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Stable Anticonvulsant Action of Benzodiazepines During Development in Rats

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Abstract—The anticonvulsant action of midazolam and clonazepam was studied in 168 immature rats in three age groups (12, 18 and 25 days old). Epileptic after-discharges of the spike-and-wave type accompanied by clonic seizures of facial and forelimb muscles induced by stimulation of sensorimotor cortex were used as a model. The solvent used for clonazepam exhibited a tendency to anticonvulsant action in 12-day-old rats. On the contrary, a proconvulsant action was seen in 25-day-old animals. The action of both benzodiazepines was identical and did not change substantially during development. The highest dose used (1 mg kg^{-1}, i.p.) shortened the duration of epileptic after-discharges, the two lower doses (0·1 and 0·02 mg kg^{-1}, i.p.) suppressed the progressive prolongation with repeated stimulations seen under control conditions. Motor correlates of stimulation remained practically uninfluenced by the two benzodiazepines, myoclonic seizures accompanying epileptic after-discharges were attenuated by the highest dose of both drugs.

Clinical data has suggested that seizure disorders predominantly affect children (Hauser & Kurland 1975); incidence of convulsion is highest in the first year of life. Generally, pharmacotherapy of seizure disorders during ontogenesis is complicated by gradual and uneven maturation of all factors involved in the action of antiepileptic drugs (Morselli 1983).

Although benzodiazepines are used exceptionally in therapy of epilepsy in adult patients (Robertson 1986; Trimble & Robertson 1986; Treiman 1990), pharmacological screening is normally in adult animals (Haefely et al 1981; Dreifuss & Sato 1982). Previous ontogenetic studies from our laboratory have demonstrated quantitative differences in the anticonvulsant actions of the benzodiazepines clonazepam (Kubová & Mareš 1989), midazolam (Kubová & Mareš 1992) and nitrazepam (Mareš & Seidl 1982). An important qualitative change was described by Barr & Lithgow (1983) as a paradoxical convulsant effect in new-born rats. However, other workers suggest that these convulsant effects in immature animals are non-epileptic myoclonic jerks (Nutt & Little 1986; Smythe et al 1988), which form a component of the normal development of rat pups (LaPointe & Nosal 1979).

In the present work we studied the effects of two benzodiazepines on epileptic after-discharges elicited by rhythmic stimulation of the sensorimotor cortex in freely-moving, developing rats. This model enabled us to study three phenomena: movements accompanying stimulation (i.e. a direct activation of the motor system), EEG after-discharges, and motor correlates of after-discharges. Clonazepam was chosen as a typical antiepileptic benzodiazepine, and midazolam as a water-soluble benzodiazepine allowing the exclusion of the solvent commercially used for clonazepam.

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Materials and Methods

Experiments were performed in 168 albino Wistar rats. Three age groups were used: 12, 18 and 25 days old, the day of birth counted as 0.

Flat silver electrodes were implanted epidurally under ether anaesthesia—two stimulation electrodes over the right sensorimotor region (AP −1 and +1; L 2 mm) and recording electrodes over the left sensorimotor region (AP 0; L 2 mm) as well as over both occipital regions. The coordinates for occipital electrodes were calculated from those for adult rats (AP 6; L4 mm) on the basis of the bregma-lambda distance. A neutral electrode was placed on a nasal bone. All electrodes were fixed to the skull by means of fast curing dental acrylic. Recording started after 1 h recovery after the surgery.

After-discharges (ADs) were elicited by 15 s stimulation of bipolar rectangular pulses (duration 1 ms, frequency 8 Hz). The stimulation current used was usually 3 mA; i.e. the suprathreshold intensity for all three age groups studied (unpublished data). Four series of stimulations were applied in all animals; the interval between the end of an AD and beginning of the next stimulation was 10 min. Five minutes after the end of the first AD, clonazepam (Roche) or midazolam (Roche) in doses of 0·02, 0·1 and 1 mg kg^{-1}, or vehicle (96% ethanol: propylene glycol: water, 2:5:3) (in an amount corresponding to the highest dose of clonazepam) was injected intraperitoneally. Control animals were stimulated without any treatment.

Electrocorticograms were recorded in reference as well as bipolar connections during stimulation, during ADs and for 1 min after the end of stimulation. The pattern and duration of ADs were evaluated. Motor correlates were observed and recorded during stimulation as well as during ADs and their incidence was counted. To quantify the intensity of motor phenomena, the five-point scale of Racine (1972) was used.

Statistical analysis of the data was performed using Biomedical Programs (BMDP) (Dixon 1988). The duration of ADs was evaluated by analysis of variance with grouping

factors dose (four levels – controls, and three doses tested) and age groups (three levels). Different control groups were used for the two drugs; a solvent group for clonazepam, an untreated group for midazolam. Simple effects in the model were computed from restricted models under the conditions of fixed levels of some factors (Winer 1971). Logarithmic transformation of AD values was used to stabilize variance in cells (Box & Cox 1964). The levels of significance were set at 5% and were adjusted according to Holm's multiple test procedure (Holm 1979).

Results

Control animals

Twelve- and 18-day-old control rats exhibited a significant progressive prolongation from the first to the fourth AD. In 25-day-old animals only the third and fourth ADs were significantly longer than the first. Treatment with solvent alone resulted in a linear significant increase in duration with repeated stimulations in all three age groups. Comparison of the control, untreated animals and those treated with the solvent demonstrated only one difference in duration of the ADs: ADs were longer in 25-day-old rats given solvent than in untreated controls after the second, third and fourth stimulation, i.e. the proconvulsant action of solvent was found in this age group. There were no differences in motor correlates of stimulation or of ADs in any age group.

Midazolam-treated rats

The highest dose of midazolam resulted in a significant shortening of all three postdrug ADs in comparison with controls in 12- and 18-day-old rats (Fig. 1). In addition, the second and third ADs were shorter than the equivalent controls after the lowest dose of midazolam in 18-day-old rats. The duration of ADs in 25-day-old animals did not differ from those in controls. Comparing the first predrug AD with the second, i.e. the AD elicited 5 min after midazolam administration, the 1 mg kg^{-1} midazolam significantly shortened the second AD in all age groups. In addition, a significant shortening of the second AD was recorded after the 0·1 and 0·02 mg kg^{-1} doses in 25-day-old animals. In other age groups the two lower doses of midazolam blocked the significant prolongation of ADs with repeated stimulations.

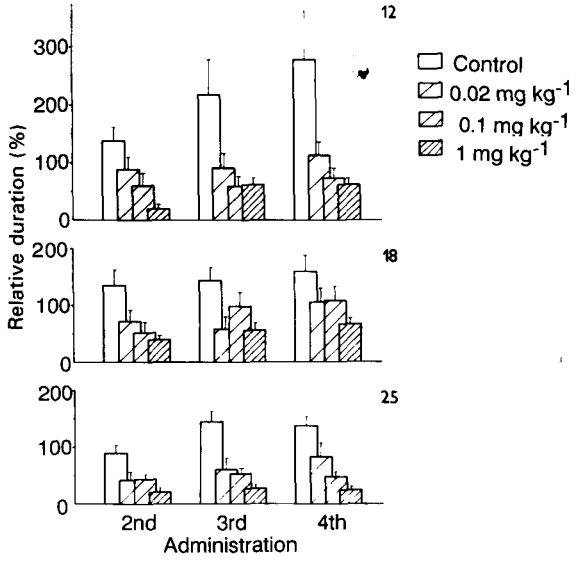

FIG. 1. Effects of midazolam on relative duration of cortical afterdischarges (mean + s.e.m.) in rats 12, 18 and 25 days old. Individual columns denote untreated controls and three groups injected with different doses of midazolam.

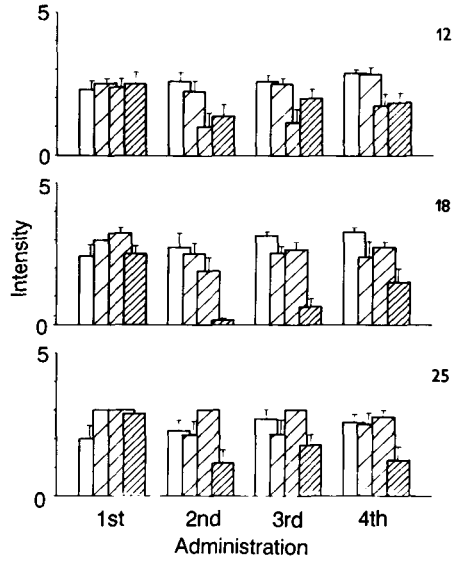

FIG. 2. Effects of midazolam on intensity of motor correlates of afterdischarges (mean + s.e.m.). Score according to Racine (1972). Columns without s.e.m. denote identical values of score (stage 3) in all animals in the group. For key, see Fig. 1.

Motor correlates of stimulation remained stable with repeated stimulation. The only effect of midazolam was a decrease in the mean score of the third stimulation after the highest dose in 18-day-old animals.

Motor correlates of ADs also did not change with repeated stimulation. They always tended to be lower than mean scores of motor phenomena accompanying stimulation. Midazolam, 1 mg kg^{-1}, significantly diminished the average score of the third ADs in all age groups; 0·1 mg kg^{-1} was effective in 12- and 18-day-old rats (Fig. 2).

Clonazepam-treated rats

Duration of the ADs was significantly shorter than in solvent-treated controls after a dose of 1 mg kg^{-1} clonazepam in 12- and 18-day-old rats and after the 0·1 mg kg^{-1} dose in 18-day-old rats (Fig. 3). The highest dose of clonazepam led to significant shortening of the second AD in comparison with the first, predrug AD in all age groups; the same effect was observed with the 0·1 mg kg^{-1} dose in 18-day-old rats and with the 0·02 mg kg^{-1} dose in 25-day-old rats. All doses abolished the progressive lengthening of ADs with repeated stimulations seen in controls.

Motor correlates of stimulation were not influenced by repeated stimulation, the action of clonazepam reached the level of statistical significance only exceptionally.

Motor correlates of ADs again did not change with

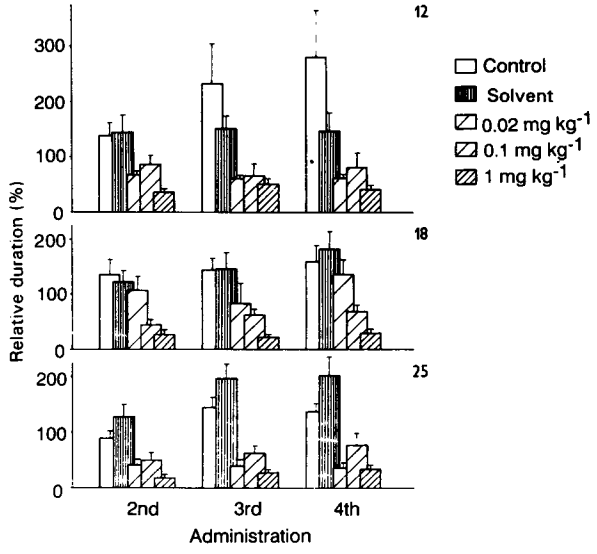

FIG. 3. Effects of clonazepam on relative duration of cortical after-discharges (mean + s.e.m.).

repeated stimulations (Fig. 4). The two higher doses of clonazepam diminished the intensity of motor phenomena in all age groups especially during the third ADs. Clonazepam significantly decreased the average score of the second AD in comparison with the corresponding first, predrug AD in 12- and 18-day-old rats.

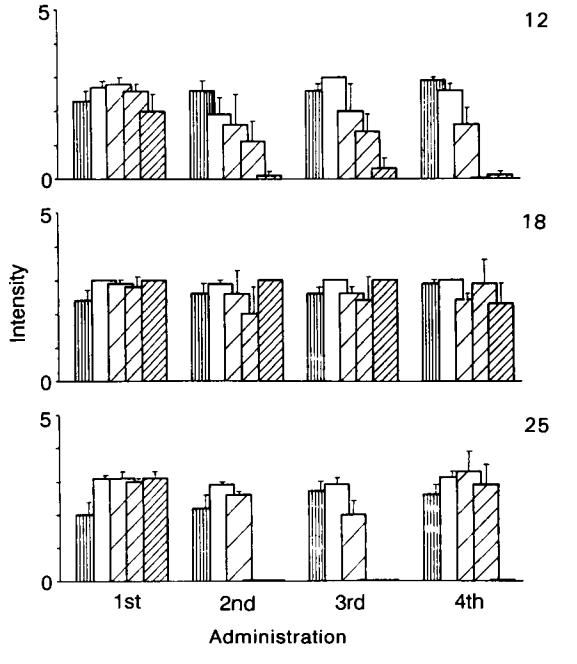

FIG. 4. Effects of clonazepam on intensity of motor correlates of after-discharges (mean + s.e.m.). The key is as in Fig. 3.

Discussion

Duration of ADs was influenced by both benzodiazepines in a dose-dependent manner. Lower doses blocked only the progressive prolongation of ADs, the highest dose used (1 mg kg^{-1}) was able to shorten ADs significantly. Motor correlates of stimulation remained practically unchanged, and only higher doses of clonazepam and midazolam were able to diminish the intensity of motor correlates of ADs. This difference might be explained by a relative insensitivity of direct activation of the motor system by electrical stimulation of the cortex to benzodiazepines in comparison with more moderate and thus vulnerable activation of this system by an epileptic AD which might be influenced by the doses used.

Cortical ADs might be taken as a model of myoclonic seizures—their motor pattern is similar to that of minimal, clonic seizures elicited by pentylenetetrazol, representing a model of myoclonic seizures (Löscher & Schmidt 1988). There is also a similarity of pharmacological profiles in rats (unpublished data) and in patients (De Vivo 1983).

EEG pattern of ADs was not changed qualitatively after the administration of the benzodiazepines. These results together with the effects of benzodiazepines on duration of ADs agree with previous findings in adult animals in acute experiments (Heidler et al 1988) as well as in freely moving rats (Kubová et al 1990).

Clonazepam and midazolam in low doses, which were unable to shorten the ADs duration, blocked progressive prolongation of ADs. This prolongation was described after repeated stimulation by Moshé & Albala (1983) and Holmes & Thompson (1987) in prepubescent animals as a sign of rapid kindling. This progressive lengthening was best expressed in our experiments in 12-day-old rats, where postictal depression is still lacking (Mareš et al 1992). Gradual prolongation of AD duration can serve as a simple model of progressive epileptogenesis; expressive effects of both benzodiazepines on this phenomenon correlate with their activity in kindled animals (Albright & Burnham 1980). The mechanisms underlying progression of ADs are unknown, but they must involve spread of epileptic activity in the central nervous system and thus they are highly sensitive to benzodiazepines. On the contrary, shortening of the duration of ADs by higher doses of both midazolam and clonazepam in comparison with control rats can be interpreted as an effect on generation of epileptic activity. The generator of the EEG spike-and-wave rhythm is probably localized in the thalamocortical system (Gloor et al 1990). The differentiated action on the generation and progression of epileptic ADs clearly speaks in favour of different mechanisms of these two epileptic phenomena.

Motor phenomena were also influenced differently: movements accompanying stimulation (elicited by direct activation of the motor system) were resistant to the action of benzodiazepines whereas the same motor pattern observed during ADs could be attenuated by benzodiazepines. Myoclonic seizures representing a correlate of ADs might be taken as a sign of spread of epileptic activity from the hypothetical generator of ADs to the generator of this type of motor seizure localized by Browning (1985) in the basal forebrain. The expressed action of benzodiazepines might again signify the effect on generalization of seizure activity (Sato 1989).

We did not find any difference between the effects of the two benzodiazepines in our model when compared with the

appropriate control groups. Clonazepam, as Rivotril, contains an amount of ethanol which could not be neglected; the injection of the vehicle resulted in a significant increase in AD duration in 25-day-old rats; this effect was completely abolished by clonazepam. On the contrary, 12-day-old rats exhibited a tendency to shortening of ADs after solvent; these findings call attention to the possible changes of the effects of ethanolic solvent during development, which would need to be analysed in a further series of experiments. Motor correlates were not influenced by solvent in any age group.

The stable anticonvulsant action exhibited by the two benzodiazepines studied at different stages of maturation is in agreement with data on development of benzodiazepine receptors in rat. These receptors could be detected in the brain of rat foetuses at embryo day 15 and the density comparable with that in adult animals is attained at postnatal day 21 (Braestrup & Nielsen 1978; Candy & Martin 1979). Type 1 benzodiazepine receptors, which are associated with the anticonvulsant and anxiolytic effects of benzodiazepines (Lippa et al 1979) are sparse at birth but increase steeply during the second postnatal week in rats (Lippa et al 1981), and our youngest group studied (rat pups 12 days old) had this type of benzodiazepine receptor in an amount sufficient for implementation of anticonvulsant action.

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