Neuroscience Letters 644 (2017) 1–4

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Which component of treatment is important for changes of cortical epileptic afterdischarges after status epilepticus in immature rats?

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HIGHLIGHTS

- Increasing intensity of stimulation led to progressive prolongation of afterdischarges in naïve rats.
- This prolongation failed to appear 3 days after lithium-pilocarpine status epilepticus.
- Similar failure was found in rats receiving only lithium chloride.
- Paraldehyde administration alone did not significantly affect the prolongation.
- Paraldehyde was found to block the effect of LiCl.

ARTICLE INFO

Article history:
Received 30 December 2016
Received in revised form 8 February 2017
Accepted 16 February 2017
Available online 21 February 2017

Keywords:
Status epilepticus
Immature rats
Pilocarpine
Lithium chloride
Paraldehyde
Cortical epileptic afterdischarges

ABSTRACT

Role of lithium chloride and paraldehyde in acute changes after lithium-pilocarpine status epilepticus (SE) induced at postnatal day 12 was studied in 15-day-old rats. In addition to SE group four other groups were formed: naïve animals without any injection, lithium chloride group, paraldehyde group and lithium-paraldehyde group. Cortical epileptic afterdischarges (CxADs) induced by increasing intensities of stimulation current were used as a measure of excitability. SE animals did not exhibit any change in duration of CxADs with increasing stimulation intensity in contrast to naïve control with a progressive prolongation of CxAD. LiCl group was similar to SE rats whereas paraldehyde and lithium-paraldehyde groups exhibited some progress in duration of ADs. Lithium chloride participates in short-term changes of CxADs after SE. Paraldehyde and combination of lithium and paraldehyde are similar to naïve controls.

1. Introduction

One of generally accepted models of status epilepticus (SE) is elicited by pilocarpine injection in rats. It is possible to administer high dose of pilocarpine (more than 300 mg/kg) in adult [1] as well as immature rodents [2] or ten times lower dose of pilocarpine after pretreatment with lithium chloride [3]. High dose of pilocarpine results in severe peripheral cholinergic effects which must be blocked by some cholinolytic drug inable to cross the blood–brain barrier. This way is not applicable in immature animals where blood–brain barrier is not yet fully functional [4] therefore we are using lithium-pilocarpine model in 12-day-old rats [5,6]. Both pilocarpine models result in high mortality of rats therefore some ways how to stop seizures are used (e.g. benzodiazepines, paraldehyde). The suppression of seizures by paraldehyde (PAR) is only transient but significantly increases percentage of animals surviving SE [7]. SE often starts epileptogenesis and spontaneous epileptic seizures appear after a long delay but changes might be found during whole silent period, i.e. even at early stages after SE.

There might be a problem because in the model of SE two other biologically active drugs (LiCl and PAR) are used in addition to PILO. The aim of our study was to analyze what is the role of individual active drugs in short-term (three days after SE) consequences of LiPILO SE. LiCl, PAR, combination of LiPAR were administered without PILO and as a basic control complete LiPILO SE was induced; the results were compared with naïve animals. Cortical epileptic afterdischarges served as a measure of excitability.

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http://dx.doi.org/10.1016/j.neulet.2017.02.043
0304-3940/

2. Methods

Experiments were performed in male Wistar albino rats. Status epilepticus was elicited by pilocarpine (40 mg/kg i.p.) in 12-day-old (P12) animals pretreated with lithium chloride (LiCl, 3 meq/kg i.p.) on P11. Injection of paraldehyde (PAR, 0.3 ml/kg i.p.) was administered after 90-min duration of SE to decrease mortality of animals. These SE rats ($n = 10$) served as a reference group. Four other groups each consisting of 10 rats were formed: (1) animals injected at P11 with LiCl and at P12 with paraldehyde (LiPAR group); (2) animals injected at P11 with LiCl (Li group); (3) animals injected at P12 with paraldehyde (PAR group); (4) naïve animals without any injection (control group). In addition to administration of LiCl at P11 in three groups all animals were taken from the nest at the age of 12 days. Saline was injected at time when pilocarpine should be administered in groups 1–3. The animals spent approximately 2 h in isolation then they were returned to their mothers. Body temperature of P12 as well as at P15 rat pups was maintained during the whole experiment by means of a plate heated electrically to 34 °C, i.e. to the temperature in the nest.

The animals were implanted under ether anesthesia with flat silver epidural electrodes on P15. Two stimulation electrodes were localized over right sensorimotor region (AP = +1 and −1; L = 2 mm) and recording electrode over left sensorimotor area (AP = 0, L = 2 mm). Reference and grounding electrodes were placed into the occipital bone. After surgery lasting 10–15 min the animals were left to recover for at least 1 h. Righting, placing and suckling reflexes were checked before the stimulation started.

Stimulator with a constant current output was used. Series lasting 15 s formed by 1-ms biphasic pulses at 8-Hz frequency were applied in 10-min intervals. Intensity of the first stimulation series was 0.2 mA, then intensities increased in 17 steps up to 15 mA. EEG digitalized at a 500-Hz frequency was recorded 10 s before stimulation, during stimulation and after stimulation during AD (if present) and at least 1 min after the end of AD and saved on a hard disk of the system (Kaminskij Biomedical Instruments, Prague).

Mean threshold intensities for elicitation of movements directly induced by stimulation, of spike-and-wave type of afterdischarges, clonic seizures accompanying this type of AD, transition into the second, limbic type of AD and of recurrent ADs were calculated from values in individual animals when the phenomena appeared for the first time and duration of ADs was measured in all five groups. Statistical evaluation (SigmaStat®, SYSTAT) of the thresholds of five groups as well as comparison of duration of ADs among individual groups was performed by one way ANOVA with subsequent pairwise comparison by Holm–Sidak test. $p < 0.05$ was taken as statistically significant.

3. Results

Movements induced by stimulation pulses, spike-and-wave ADs and clonic seizures accompanying this type of AD were elicited in all animals. Exceptionally, even the 15-mA intensity of stimulation was in some animals not high enough to induce transition into the limbic type of ADs, and appearance of recurrent ADs was far from being constant.

3.1. Threshold intensities (Fig. 1)

All animals exhibited movements during stimulation and generated spike-and-wave type of ADs accompanied by clonic seizures usually restricted to head and forepaw muscles. All four experimental groups exhibited significantly higher threshold intensities necessary for elicitation of movements directly elicited by stimulation of sensorimotor cortex than control naïve rats. In addition,

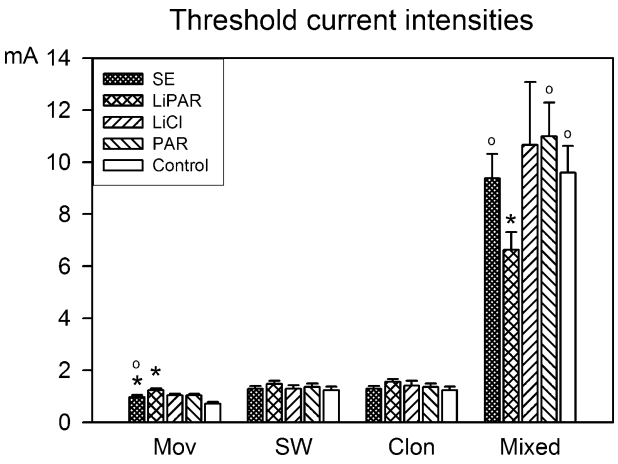

Fig. 1. Average threshold intensities for four phenomena elicited by cortical stimulation. Ordinate: current intensity in mA. Abscissa: Mov – movements induced directly by stimulation; SW – spike-and-wave discharges; Clon – clonic seizures accompanying SW afterdischarges; Mixed – transition to the other, limbic type of afterdischarge. Individual columns – see inset. Asterisks denote significant difference vs. controls; o – significant difference vs. LiPAR group.

threshold intensity in LiPAR group tended to be higher than in SE group ($p = 0.077$). No significant differences were found among the five groups in thresholds for spike-and-wave ADs as well as for clonic seizures accompanying this type of ADs. Outlined differences between SE and LiPAR groups (tendency to higher thresholds in LiPAR group) did not reach the level of statistical significance.

The incidence of mixed type of ADs in 15-day-old rats was low. In addition to control naïve animals (transition of spike-and-wave ADs into the limbic type ADs was present in nine out of 10 rats), only SE group exhibited limbic type of ADs in relatively high percentage of rats (seven out of 10 animals). In the three remaining groups this transition was observed in only two to four out of 10 rats. As threshold intensities for this type of ADs were concerned, significantly lower stimulation intensity was found in LiPAR than in SE, LiCl and control naive groups. Due to a low presence of the second, limbic type of ADs in LiPAR and LiCl groups, these tendencies have to be taken cautiously.

3.2. Duration of ADs (Fig. 2)

Control naïve animals exhibited marked prolongation of total ADs duration with increasing intensity of stimulation current. No similar prolongation was seen in SE rats, there was no difference among total duration of ADs elicited by different intensities of stimulation current. LiCl group was close to SE rats, only ADs elicited by the two highest stimulation intensities exhibited a tendency to longer ADs if compared with SE animals. A progressive prolongation of ADs with increasing stimulation was found in LiPAR group (with the exception of high stimulation intensities) and in a less extent in PAR group. For comparison Table 1 demonstrates average durations of ADs elicited with 2.2-mA (the lowest intensity when all animals exhibited ADs) and 15-mA intensity, i.e. the highest intensity of stimulation current.

Nearly the same results were obtained when duration of spike-and-wave ADs only was evaluated, a marked difference was found in a loss of significance between control and LiPAR groups in duration of ADs elicited by 12 and 15 mA.

Incidence of mixed type of ADs in three treatment groups (only two LiCl rats and four animals in either PAR or LiPAR groups) was so small that reliable statistics of duration of this type of ADs could not be calculated.