Physiol. Res. 48: 389-394, 1999

Dizocilpine Pretreatment Suppresses the Action of Hypoxia on Hippocampal Epileptic Afterdischarges in Immature Rats

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Received February 15, 1999
Accepted June 16, 1999

Summary

Effect of dizocilpine (0.5 mg/kg i.p.) on epileptic afterdischarges elicited by low-frequency electrical stimulation of the dorsal hippocampus was studied in rat pups aged 12 and 18 days. Repeated elicitation of afterdischarges (ADs) in control animals resulted in a progressive increase of the duration of ADs in both age groups. Dizocilpine (MK-801) injected after the first afterdischarge suppressed this prolongation in 12-day-old rats only. Hypobaric hypoxia (simulated altitude of 9000 m for one hour) led to a marked prolongation of the first afterdischarge in both age groups with a tendency to shorter ADs after repeated stimulations. Dizocilpine potentiated this tendency in 12-day-old rat pups so that it became statistically significant. Administration of dizocilpine before hypoxia prevented the increase in duration of the first afterdischarge in both age groups.

Key words

Epileptic seizures • Ontogeny • Hypoxia • Dizocilpine • Rats

Introduction

Dizocilpine (MK-801) was found to exhibit a protective action against hypoxia as well as ischemia in experimental animals and *in vitro* (Gill *et al.* 1987, Meldrum 1990, Domenici *et al.* 1993, Yassin and Scholfield 1994). In comparison with the data from adult animals, the results from immature animals are less numerous (McDonald *et al.* 1987, Hattori *et al.* 1989, Olney *et al.* 1989) in spite of the fact that the resistance against hypoxia changes substantially with age (Jílek 1970). In addition, N-methyl-D-aspartate receptors, the site of noncompetitive antagonistic action of dizocilpine (Kemp *et al.* 1987, MacDonald and Nowak 1990), are more sensitive in immature than in mature rats (Schoepp *et al.* 1990, Mareš and Velíšek 1992). We have described marked changes of hippocampal epileptic afterdischarges after the exposure of rat pups to hypobaric hypoxia – namely significant prolongation of the first afterdischarge and loss of progressive lengthening of afterdischarges with stimulations repeated in 10 min intervals (Marešová and Mareš 1991). We therefore decided to use this model to study the possible protective effects of dizocilpine against hypoxia.

Methods

All experiments were approved by the Ethical Committee of the Institute of Physiology AS CR (Prague) and were in agreement with guidelines of the Animal

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Protection Law of the Czech Republic. Experiments were performed in 97 male Wistar rats aged 12 and 18 days. Under ether anesthesia, stimulation electrodes were implanted into the dorsal hippocampus. The adult coordinates AP=3, L=3 and H=3 mm according to Fifková and Maršala (1960) were recalculated for immature rats on the basis of the bregma-lambda distance which was taken as 8 mm for adult rats. The coordinates used in our experiments were similar to those given by Vožeh *et al.* (1979). Flat silver epidural recording electrodes were placed over sensorimotor and visual areas of both hemispheres. An indifferent electrode was placed on the nasal bone. After at least one hour of recovery, the animals were fed with 5 % solution of sucrose, their righting and placing reflexes being examined before the onset of the experiments. During the recovery as well as during the recordings, the animals were placed on a pad heated to 34 °C, i.e. the temperature of the nest.

Stimulation was performed with rectangular stimuli of 0.3 ms duration in 15 s series at a frequency of 8 Hz. The threshold for hippocampocortical evoked potentials in the ipsilateral sensorimotor area was always found and then a twofold value was used throughout the experiments.

In all experimental groups, 12- and 18-day-old rats were used. In Experiment 1, animals not exposed to hypoxia were stimulated four times at 10 min intervals between the end of an afterdischarge and the beginning of the subsequent stimulation. This group served as the controls. Rats in the other groups received an intraperitoneal injection of dizocilpine one minute after the end of the first afterdischarge. In Experiment 2, the rats were exposed for one hour to simulated altitude of 9000 m after the recovery following the surgical preparation. Fifteen minutes after the end of this exposure, stimulation was started and the same groups as in Experiment 1 were investigated. In Experiment 3, only one group of rats was used –surgically prepared animals were allowed to recover, then they were administered dizocilpine and exposed to hypobaric hypoxia (again simulated altitude of 9000 m) which started 10 min later. Fifteen minutes after the end of the exposure, four stimulation series were applied at 10 min intervals. Dizocilpine maleate (a generous gift of Research Institute of Pharmacy and Biochemistry, Prague), freshly dissolved in physiological saline in the concentration of 0.5 mg/ml, was administered intraperitoneally in a dose of 0.5 mg/kg. Control animals did not receive any injection because pilot experiments had demonstrated that an injection of physiological saline had no influence on afterdischarges in rat pups.

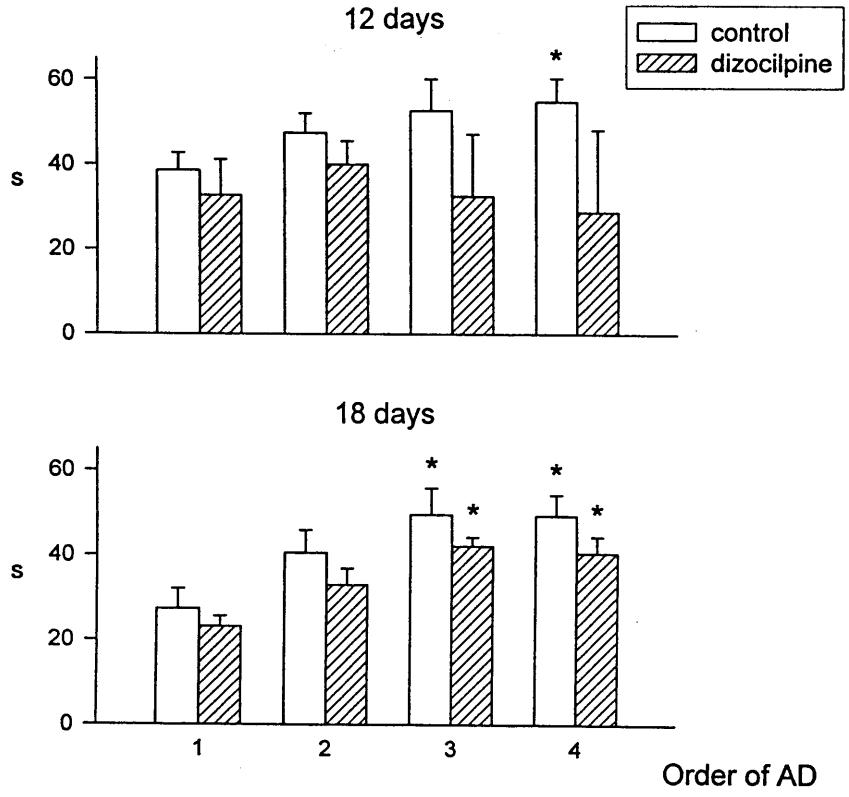

Fig. 1. *Influence of dizocilpine (0.5 mg/kg i.p. injected between the first and the second stimulation) on the duration of hippocampal afterdischarges (mean ± S.E.M.) in 12- and 18-day-old rats. Abscissa: the first to fourth afterdischarge of control and experimental rats (see upper right corner). Ordinate: duration in seconds. An asterisk denotes a significant difference in comparison with the appropriate first afterdischarge.*

After establishing the stimulation voltage spontaneous EEG was recorded for at least two minutes, then the first stimulation started. EEG was continuously recorded during the stimulation, during an afterdischarge and one minute after the end of the afterdischarge. The duration of afterdischarges was measured and statistically evaluated by means of two-way analysis of variance with a subsequent multiple comparison according to Holm (1979). $P<0.05$ value was taken as the level of statistical significance.

Each age and dose group consisted of 8 to 11 rat pups. Animals were used once only. After the end of the experiment, they were sacrificed by an overdose of ether anesthesia and the localization of hippocampal electrodes was histologically controlled. Only animals with electrodes in the dorsal hippocampus were included in this study.

Results

Experiment 1

The duration of ADs in 18-day-old animals not exposed to hypoxia was not influenced by dizocilpine and both untreated and dizocilpine-treated rats exhibited a significant prolongation of ADs with repeated stimulation (Fig. 1). On the contrary, in 12-day-old animals, in which a similar progressive increase in duration was observed under control conditions, dizocilpine in the dose used (0.5 mg/kg) was able to block this prolongation, so that the duration of afterdischarges remained at the same level during the whole session. In addition, dizocilpine increased the variability of results in this age group.

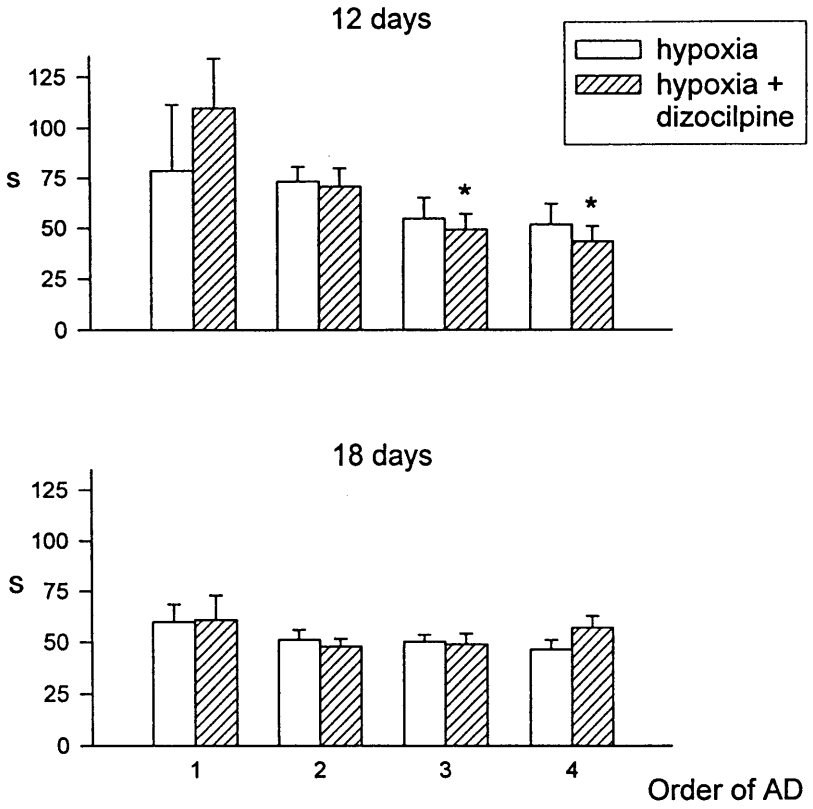

Fig. 2. *Effect of dizocilpine on hippocampal afterdischarge duration in rats exposed to hypoxia. Hypoxia + dizocilpine group represents animals injected with dizocilpine between the first and second stimulation. For details see Figure 1.*

Experiment 2

Hypoxia markedly prolonged duration of the first afterdischarge in both age groups (Figs 2 and 3). With repeated stimulation there was no significant change in duration of ADs in control 12- and 18-day-old rats and in dizocilpine-treated 18-day-old rats. On the contrary, dizocilpine significantly shortened the third and fourth ADs in comparison with the first (predrug) response in 12-day-old rat pups. However, no significant difference was found when these ADs were compared with the corresponding 3rd and 4th ADs in the control animals.

Experiment 3

Dizocilpine tended to restore the original duration of the first ADs in both age groups to the control, non-hypoxic level; statistical significance was

observed in 18-day-old animals only. The high variability of results in 12-day-old animals resulted in the absence of a significant difference between dizocilpine-pretreated youngs and non-exposed controls or rat pups exposed to hypoxia (Fig. 3).

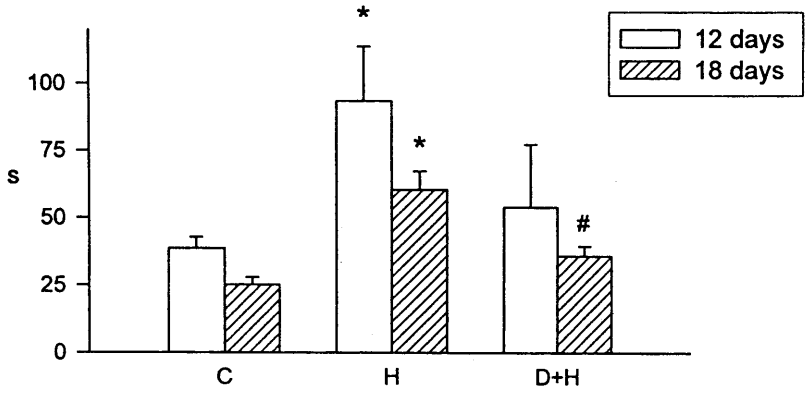

Fig. 3. *Effect of hypoxia and dizocilpine on the duration of the first afterdischarge (mean ± S.E.M.) in 12- and 18-day-old rats (see upper right corner). Abscissa: C = controls. H = rats exposed to hypoxia. D+H = rats pretreated with dizocilpine and then exposed to hypoxia. Ordinate: duration in seconds. Asterisks denote significant differences in comparison with the control group. # significant difference in comparison with the hypoxia group.*

Dizocilpine administered before the exposure to hypoxia resulted in a significant shortening of all afterdischarges in comparison with hypoxia-exposed control 18-day-old animals (this group was taken from Experiment 2). At the same time, a progressive increase in duration, seen in animals not exposed to hypoxia, failed to appear in this group. The third afterdischarge was significantly shorter than the corresponding afterdischarges in both control and dizocilpine-treated nonexposed animals. Twelve-day-old rat pups pretreated with dizocilpine did not exhibit significant changes in comparison with hypoxia-exposed controls due to a high variability of the duration of the first ADs (Fig. 4).

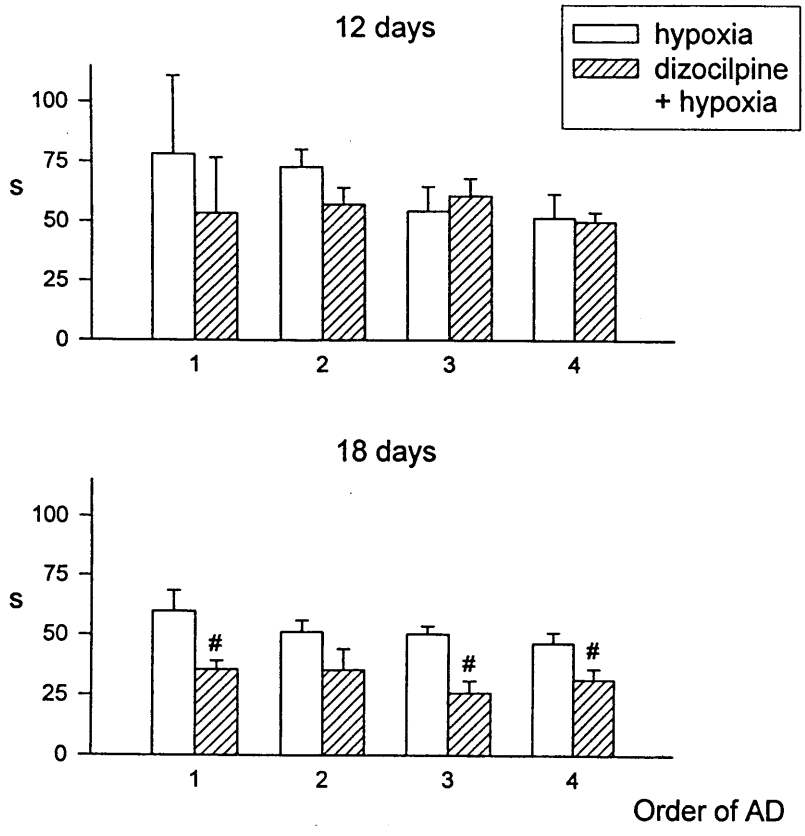

Fig. 4. *Effect of dizocilpine pretreatment on the effect of hypoxia on hippocampal afterdischarge duration. # denotes significant difference between hypoxia and dizocilpine + hypoxia groups. Other details as in Figures 1 and 2.*

Discussion

The dose of dizocilpine used in our experiments (0.5 mg/kg) was high enough to exhibit anticonvulsant action against pentylenetetrazol-induced generalized tonic-clonic seizures in immature as well as adult rats (Velíšek *et al.* 1991). Plasma and brain levels were measured in adult rats and high levels were found up to two hours after the administration of a 1 mg/kg dose (Hucker *et al.* 1983). Locomotor hyperactivity was also maintained after a 0.3 mg/kg dose for at least two hours (Dai *et al.* 1995). Therefore, the stimulations in our experiments were performed during the period of the marked effect of dizocilpine.

The effect of dizocilpine on hippocampal afterdischarges in control rats (not exposed to hypoxia) was only moderate in 12-day-old rats (blockade of progressive prolongation of afterdischarges with repeated stimulation) and was missing in the 18-day-old group. The difference between these two age groups is in agreement with our data on ketamine action against hippocampal as well as cortical epileptic afterdischarges – it exhibited a very feeble action, if any, in 18-day-old rats (Mikolášová *et al.* 1994, Kubová and Mareš 1995), but in contradiction with the effects of dizocilpine on cortical epileptic afterdischarges, as well as on pentylenetetrazol-induced motor seizures. Here, the dose of 0.5 mg/kg suppressed cortical afterdischarges (Šlamberová and Mareš, submitted) and generalized tonic-clonic seizures (Velíšek *et al.* 1991) in both age groups. Surprisingly, dizocilpine pretreatment before hypoxia was more effective in 18- than in 12-day-old rat pups. Suppression of the progressive prolongation of afterdischarges with repeated stimulation observed in 12-day-old rat pups is in agreement with data on dizocilpine and kindling in both adult (Giorgi *et al.* 1991, Minabe and Emori 1992) and immature rats (Trommer and Pasternak 1990), as well as with the finding that dizocilpine binds to the activated state of the NMDA regulated channel (Foster and Wong 1987).

The effects of hypoxia were the same as in our previous experiments: prolongation of the first afterdischarge with a failure of progressive increase with repeated stimulations (Marešová and Mareš 1991). Dizocilpine was found to block the most marked effect of hypoxia, i.e. prolongation of the first afterdischarge, thus demonstrating its protective action against hypobaric hypoxia. This finding is in agreement with the data which reported that dizocilpine is able to prevent hypoxic-ischemic damage of the brain in both adult and immature animals (Gill *et al.* 1987, McDonald *et al.* 1987, Meldrum 1990). In addition, dizocilpine exhibited a marked anticonvulsant action (shortening of afterdischarges) in our animals exposed to hypoxia. This effect was present in spite of a markedly compromised binding of dizocilpine after hypoxia (Hoffman *et al.* 1994). A possible effect on progressive epileptogenesis, i.e. prolongation of afterdischarges with repeated stimulations, could not be measured in our model, because of the absence of this phenomenon in hypoxia-exposed rat pups. The neuroprotective action, which was described in immature animals when dizocilpine was administered after hypoxic-ischemic insult (Hattori *et al.* 1989), is in agreement with the preserved action of dizocilpine in our 12-day-old rats given the drug after the exposure to hypoxia and first stimulation. A detailed analysis of dizocilpine action after hypoxia should be the next step in our studies concerning the protective action of NMDA antagonists.

Acknowledgements

This study was supported by grant No. 1504/94/309 of the Grant Agency of the Czech Republic. The authors would like to thank M. Langmeier, M.D., Ph.D. for histological control of the localization of deep electrodes.

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