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## SHORT COMMUNICATION

# Clobazam Exerts an Anticonvulsant Action in Immature Rats

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## Summary

The anticonvulsant action of 1,5-benzodiazepine clobazam was studied in 12-, 18-, and 25-day-old rats. Cortical epileptic afterdischarges (ADs) elicited by rhythmic electrical stimulation of the sensorimotor cortical area were used as a model in animals with implanted electrodes. As far as the duration of ADs is concerned, clobazam in doses of 1 or 5 mg/kg i.p. blocked the progressive increase with repeated stimulations in all age groups and the higher dose significantly shortened ADs in 25-day-old rats. The intensity of movements accompanying stimulation was decreased only by the 5 mg/kg dose in 25-day-old animals, whereas clonic seizures were less intense after both doses in 12- and 25-day-old rat pups. Clobazam exerted an anticonvulsant action at all the developmental stages studied; the lower efficacy in 18-day-old rats (described also for clonazepam) remains to be analyzed.

**Key words**
Clobazam – Epileptic afterdischarges – Cortex – Rat – Ontogeny

The mechanism of action of benzodiazepines (BDZs) was elucidated by the discovery of benzodiazepine receptors and their structural and functional coupling with $\gamma$-aminobutyric acid$_A$ ($GABA_A$) receptors (Mohler and Okada 1978, Squires and Braestrup 1978). BDZs modulate the $GABA_A$ receptor current by increasing the opening frequency of chloride channels without altering channel conductance or duration of opening (Macdonald and Kelly 1993).

Benzodiazepines have a traditional place in antiepileptic therapy. The clinical use of BDZs can be divided into two categories. First, in the acute treatment of seizures as drugs of choice in *status epilepticus* and also in some cases of febrile seizures. Second, the BDZs are utilized in long-term therapy of certain seizure types, primarily in the paediatric population (Rogawski and Porter 1990, Shorvon 1995). The marked anticonvulsant action of BDZs is compromised by the rapid development of tolerance, hence new drugs are being synthesized. One of these newer BDZs is clobazam. In contrast to classical 1,4-benzodiazepines, it has nitrogen atoms in the heterocyclic ring in the 1,5-position. (Trimble and Robertson 1986). There are some differences between the effects of 1,5- and 1,4-benzodiazepines. A greater therapeutic potential and the lower incidence of side-effects was described for clobazam when compared with 1,4-benzodiazepines (Trimble and Robertson 1986). Therefore, clobazam is used as adjunctive therapy in resistant cases of epilepsies (Fisher and Blum 1995, Shorvon 1995).

BDZs exhibit a potent anticonvulsant action in a wide variety of animal seizure models. They are particularly effective against seizures induced by electroshock (Stéru *et al.* 1986), various chemoconvulsants (pentylentetrazol, bicuculline, picrotoxin, strychnine), in kindled seizures and in absence seizures (Shenoy *et al.* 1982, Rogawski and Porter 1990). Unfortunately, tolerance to the anticonvulsant effects was also described for clobazam (Vajda *et al.* 1987, De Sarro *et al.* 1992, Ramsey-Williams *et al.* 1994). All these data were obtained in adult experimental animals. Because of the clinical use of BDZs in paediatric patients where the incidence of tolerance may be lower, our laboratory started to study the anticonvulsant effects of benzodiazepines in immature rats. The effects of clonazepam and midazolam on pentylenetetrazol-induced motor seizures are stable during ontogenesis of rats (Kubová and Mareš 1989, 1992). The same was found for a model of myoclonic seizures induced by electrical stimulation of the cerebral cortex (Kubová *et al.* 1993); this model of cortical epileptic afterdischarges (ADs) where EEG and motor phenomena may be evaluated simultaneously, was chosen for our present study.

Three age groups of Wistar albino rat pups of both sexes were used (12, 18 and 25 days old). Animals were kept at 12/12 h light-dark conditions under constant temperature (24±1 °C). The day of birth was taken as 0, the litters were culled to 8–10 pups. Flat silver electrodes (approximately 1 $mm^2$) were implanted epidurally under ether anaesthesia; two stimulation electrodes over the right sensorimotor, frontal cortical area (coordinates AP = −1 and +1, L = 2 mm in relation to the bregma), recording electrodes over the left sensorimotor region (AP = 0, L = 2 mm), right and left visual, occipital areas. The coordinates for frontal electrodes were the same in all age groups, those for occipital electrodes were calculated from the adult values of AP = 6 and L = 4 mm. The recalculation was based on the actual bregma-lambda distance, the background value of 8 mm was taken for adult rats. An indifferent electrode was inserted into the nasal bone. All electrodes were cemented to the skull by dental acrylic. Recording started after one hour recovery from ether anaesthesia.

Fifteen-second series of biphasic rectangular pulses of 1 ms duration and 8 Hz frequency were generated by a constant current stimulator. The intensity of electric stimulation equalled the threshold for elicitating clear-cut ADs (from 2.5 to 5 mA). The stimulation series were repeated four times at 10 min intervals. Clobazam (a gift from Hoechst Marrion Roussel) was dissolved in a three-component solvent (propylenglycol, alcohol and distilled water in a ratio of 5:2:3). The freshly prepared solution was administered intraperitoneally 5 min after the end of the first AD in doses of 1 or 5 mg/kg, respectively. Control animals received the solvent in a corresponding volume, i.e. 1 ml/kg. Each age and dose group consisted of 8–10 animals.

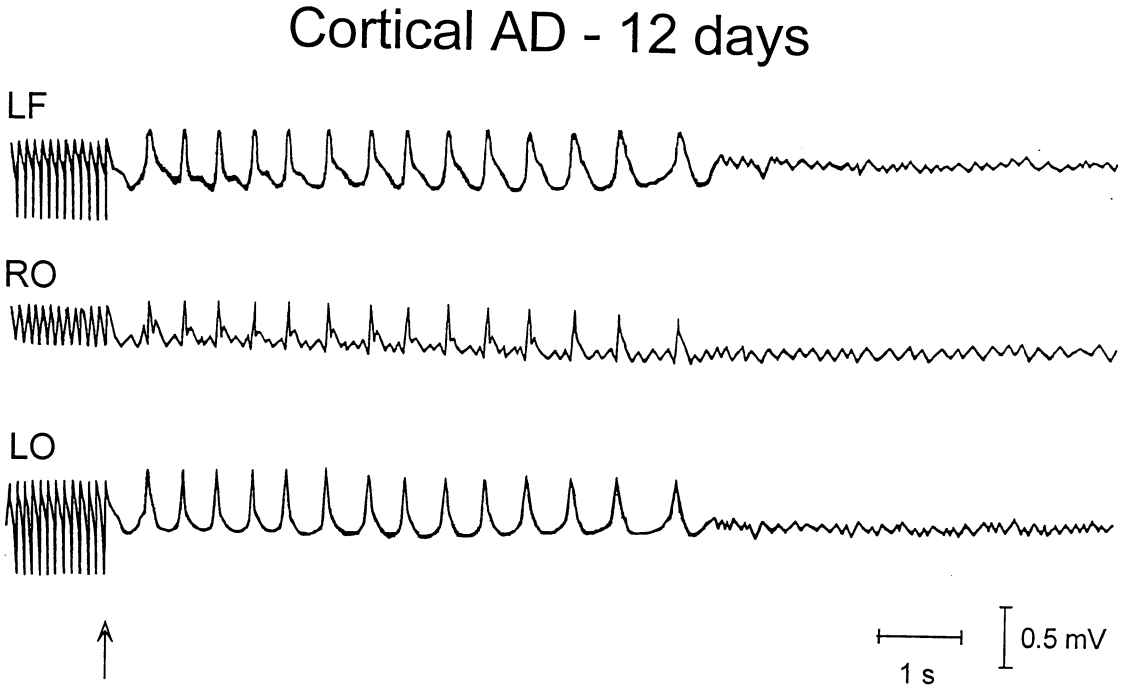


**Fig. 1.** *Electrocorticographic recording of an afterdischarge in 12-day-old rat. Individual leads from top to bottom: left frontal (LF), right occipital (RO) and left occipital (LO) in reference connections. An arrow denotes the end of stimulation. Amplitude calibration 0.5 mV, time mark 1 s.*

The electroencephalogram was recorded in both the reference and bipolar connection during stimulation, during ADs and one minute after their termination. Motor phenomena accompanying both stimulation and ADs were marked into the EEG recordings. The duration of ADs was evaluated and motor phenomena were quantified using a five-point scale of Racine (1972) modified only in point 1 in which all phenomena not synchronous with the stimuli and/or sharp EEG graphoelements were classified. Points 2 to 5 remained unchanged (head jerks, forelimb clonus, rearing, rearing and falling). The duration as well as the motor scores were statistically evaluated by means of three-way ANOVA with factors: age – levels 12, 18, 25 days, dose – levels control, clobazam 1 mg/kg, clobazam 5 mg/kg, repeated measurements – levels first to fourth stimulation. As there were no significant third order interactions found but all other interactions were significant at the 5 % level, less complex two-way analysis for each age level as well as for each dose level was performed. Tukey's significant difference method was used for multiple comparison. Some specific contrasts were computed to test the planned comparison and linear trend. Computations were performed by means of BMDP7D and BMDP2V computer programmes (Dixon 1988).

The first cortical stimulation always evoked an afterdischarge in all rats. These afterdischarges were characterized by EEG spike-and-wave rhythm in 18- and 25-day-old rats and by rhythmic sharp delta waves in the youngest group (Fig. 1). Duration of the first ADs was: 19.8±2.9 s (mean ± S.E.M.) in 12-day-old pups; 10.5±1.1 s in 18-day-old rats; 10.0±1.6 s in 25-day-old animals, i.e. the first AD in 12-day-old pups was significantly longer than in the two older groups. Stimulation was always accompanied by rhythmic movements of their head and forelimbs synchronous with individual stimuli. Rearing and falling of animals was occasionally observed. The motor correlate of ADs comprised identical clonic movements synchronous with sharp EEG elements. There were no significant differences among the three age groups as concerns the intensity of stimulation-bound movements or clonic seizures.

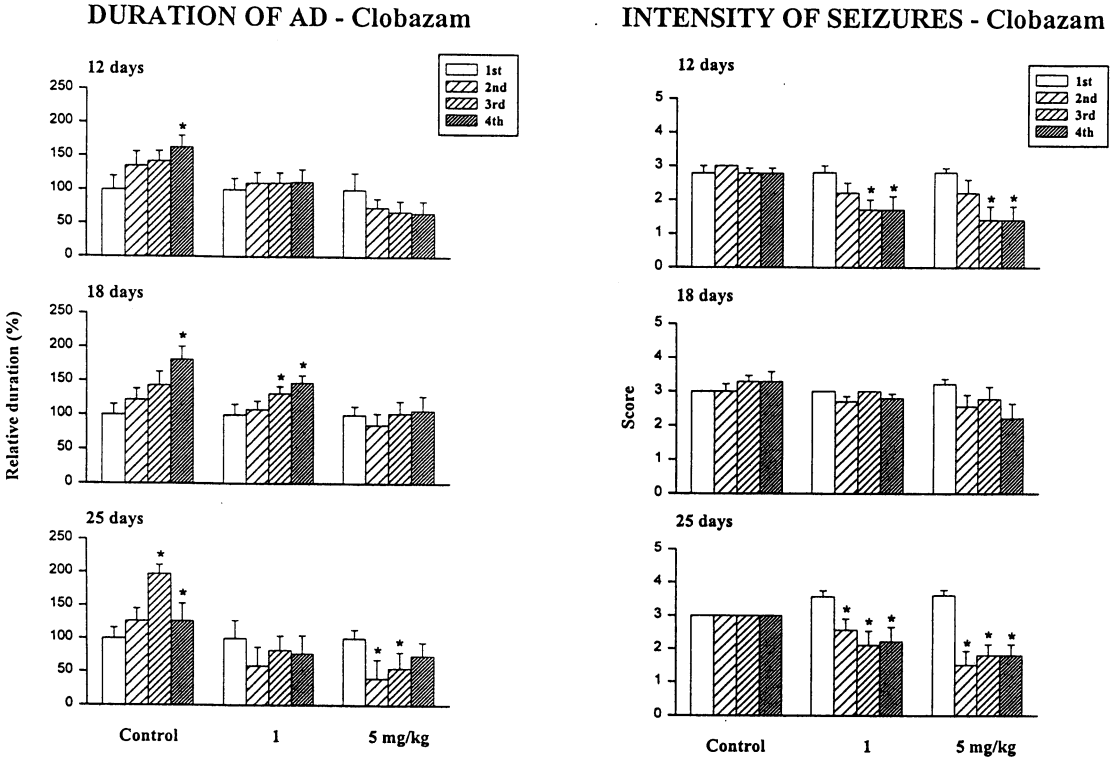


**Fig. 2a.** *Effects of clobazam on duration of afterdischarges (mean + S.E.M.) in rats 12, 18 and 25 days old (from top to bottom). Abscissa – control groups, doses – 1 mg/kg, 5 mg/kg; ordinates – relative duration of the ADs, the first AD was taken as 100 %. Explanation of individual columns is on the right. Asterisks denote a significant difference in comparison with the corresponding first stimulation, circles with the corresponding stimulation in the control group.*
**b.** *Effects of clobazam on movements accompanying afterdischarges (mean intensity + S.E.M.). S.E.M. is missing in such cases in which all animals exhibited the same seizure severity (point 3). Ordinates – five-point scale of intensity of motor phenomena according to Racine (1972).*

*Twelve-day-old rats:* Repeated stimulations led to a progressive increase in the duration of ADs in the control group. Both doses of clobazam blocked this progressive prolongation of ADs (Fig. 2a) and decreased the intensity of seizures during the third and fourth ADs (Fig. 2b). The 5 mg/kg dose tended to shorten all postdrug ADs. The intensity of movements accompanying stimulation did not change after administration of any dose (data not shown).

*Eighteen-day-old rats:* Repeated stimulations again resulted in a progressive prolongation of ADs in the control group. The higher dose blocked the progressive increase in duration of ADs (Fig. 2a) but neither dose influenced the intensity of movements accompanying stimulations or ADs. The higher dose tended to decrease the intensity of seizures but the difference was not statistically significant.

*Twenty-five-day-old rats:* Repeated stimulations also increased the duration of ADs under control conditions in this age group. Both doses of clobazam blocked the progressive prolongation of ADs. In addition, there was a shortening of the duration of the second and third ADs after the higher dose of clobazam (Fig. 2a). The dose of 1 mg/kg led to a suppression of the intensity of seizures (Fig. 2b) but failed to influence the intensity of movements accompanying electrical stimulation. The higher dose (5 mg/kg) decreased the intensity of both movements accompanying stimulation and clonic seizures.

Clobazam exhibited an anticonvulsant action in our model. It blocked the progressive prolongation of ADs with repeated stimulations in all age groups. In addition, it significantly shortened the ADs in 25-day-old rats. These results are qualitatively the same as with clonazepam and midazolam but the effective doses of the two classical benzodiazepines were lower – the 1 mg/kg and lower doses of both significantly shortened the ADs. On the contrary, clobazam exhibited a better ratio between the anticonvulsant and sedative effect than clonazepam (Stéru *et al.* 1986). This anticonvulsant action of midazolam and especially clonazepam and clobazam was best expressed in 25-day-old rats (Kubová *et al.* 1993). The effects of all benzodiazepines on the intensity of clonic movements accompanying stimulation were negligible, whereas clonic seizures were attenuated by clobazam and clonazepam in the 12- and 25-day-old rats, and by midazolam in all age groups (present results and Kubová *et al.* 1993). At the moment, we do not have an adequate explanation for the lack of the effect of clonazepam and clobazam on motor seizures in 18-day-old rats. It is neither a general feature of benzodiazepines (midazolam was effective - Kubová *et al.* 1993) nor drugs potentiating GABAergic inhibition (valproate and phenobarbital suppressed these clonic seizures in all age groups - Polášek *et al.* 1996). The density of benzodiazepine receptors in rat brain increases up to postnatal day 21 (Braestrup and Nielsen 1978, Candy and Martin 1979). Both basic types of benzodiazepine receptors (the difference is due to the existence of $alpha_1$ or $alpha_2$ subunits – Prichett *et al.* 1989) are present in sufficient number in 18-day-old rats because there is a steep increase of BZ1 type of receptors during the second postnatal week (Lippa *et al.* 1981). This is why the lower efficacy of clobazam in this age group might be due to more subtle developmental changes in subunit composition of $GABA_A$ receptor complexes.

Our data led us to the conclusion that there are no marked differences between clobazam and 1,4-benzodiazepines in the model of myoclonic seizures. The stable anticonvulsant action of clobazam at different stages of postnatal maturation of rats may predict the safe clinical use of clobazam in paediatric neurology.

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