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SHORT COMMUNICATION

The Action of Pramiracetam on Consequences of Hypobaric Hypoxia is Only Moderate

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Summary

The possible protective action of pramiracetam, a pyrrolidinone nootropic drug, against hypobaric hypoxia was studied in two age groups of immature rats with implanted electrodes. Epileptic afterdischarges induced by hippocampal stimulation were used as a measure of hypoxic damage. Pramiracetam did not substantially change these afterdischarges in 12- and 18-day-old rat pups which were not exposed to hypoxia. Hypobaric hypoxia (simulated altitude of 7000 m for one hour) led to prolongation of the first afterdischarge in both age groups. Pramiracetam did not influence this prolongation in 12-day-old rats. The first afterdischarge was shortened significantly in 18-day-old animals but not to the level of rats not exposed to hypoxia. The afterdischarges elicited by repeated stimulations (four times at 10 min intervals) did not differ in pramiracetam-treated and control rats.

Key words

Pramiracetam – Hypoxia – Epileptic afterdischarges – Hippocampus

Hypoxia exhibits a marked action on epileptic afterdischarges (ADs) elicited by hippocampal stimulation in immature rats (Marešová and Mareš 1991). After exposure to 1-hour hypobaric hypoxia (simulated altitude of 7000 m) hippocampally elicited afterdischarges were significantly prolonged in both 12- and 18-day-old rat pups in comparison with age-matched controls. When the stimulation was repeated at 10-min intervals, the afterdischarges did not increase in duration in contrast to control animals where the progressive prolongation of afterdischarges could be reliably elicited.

These changes in AD duration were used as a measure for testing of the protective action of pramiracetam, N-(2-(diisopropylamino)ethyl)-2-oxo-1-pyrrolidineacetamide (Research Institute for Pharmacy and Biochemistry, Prague), a congener of piracetam (Poschel *et al.* 1983). Our hypothesis that this type of nootropics may also protect against hypobaric hypoxia was based on the ability of nootropic drugs to restore brain functions after insults such as electroshock, scopolamine administration or hypoxia (Martin and Haefely 1993).

Experiments were performed on 98 immature rats of two age groups – 12 and 18 days old. All animals were under ether anaesthesia implanted with stimulation and recording electrodes. Stimulation electrodes (two isolated stainless steel wires sharpened electrolytically to the tip diameter of approximately 50 μm, isolated up to the tips) were introduced stereotaxically into the dorsal hippocampus. Coordinates for adult rat hippocampus (AP = 4 mm, L = 4 mm, H = 4 mm – Fifková and Maršala 1960) were recalculated according to the bregma-lambda distance

which was 8 mm in adult animals. Histological control in pilot experiments demonstrated that a correction must be made for the horizontal coordinate – approximately 10–15 % had to be added. Recording cortical electrodes (flat silver wires) were implanted epidurally over sensorimotor and visual areas of both hemispheres as described previously (Schickerová *et al.* 1984).

After the interruption of ether anaesthesia, the animals were allowed to recover for at least one hour, then righting and placing reflexes were examined, animals were fed with a 5 % solution of glucose (and thus the suckling reflex was controlled) and the experiment was started.

Animals exposed to hypobaric hypoxia were put into a hypobaric chamber and then maintained at a simulated altitude of 7000 m for one hour. Stimulations began fifteen minutes after the end of this exposure.

Stimulation with 15-s series of rectangular pulses of 1 ms duration and 8 Hz frequency was applied four times at 10-min intervals between the end of an afterdischarge and the beginning of the subsequent stimulation. The voltage of stimulation was always equal to double of the value necessary for elicitation of evoked responses in ipsilateral sensorimotor cortical region. With the electrode resistance of 30–50 kΩ intensities of stimulating current ranged from 200 to 500 μA.

Five groups were formed: two control groups without exposure to hypoxia (one injected with physiological saline and the other was administered pramiracetam in the dose of 100 mg/kg i.p. 5 min after the end of the first AD) and three groups exposed to hypoxia – one control and two received an injection of pramiracetam again in a dose of 100 mg/kg i.p. One experimental group received pramiracetam again between the first and second stimulation, the second experimental group was administered pramiracetam 30 min before the exposure to hypobaric hypoxia. Pramiracetam was always freshly dissolved in distilled water in a concentration of 100 mg/ml, so that the volume of 1 ml/kg was administered intraperitoneally to rat pups. The dose was chosen according to the therapeutic window described by Poschel *et al.* (1985) and Hall and von Voigtlander (1987).

All experimental groups consisted of six to 12 rat pups. Body temperature of rats was maintained by means of an electrically heated pad during surgical preparation, the rest period and registration, the hypobaric chamber was also heated to 34 °C (i.e. the temperature of the nest). The animals were used only once and after the end of experiment they were sacrificed by an overdose of general anaesthesia.

The duration of afterdischarges was measured and statistically evaluated by means of analysis of variance (BMDP Programs). Level of significance was set at 5 %. In addition, the number of most expressive behavioural automatisms – wet dog shakes – was counted.

Control rats without hypoxia

Twelve-day-old rats exhibited a progressive prolongation of afterdischarges with repeated stimulations. Pramiracetam did not block this progress, but slowed it down so that the third and fourth ADs in treated animals were shorter than the corresponding ones in naive rats. Eighteen-day-old rats did not exhibit such a marked progressive lengthening under control conditions. After pramiracetam the progression was more marked. Number of wet dog shakes remained stable in all four afterdischarges in all groups.

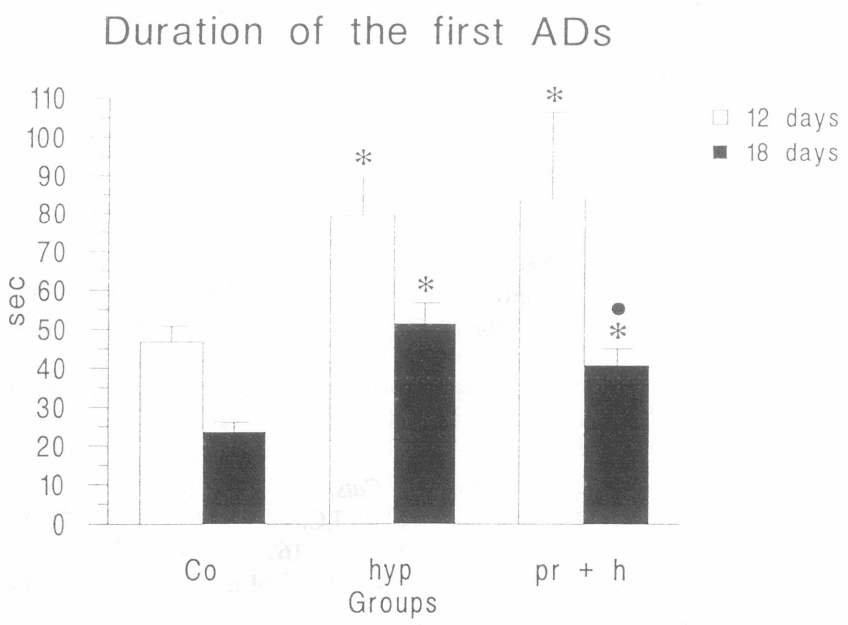

Fig. 1
Duration of the first afterdischarge (Mean ± S.E.M.) in rat pups aged 12 days (white columns) or 18 days (black columns) under the influence of hypoxia and pramiracetam. Co – control rats, hyp – rats exposed to hypoxia, pr + h – rats exposed to hypoxia and given pramiracetam before electrical stimulation. Asterisks denote significant difference against control age-matched rats, circle denotes significance in comparison with corresponding value in rats exposed to hypoxia.

Animals exposed to hypobaric hypoxia

Hypobaric hypoxia led to significant prolongation of the first AD in both age groups (Fig. 1). In contrast to age-matched controls without hypoxia, 12-day-old rat pups did not exhibit further lengthening of ADs with repeated stimulations. Similar results were found in 18-day-old rats, where the duration of all ADs remained at the higher level than in rats not exposed to hypoxia. Pramiracetam injected between the first and second stimulation did not change the duration of any AD or the number of wet dog shakes in 12- as well as in 18-day-old animals.

Pretreatment with pramiracetam before the exposure to hypobaric hypoxia did not prevent the prolongation of the first AD in both age groups, but the first AD in pramiracetam-pretreated 18-day-old rats was shorter than the corresponding AD in control animals exposed to hypoxia (i.e. the average duration was between those of naive and hypoxia-exposed rats – Fig. 1). Duration of subsequent ADs was identical in the control and pramiracetam-pretreated groups. No differences were found in the number of wet dog shakes.

The action of hypoxia on the duration of the first afterdischarge was the same as in our previous report (Marešová and Mareš 1991). The longer duration of the first AD might signify that the mechanisms arresting epileptic seizures are compromised. Under normal conditions, seizures are arrested by active inhibitory mechanisms (Engel 1989), among which the opioid (Frenk *et al.* 1979) and GABAergic systems (Mareš and Makal 1994) play a role. Selective suppression of GABAergic mechanisms by hypoxia was demonstrated *in vitro* by Sher (1990) because GABA uptake and clonazepam-displaceable benzodiazepine binding were more depressed than cholinergic markers.

The effect of pramiracetam was unexpectedly moderate, only the preventive treatment resulted in a significant effect. Our hypothesis that pyrrolidinone nootropics could protect against consequences of hypobaric hypoxia was not proven. There are some possible reasons for this result:

1. Differences among models of hypoxia and ischaemia used in the literature as demonstrated by Sakurai *et al.* (1990).
2. Different sensitivity of the immature brain to pramiracetam so that the therapeutic window would be shifted towards higher or lower doses. Data to this point are missing in the literature. This problem should be addressed in our future studies.
3. Immaturity (or absence) of mechanisms of action of pyrrolidinone nootropics in 12- and 18-day-old rats.

The exact mechanism of action of this class of nootropics is not known. Mondadori *et al.* (1990) demonstrated an involvement of the adrenals because adrenalectomy and blockade of aldosterone receptors abolished the memory enhancing effects of piracetam, pramiracetam, oxiracetam and aniracetam.

Another possible mechanism of action might be an increase of high affinity choline uptake (Pavlík *et al.* 1987, Pepeu and Spignoli 1989). The cholinergic system develops at early stages of development in rats (Coyle and Yamamura 1976) so that this system cannot be responsible for developmental changes.

Recent data speak in favour of the action of pyrrolidinone on excitatory amino acid receptors of the AMPA type (Copani *et al.* 1992). The density of these receptors in the rat forebrain is higher in immature than in adult rats (Insel *et al.* 1990), so that an opposite effect, i.e. higher efficacy of pramiracetam should be expected.

It can thus be concluded that the third possibility is less probable whereas the first two points remain to be elucidated.

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Reprint Requests

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