RAPID COMMUNICATION

Effects of Alprazolam on a Model of Human Absences — Rhythmic Metrazol Activity in Rats

H. KUBOVÁ¹², P. MAREŠ¹³

1Institute of Physiology, Academy of Sciences of the Czech Republic, Departments of 2Pharmacology and 3Pathophysiology, 3rd Medical Faculty, Charles University, Prague

Received April 6, 1993
Accepted June 10, 1993

Summary
Two doses of alprazolam (0.1 and 0.5 mg.kg⁻¹) were tested against a model of human absences — rhythmic EEG activity elicited by low doses of pentylenetetrazol (35 mg.kg⁻¹) — in 10 unrestrained rats with implanted cortical electrodes. Alprazolam delayed the onset of epileptic EEG activity, decreased the number of rhythmic episodes and shortened the total duration of rhythmic activity in a dose-dependent manner. The average duration of episodes of rhythmic activity remained unchanged; other benzodiazepines studied previously were able to influence this measure.

Key words
Epileptic seizures — EEG — Pentylenetetrazol — Alprazolam — Rat

Benzodiazepines are used in the treatment of various types of human epilepsies-absences, atypical absences, Lennox-Gastaut syndrome and myoclonic epilepsies (Burdette and Browne 1990). They are also efficient against many models of epileptic seizures in experimental animals (for review Schmidt 1989, Sato 1989), including models of human absences (Marescaux et al. 1984, Brabcová et al. in press). A new wave in benzodiazepine research was initiated by the possibility to find the broad spectrum from full agonists through antagonists to full inverse agonists (Haefely 1989). Partial agonists are of special interest (Haefely et al. 1990). We had the possibility to test partial agonists clonazepam and bretazenil (Brabcová et al. in press) as well as a full agonist midazolam (Kubová and Mareš in preparation) against spike-and-wave episodes induced by low doses of pentylenetetrazol (rhythmic metrazol activity, RMA) which represent one of the models of human absences (Schickerová et al. 1989). Alprazolam according to experimental data represents an intermediate step between full agonists (midazolam, diazepam) sharing with them the distribution of binding sites in the central nervous system and partial agonists (clonazepam, bretazenil) exhibiting similar high potency in proconflict test (Giusti et al. 1992). Because of this intermediate position we decided to test this benzodiazepine against rhythmic metrazol activity as the first step in delineation of its anticonvulsant activity.

Experiments were performed in 10 male albino rats of the Wistar strain. Body weight of the animals at the beginning of experiments ranged from 250 to 280 g. Electrode implantation was made under pentobarbital anaesthesia (50 mg.kg⁻¹ i.p.). Cortical silver ball electrodes were placed on the undamaged dura mater over sensorimotor and visual regions of both hemispheres (coordinates AP = 0, L=2.5 mm and AP=6, L=4 mm in relation to bregma). An indifferent electrode was localized on the nasal bone. The electrodes were fixed to a connector and the whole electrode arrangement was cemented to the skull by dental acrylic. The animals were allowed to recover one week after the operation, then the experiments started. Each animal passed four recording sessions: the first, control one, then two drug sessions and as the last session a control with administration of solvent. After a short recording of spontaneous EEG pentylenetetrazol (PTZ, Sigma) was injected intraperitoneally in a dose of 35 mg.kg⁻¹ and EEG and behaviour of the rats was recorded for 30 min. In the
drug sessions after control EEG recording alprazolam (a gift from Chemopharma, Ústí nad Labem) was injected intraperitoneally in a dose of 0.1 or 0.5 mg.kg\(^{-1}\), EEG was recorded between 8 and 10 min after alprazolam. Half of the animals received the lower dose of alprazolam in the second session and the higher dose in the third session, the other five rats were given the two doses in a reverse order. The fourth session was again a control one, the animals were injected with solvent (propyleneglycol, ethanol, water) in the amount corresponding to the higher dose of alprazolam. Pentylenetetrazol was administered 10 min after alprazolam or solvent and recording proceeded again for 30 min.

The latencies of the first episode of rhythmic metrazol activity (RMA) and of the first RMA registered in all four cortical regions (generalized RMA) were measured. Between the 10th and 15th min after PTZ injection, all episodes of RMA were measured and they were quantified by their number and duration, so that the total and average duration of RMA could be calculated. The results were statistically evaluated by means of analysis of variance. Multiple comparisons were made by means of paired t-test with reduced level of significance of single tests according to Holm's sequential procedure (Holm 1979). The level of statistical significance was set at 5 %. Absolute as well as relative (i.e. the control value for each measure in each animal was taken as 100% and drug and solvent values were related to this basis) values were used for evaluation. There were no differences between the two methods of calculations.

### Latency of RMA - Alprazolam

![Fig. 1](image.png)

Latencies (mean ± S.E.M.) of the first RMA (left) and of the first generalized RMA (right). Abscissa - control groups and different doses of alprazolam (see explanation of individual columns in the right upper corner); ordinate - latencies in seconds.

The first RMA as well as the first generalized RMA appeared under control conditions in less than two minutes after PTZ injection (Fig. 1), the difference between the two latencies was very small. Solvent tended to increase these latencies, but statistical significance was not reached. Alprazolam prolonged both latencies in a dose-dependent manner, the values obtained after the 0.5 mg.kg\(^{-1}\) dose were significantly different from those after solvent. One rat did never exhibit generalized RMA after administration of the higher dose of alprazolam.

Quantification of RMA periods between the 10th and 15th min after PTZ (Fig. 2) demonstrated a lack of effect of solvent on number of episodes whereas alprazolam decreased their number again in a dose-dependent manner, both doses resulted in a significant change. An average duration of RMA episodes was not changed by alprazolam in comparison with solvent control. On the other hand, the total duration of RMA was significantly and dose-dependently decreased by alprazolam reflecting thus a decrease in the number of episodes.
Our results demonstrate the efficacy of alprazolam in a model of human absences—rhythmic metrazol activity. This activity as well as other rhythmic cortical EEG activities are generated by thalamocortical mechanisms (Steriade and Deschenes 1984, Gloor and Fariello 1988). We have demonstrated this mechanism for RMA in previous papers (Pohl and Mareš 1983, 1986). The key role in generation of rhythmic thalamocortical activities is played by the thalamic reticular nucleus (Mulle et al. 1986, Avanzini et al. 1992), where GABAergic inhibition was demonstrated (Houser et al. 1980).

The action of alprazolam is basically similar to other benzodiazepines tested against RMA—clonazepam and bretazenil (Brabcová et al. in press) as well as midazolam (Kubová and Mareš unpublished results). In contrast to the basic similarity of action, there is one difference between alprazolam and all other benzodiazepines tested: alprazolam was unable to shorten the mean duration of RMA episodes in spite of the fact that the dose of 0.5 mg.kg⁻¹ was given meanwhile the 0.1 mg.kg⁻¹ dose was the highest dose of clonazepam and bretazenil. This result signifies that alprazolam is able to suppress RMA in an "all-or-nothing" manner, i.e. to block the appearance of RMA without changing the structure of individual rhythmic episodes. The same effect was described for ethosuximide (Brabcová et al. in press). Benzodiazepines exert their anticonvulsant action through specific receptors, tightly bound to GABAₐ receptors (for review Haefely 1989). It might be thus hypothesized that the mode of action of alprazolam on the supramolecular complex GABAₐ receptor/benzodiazepine receptor/chloride ionophore might be different from those exhibited by clonazepam and bretazenil.

Before the clinical trials with alprazolam as an antiepileptic drug could start it will be necessary to study if the tolerance to its anticonvulsant effects develops or not. This unwanted side effect (Nutt 1990) render impossible the use of nearly all benzodiazepines for long-term treatment of epilepsies (Burdette and Browne 1990).
References


Reprint Requests
H. Kubová, PharmD, PhD, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic.