *et al.* 1990, Marescaux *et al.* 1992, Snead 1992). There are pharmacological data obtained in experimental animals indicating that this spike-and-wave rhythm in feline generalized penicillin epilepsy (Gloor 1979), in genetically determined absence seizure rats (Marescaux *et al.* 1984), and in gamma-hydroxybutyrate induced model (Snead 1978) could be suppressed by antiepileptics effective against human absences. All these models are characterized by behavioral arrest and only minimal motor phenomena (twitches of facial muscles). We started a study on pharmacological sensitivity of rhythmic spike-and-wave activity induced by low doses of pentylenetetrazol (rhythmic metrazol activity) and confirmed the above findings in this model with ethosuximide, valproate, and two benzodiazepines – clonazepam and bretazenil (Brabcová et al. 1993). The rhythmic metrazol activity is similar to the above mentioned models also in motor pattern, because arrest of locomotion and minute twitches of vibrissae or ears could be observed (Schickerová *et al.* 1989).

Another approach how to elicit spike-and-wave rhythm in experimental animals was recently demonstrated. Low-frequency cortical stimulation

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induced in rats epileptic afterdischarges characterized by an EEG spike-and-wave rhythm. It is accompanied by marked clonic seizures of facial and forelimb muscles and therefore it is highly improbable that it represents a model of absence seizures. This spike-and-wave rhythm could be suppressed by clonazepam (Kubová *et al.* 1990) and valproate (Kubová *et al.* 1996), but – in contrast to rhythmic metrazol activity – not by ethosuximide (Mareš *et al.* 1997). Just opposite data were obtained with ketamine, which suppressed cortical afterdischarges (Mareš *et al.* 1992) but not rhythmic metrazol activity (Velíšek *et al.* 1993). These findings led us to a conclusion that the presence of EEG spike-and-wave rhythm is not sufficient to classify experimental seizures as a model of absence seizures, because it accompanies models of at least two different types of human epileptic seizures. To test this hypothesis we started a systematic pharmacological study of these two spike-and-wave models. Some of these results were already published (Brabcová *et al.* 1993, Kubová *et al.* 1996, Mareš *et al.* 1997). A water soluble benzodiazepine, midazolam, was chosen to verify the action of benzodiazepines against both models in question and to exclude a possible facilitating action of solvent for other benzodiazepines (this solvent contains an amount of ethanol which is far from being negligible). In addition to the two models mentioned, the third model characterized by spike-and-wave rhythm – minimal metrazol seizures – was included. Originally, it was taken as a model of absences, but it represents rather a model of human myoclonic seizures (Löscher and Schmidt 1988). This classification is supported by a motor pattern of these seizures, which is indistinguishable from that accompanying cortical afterdischarges, and by its sensitivity to phenobarbital (Kubová and Mareš 1991). In addition to phenobarbital, clonazepam and valproate suppress these minimal motor seizures (Mareš *et al.* 1981), whereas other drugs potentiating GABAergic inhibition do not have such effect (Staňková *et al.* 1997, Kubová *et al.* 1997).

Method

The experiments were performed in 36 adult male Wistar rats three months old at the time of surgery (their body weight ranged from 250 to 280 g). Surgical procedure was the same for all three series of experiments. Epidural cortical electrodes were implanted under Nembutal anesthesia (50 mg/kg i.p.), connected to a six-plug female connector and the whole assembly was attached to the skull by means of fast curing dental acrylic. After surgery, the rats were allowed to recover for one week before the experiments started. The experiments were performed in agreement with the Animal Protection Law of the Czech Republic and were approved by the Ethical Commission of the Institute of Physiology of the Academy of Sciences of the Czech Republic.

Experiment 1: Rhythmic metrazol activity (RMA, n=11)

Four cortical recording electrodes were implanted over sensorimotor (AP=0; L=2.5 mm in relation to bregma) and visual (AP=6; L=4 mm) areas of both hemispheres. A stainless steel screw in the nasal bone was used as an indifferent electrode and it served also to anchor the electrode and connector assembly.

Each animal underwent three or four experimental sessions with an interval of at least three days between the sessions. The first session was always a control one: after a short recording of spontaneous electrocorticogram (ECoG) pentylenetetrazol (PTZ) was injected in a dose of 40 mg/kg i.p. (i.e. the dose effective in Wistar rats of our breeding colony) and recording continued for 30 min. If the 40 mg/kg dose of PTZ did not elicit numerous RMA episodes, the control session was repeated with a dose of 45 or 50 mg/kg. In the two following drug sessions, we used the effective dose of PTZ, which was established in the control experiments. In both drug sessions, midazolam (Dormicum® Roche, a gift from Hoffmann La Roche Co.) was injected intraperitoneally 10 min before PTZ so that EEG was recorded between the 10th and 40th min after the pretreatment. This time schedule was based on our data concerning the time profile of midazolam anticonvulsant action – incidence of generalized tonic-clonic seizures was decreased by midazolam even if PTZ was administered 30 min later (Kubová and Mareš 1992). The doses of 0.1 and 1 mg/kg were used; half of the animals received at first the higher dose and in the next session a lower one, the other half of rats was treated in a reverse order.

RMA was evaluated from paper EEG recordings. Latency of the first RMA and of the first generalized RMA (i.e. recorded in all four cortical areas) was measured. Furthermore, all RMA episodes between the 10th and 15th min after PTZ administration, when the activity was stabilized under control conditions, were measured, their number and total duration were counted and the average duration was calculated. In addition,

frequency of spike-and-wave complexes was counted in twenty RMA episodes to calculate an average.

Experiment 2: Minimal metrazol seizures (n=11)

Surgical preparation of the animals was the same as in Experiment 1. Experimental design was again the same as in the first experiment, only the initial dose of PTZ was 60 mg/kg and it could be increased to 65 or 70 mg/kg i.p. The dose of PTZ eliciting minimal metrazol seizures under control conditions was used in drug sessions.

Incidence of minimal seizures was recorded and if present, their latency, duration and electroclinical correlation were evaluated.

Experiment 3: Cortical epileptic afterdischarges (ADs, n=14)

Surgical preparation was the same as in Experiment 1, only the recording electrode over the right sensorimotor cortex was replaced by two silver ball stimulation electrodes, localized at coordinates AP = −1 and +1, L=2.5 mm.

Stimulation procedure: 15-s series of 1-ms biphasic rectangular pulses with a frequency of 8 Hz were used. Threshold intensity for elicitation of clear-cut afterdischarge was established at first; it varied widely in the same range as in our previous study (7.2±0.8 mA; Makal *et al.* 1993). The stimulation with this intensity was then repeated four times, an interval between the end of the AD and the beginning of subsequent stimulation being equal to 10 min. The same intensity as in the first, control session was used in the following two drug sessions. Midazolam in the doses of 0.1 or 1 mg/kg i.p. was administered 5 min before the second stimulation so that the first stimulation represented a control, predrug one even in midazolam sessions. Postdrug stimulations took thus place 5, 15, and 25 min after midazolam injection.

Incidence, ECoG pattern, and duration of ADs were evaluated. Motor correlates of stimulation as well as of ADs were registered and quantified according to a modified five-point scale of Racine (Racine 1972, Kubová *et al.* 1996).

Statistical evaluation

One-way ANOVA was used for statistical comparison of RMA parameters and minimal seizure latency with subsequent comparison according to Holm (1979). The incidence of minimal seizures was evaluated by means of the four-pole table (Fisher's F). Two-way ANOVA served for evaluation of afterdischarge duration with variables dose and number of ADs, again with post-hoc comparison. Scores of motor correlates of stimulation and ADs were evaluated by non-parametric Kruskal-Wallis test. All the computations were performed by means of BMDP programs (Dixon 1983). Data were expressed as means±SEM. The level of statistical significance was set at 5 %.

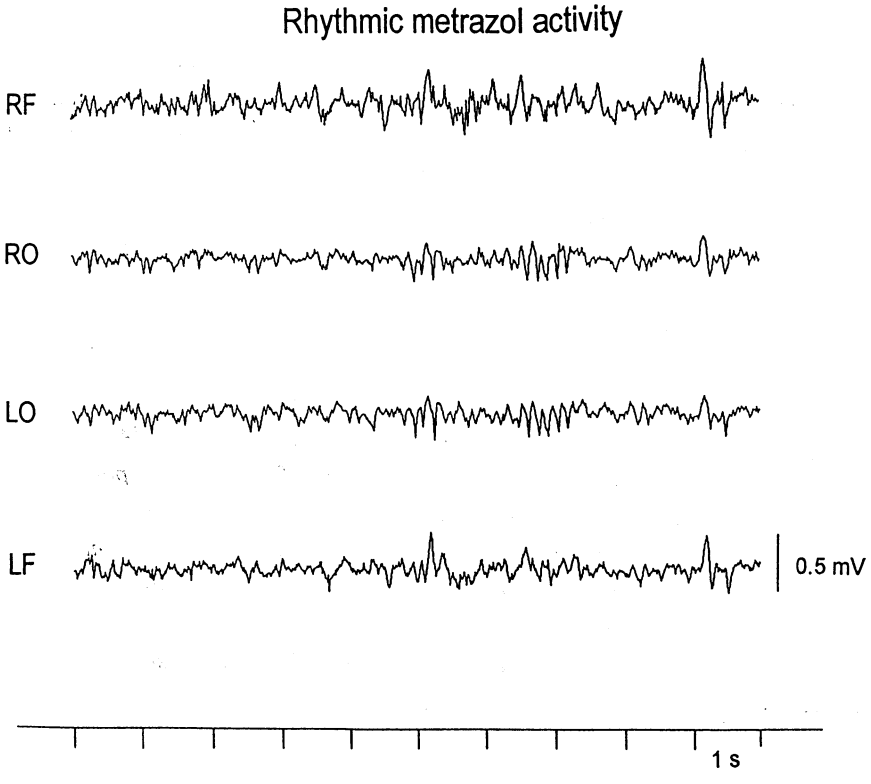

Fig. 1. *Two short episodes of rhythmic EEG activity (theta waves in occipital leads) induced by a low dose of pentylenetetrazol. Individual leads from top to bottom: RF - right frontal, RO - right occipital, LO - left occipital, LF - left frontal cortical region in a reference connection. Amplitude calibration 0.5 mV, time marks 1 s.*

Results

Experiment 1: Rhythmic metrazol activity

Pentylenetetrazol in the doses given induced marked rhythmic activity (theta waves occipitally, Fig. 1, spike-and-wave complexes frontally) in less than two minutes. The difference between the latencies to the first RMA and the first generalized RMA episode was negligible (Fig. 2). Number of RMA episodes, their total and mean duration were remarkably stable (Fig. 3). The frequency of complexes in the episodes was about 6 Hz and the variation was very small (data not shown).

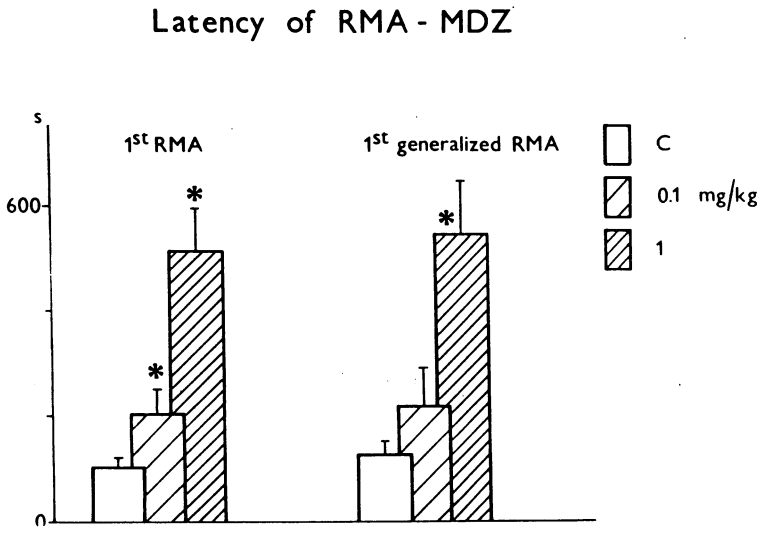

Fig. 2. *Influence of midazolam on latency of the first RMA (left) and of the first generalized RMA (right) recorded in all four cortical areas (mean ± S.E.M.). Abscissa - control recordings, midazolam 0.1 and 1 mg/kg i.p. Ordinate - latency in seconds. Asterisks denote a significant difference in comparison with controls.*

A marked dose-dependent effect of midazolam was observed. Latencies to the first RMA episode were significantly prolonged after both doses of midazolam, to the first generalized RMA only after the 1 mg/kg dose, the effect of the 0.1 mg/kg dose nearly reached the level of statistical significance (Fig. 2). Number of episodes was significantly decreased only by the higher dose, whereas the total duration was shortened by both doses of midazolam. Mean duration of episodes remained unchanged after the lower dose, but it decreased significantly after the higher dose (Fig. 2). Frequency of spike-and-wave complexes was significantly increased by both doses to 7.5±0.3 Hz and 8.1±0.1 Hz, respectively.

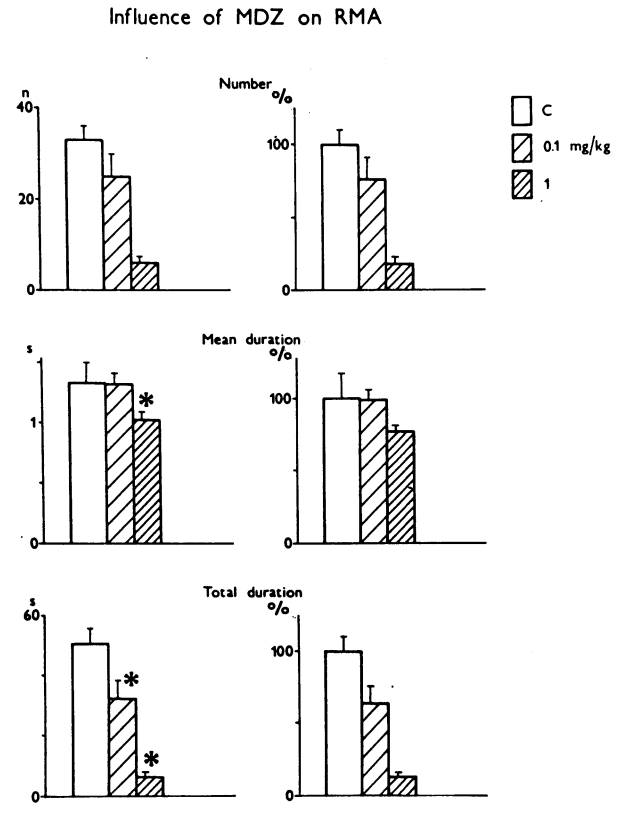

Fig. 3. *Influence of midazolam on rhythmic metrazol activity (RMA.. Upper part - number of RMA episodes; middle part - mean duration of RMA; lower part - total duration of RMA (means ± S.E.M.). Left graphs - absolute numbers; right graphs - relative numbers, control value (i.e. without midazolam) is always taken as 100%. Ordinates: upper graph - number of episodes, middle and lower graphs - duration in seconds, other details as in Figure 2.*

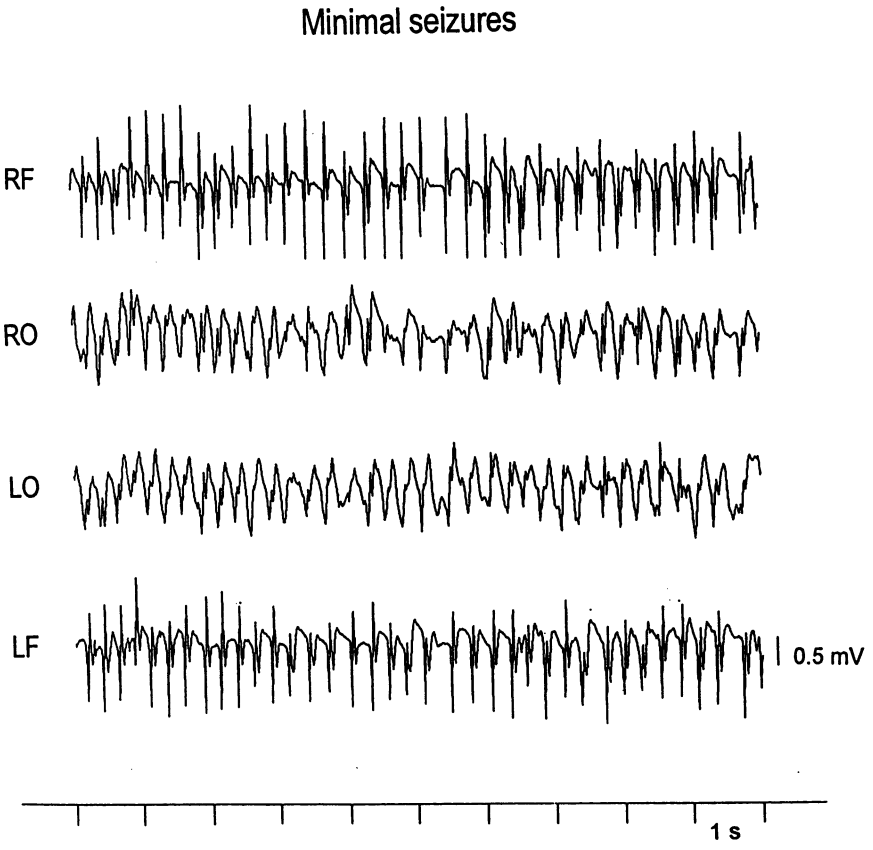

Fig. 4. *EEG recording of minimal seizures induced by pentylenetetrazol. Details as in Figure 1.*

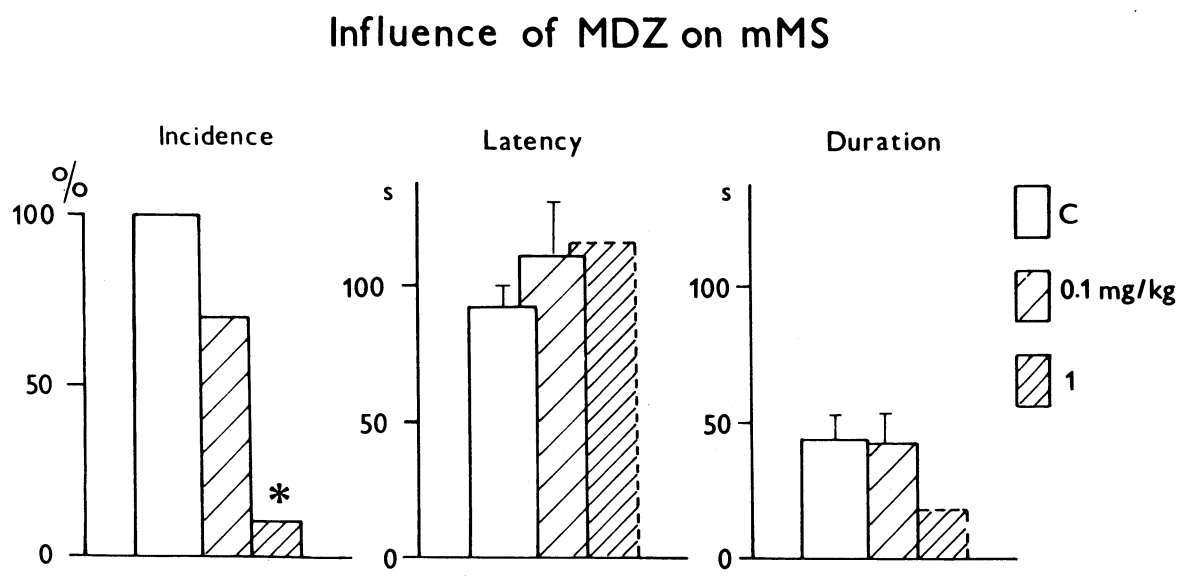

Fig. 5. *Influence of midazolam on minimal metrazol seizures. From left to right - relative incidence (control values without midazolam are taken as 100 %); latency (mean ± S.E.M.) and duration (mean ± S.E.M.). Left ordinate - percentage of animals exhibiting minimal seizures; middle and right ordinates - time in seconds. Dashed columns in the middle and right graph represent values from one rat. Other details as in Figure 2.*

Experiment 2: Minimal metrazol seizures

Motor pattern of minimal metrazol seizures elicited in control sessions consisted of clonic movements of head and forepaws lasting 43.4±9.6 s on the average. There was only a moderate tonic component in these seizures expressed as a dorsiflexion of head or torsion of

head and body. Righting reflexes were always preserved; if the rats fell down they righted immediately. These seizures were accompanied by spike-and-wave rhythm in the ECoG, this activity was better expressed in frontal (sensorimotor) than in occipital (visual) region (Fig. 4).

The lower dose of midazolam only tended to decrease the incidence of minimal seizures, leaving untouched their duration and latency (Fig. 5). The 1 mg/kg dose of midazolam blocked minimal seizures in nine out of 10 animals.

Frequency of the spike-and-wave rhythm was 3.3±2.0 Hz under control conditions and it was not changed by the 0.1 mg/kg midazolam (3.3±2.5 Hz).

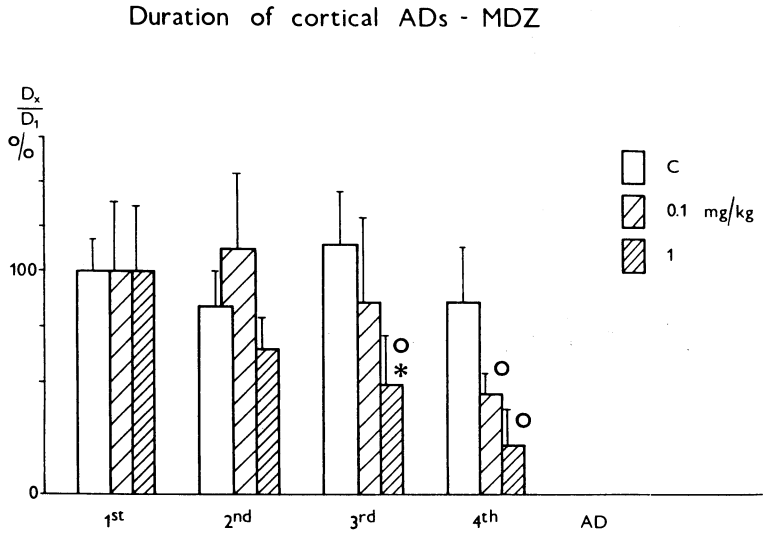

Fig. 6. *Influence of midazolam on the duration of cortical afterdischarges (mean ± S.E.M.). Abscissa - first to fourth afterdischarge (AD); ordinate - relative duration of ADs, 100% represents the duration of the first AD. Midazolam was injected between the first and the second stimulations. Circles denote significant differences in comparison with the first ADs, asterisks in comparison with the corresponding ADs in the control group. Other details as in Figure 2.*

Experiment 3: Cortical epileptic afterdischarges (ADs)

In control animals, the stimulation was accompanied by clonic movements of head and forelimbs synchronous with individual stimuli. ADs were recorded after all four stimulations. ADs were formed by spike-and-wave rhythm with the frequency of about 2 Hz. The frequency did not change with repeated elicitation of ADs. Afterdischarges were accompanied by clonic seizures the motor pattern of which was identical with that of minimal metrazol seizures described above. The only difference was in the tonic component of seizures: the dorsiflexion and torsion of the head were rather exceptional, but sometimes the animals reared (stage 4 according to Racine (1972)) as it was observed even during stimulation. Four repeated stimulations did not change significantly the duration of ADs and intensity of clonic movements (Figs 6 and 7).

There was a tendency to a dose-dependent effect of midazolam (Figs 6 and 7). The 0.1 mg/kg dose of midazolam exhibited some effects only in the fourth stimulation: the intensity of movements accompanying stimulation was lower than under control conditions and the ADs were shortened in comparison with both the first AD in the midazolam session and the fourth AD in the control session. Higher dose of midazolam exhibited a marked effect against ADs and their motor correlates. The third and fourth ADs exhibited significantly shorter duration in comparison to predrug afterdischarge or corresponding ADs in control session and the intensity of motor seizures accompanying all postdrug ADs was lower than that of the corresponding ADs in the control session. Movements related to stimulation were not significantly different from those in control session, but their intensity was lower than that of the first, predrug stimulation. Frequency of spike-and-wave discharges progressively increased in sessions with the 1 mg/kg dose of midazolam from the predrug value 2.1±0.4 Hz up to 3.3±2.6 Hz in the fourth AD, but due to high variability this difference did not reach the level of statistical significance in spite of the fact, that 12 out of 14 animals exhibited an increase in frequency.

Discussion

Our results demonstrated similar action of midazolam against all three models of epileptic seizures characterized by spike-and-wave rhythm in the EEG. This is in agreement with our data on clonazepam (Brabcová *et al.* 1993, Kubová *et al.* 1990). These data suggest the identical mechanism of generation of spike-

and-wave rhythm in the three models tested. Analysis of spike-and-wave rhythm demonstrated thalamocortical mechanism of its generation (Gloor *et al.* 1990) with a marked role of thalamic reticular nucleus (Avanzini *et al.* 1992). This nucleus contains GABAergic inhibitory cells, which synchronize the input to the cerebral cortex (Jones 1975, Mulle *et al.* 1986, Spreafico *et al.* 1988). The role of GABAergic system is not simple; benzodiazepines, facilitating GABAergic inhibition (Haefely 1989), are able to block spike-and-wave rhythm (e.g. our present data), but GABA agonists augment this rhythm (Vergnes *et al.* 1984). The suppression of oscillations in the thalamocortical system by benzodiazepines is probably due to their facilitatory action on recurrent inhibition in the thalamic reticular nucleus and resulting decrease of inhibitory output onto relay neurons (Huguenard and Prince 1994).

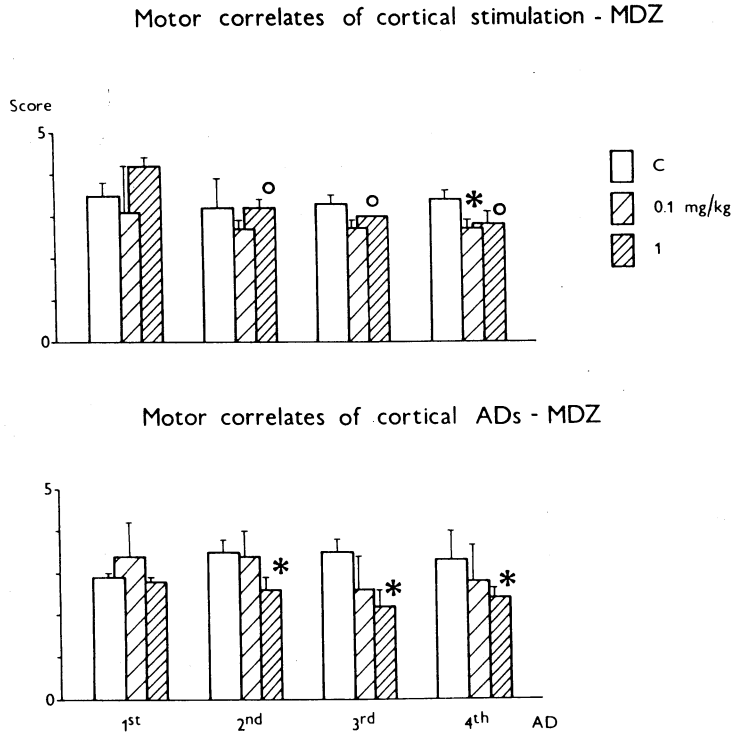

Fig. 7. *Influence of midazolam on score (mean ± S.E.M.) of clonic movements accompanying stimulation (upper part) and clonic seizures accompanying afterdischarges (lower part). Abscissa - first to fourth stimulation or AD. Ordinates - five-point scale according to Racine (1972). Details as in Figures 2 and 6.*

The identity of spike-and-wave rhythm in our models was denied by our data with ethosuximide, which was found to block rhythmic metrazol activity (Brabcová *et al.* 1993) and spike-and-wave rhythm accompanying minimal metrazol seizures, but to be inefficient against spike-and-wave type of cortical afterdischarges (Mareš *et al.* 1997). In addition, there is a different effect of ethosuximide on rhythmic metrazol activity and minimal metrazol seizures in immature rats (Mareš 1998). These doubts were further substantiated by results with ketamine, which exhibited opposite effects, i.e. blocking cortical afterdischarges (Mareš *et al.* 1992) leaving intact rhythmic metrazol activity (Velíšek *et al.* 1993) and minimal metrazol seizures (data only on motor seizures – Velíšek *et al.* 1989). Even more marked difference was found with $GABA_B$ antagonist CGP 35348 – rhythmic metrazol activity was efficiently suppressed but cortical afterdischarges were potentiated (Mareš and Šlamberová, submitted for publication). On the other hand, spike-and-wave rhythm as well as other rhythmic EEG activities are generated by thalamocortical system (recruiting and augmenting responses, rhythmic afterdischarges as the late component of cortical evoked potentials, sleep and barbiturate spindles – Steriade and Deschenes 1984). What has to be different, is the spread of this activity into other parts of the brain – especially into the motor system. Rhythmic metrazol activity is accompanied by very feeble motor phenomena (minute jerks of facial muscles – Schickerová *et al.* 1989), whereas the other two models by intense clonic seizures involving muscles of the head and forelimbs at least (Kubová *et al.* 1990, Swinyard *et al.* 1989). It means that the low dose of pentylenetetrazol (40 mg/kg in our rats) was able to set into action only the thalamocortical generator of spike-and-wave rhythm, to arrest a locomotor activity and to prevent a highly motivated behavior (Schickerová *et al.* 1989), but it was not capable to activate markedly the motor system. In contrast, in the case of minimal

metrazol seizures and cortical afterdischarges of the spike-and-wave type, the epileptic activity had to spread into motor system to set into action a generator of minimal seizures which was localized into basal forebrain structures (Browning and Nelson 1985, 1986). Thus both higher doses of pentylenetetrazol and stimulation of sensorimotor cortex resulted in a widespread activation of the central nervous system. What are the reasons for such a difference, remains to be addressed in future. The difference in frequencies of spike-and-wave rhythm found in Experiments 1 (about 6 Hz) and Experiments 2 and 3 (2 and 3 Hz, respectively) might be important for the penetration of epileptic activity into other structures as well as for pharmacological reactivity. The transformation of rhythmic afterdischarges ("thalamocortical spindles") into epileptic spike-and-wave rhythm is characterized by a marked decrease in frequency (Gloor 1984). In any case, it is untenable to take spike-and-wave rhythm as the only measure to define models of human absence seizures. This conclusion is supported by the clinical data demonstrating that spike-and-wave rhythm may occur not only in human absence seizures but also in myoclonic and other types of epileptic syndromes (Dreifuss 1990, Janz 1990, Roger *et al.* 1990).

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