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ARE MORPHOLOGIC AND FUNCTIONAL CONSEQUENCES OF STATUS EPILEPTICUS IN INFANT RATS PROGRESSIVE?

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Abstract—The present study examined whether status epilepticus (SE) induced by LiCl-pilocarpine in immature rats (postnatal day [P]12) interferes with normal development; leads to progressive epileptogenesis, or cognitive decline and to pathology similar to that seen in human temporal lobe epilepsy. We correlated the extent of pathologic changes with the severity of functional alterations or epilepsy. SE-induced changes were compared with those of rats with SE induced at P25. Animals of both ages were exposed to a battery of behavioral tests for up to 3 months after SE. Rats with SE at P12 showed mild retardation of psychomotor development and delayed habituation, whereas rats with SE at P25 showed no habituation. Assessment in adulthood using the Morris water maze test revealed that SE at both P12 and P25 led to cognitive impairment and that the severity of the impairment increased with age. A handling test revealed increased aggression in rats with SE at P25, but not in rats with SE at P12. Epilepsy was diagnosed with continuous video-electroencephalographic (EEG) monitoring for up to 7 d. P25 rats were monitored at 5 months after SE and seizures were detected in 83.3% of animals. P12 animals were divided into two groups and monitored at 5 or 7 months after SE. Both the severity and incidence of spontaneous recurrent seizures tended to progress with time, and their incidence increased from 50% to 87.5% at 5 and 7 months, respectively. Morphometric analysis and stereologic assessment of hilar neurons performed after video-EEG monitoring revealed atrophy of temporal brain structures, enlargement of lateral ventricles, and loss of hilar neurons in both age groups. In P12 rats, morphologic damage also tended to progress over time. Performance of animals in the Morris water maze correlated with the severity of damage, but not with seizure parameters.

Key words: development, status epilepticus, cognitive function, epilepsy, brain damage, progressive atrophy.

INTRODUCTION

Epilepsy is the second most common neurologic disease (Hauser et al., 1993), and according to the World Health Organization, approximately 50 million people worldwide have epilepsy. Population studies suggest that seizure incidence is highest during the first year of life (Forsgren, 2004) and that early-life seizures frequently result in the development of epilepsy and behavioral alterations later in life (LaFrance et al., 2008). Aside from epilepsies caused by genetic or developmental abnormalities, most early-life epilepsies are caused by external insults such as stroke, hypoxia–ischemia, and trauma. These early-life insults often lead to epileptogenesis, a process in which initial brain injury of various etiologies triggers cascades of molecular, cellular, and network changes, and eventually spontaneous seizures. Recent ontogenetic data suggest that epileptogenesis is developmentally regulated and that age at the time of injury determines the mechanisms responsible for epileptogenesis (for rev. Rakhade and Jensen, 2009).

Experimentally induced status epilepticus (SE) is the most frequently used epileptogenic insult in research, and can be induced by injection of kainate or pilocarpine at very early developmental stages in rodents. Injection of LiCl followed by pilocarpine induces convulsive SE as early as postnatal day (P)7–10 (Hirsch et al., 1992). There are remarkable differences in the severity of LiCl–pilocarpine-induced SE depending on the age group (Hirsch et al., 1992), and both the severity and duration of SE increase with age (Kubová et al., 2004). The lower severity of initial insult documented in rats younger than 2 weeks might be one factor associated with less extensive structural damage and milder functional impairment compared to older age groups. Other factors, such as the remarkable plasticity and adaptability of the immature brain, however, might also contribute to the age-related differences in both the functional sequelae and epileptogenesis.

Recent studies demonstrated that the immature brain is not resistant to SE-induced brain injury and that chronic changes, such as anatomic alterations, functional impairments, and epileptogenesis are seen in rats after SE at P14 or younger (Sankar et al., 1998; Wu et al., 2001; Kubová et al., 2004; Nairismägi et al., 2006). In adult animals, spontaneous seizures generally occur after a latent period, which is highly variable in duration, from days to a few weeks after SE (Leite et al., 1990). Furthermore, the severity of both epilepsy and brain

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Abbreviations: ANOVA, analysis of variance; CE, coefficient of error; EEG, electroencephalograph; FJB, Fluoro-Jade B; SDs, standard deviations; SE, status epilepticus.

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damage progresses with time after SE (Persinger et al., 1998; Nairismägi et al., 2004; Williams et al., 2009). The progressive nature of epilepsy and brain damage has not been studied in animals with SE early in life and there are no data on possible age-related differences in the latent phase. In our previous studies, we reported brain atrophy in the temporal lobe 3 months after Lithium/pilocarpine SE at P12 (Nairismägi et al., 2006), and at the same time point epilepsy was observed in 25% of animals (Kubová et al., 2004). We hypothesize that after early SE, both the incidence and severity of epilepsy increase with time and that brain damage will progress for a longer time. An important question is whether early SE affects normal development and whether developmental retardation or behavioral alterations predict the development of epilepsy or its severity later in life.

We designed a series of experiments to address the following questions: (1) Does early SE delay normal development, leading to cognitive impairment later in life? (2) Does the risk of developing epilepsy increase with time after SE? (3) Does the severity of epilepsy and brain atrophy progress with time after SE? (4) Is there any association between the severity of cognitive impairment, the severity of epilepsy, and the extent of brain atrophy? To address these questions, SE was induced by LiCl–pilocarpine in 12-day-old rats and animals were followed up for 5 or 7 months. Development of motor skills was repeatedly tested in immature animals. Spontaneous locomotion was followed in an open field and repeated exposure was used to test the development of habituation. Cognitive abilities were tested in the Morris water maze. Video-electroencephalographic (EEG) monitoring was used to check for the presence of spontaneous seizures, i.e., the development of epilepsy. After the end of EEG monitoring, the brains of the animals were subjected to morphometric and stereologic analyses. To determine a role of age at the time of the insult, the results were compared with data obtained from rats that experienced SE at P25.

EXPERIMENTAL PROCEDURES

Animals

SE was induced in male Wistar albino rats on P12 (n = 23) or P25 (n-25). The day of birth was defined as day 0. Rats were housed under a controlled environment (temperature 22 ± 1 °C, humidity 50–60%, lights on 6 a.m. to 6 p.m.), with free access to food and water. Experiments were approved by the Animal Care and Use Committee of the Institute of Physiology of the Academy of Sciences of the Czech Republic. Animal care and experimental procedures were conducted in accordance with the guidelines of the European Community Council directives 86/609/EEC and NIH Guidelines (Assurance No. #A5820-01; valid till 1/31/2014).

Induction of SE

Rats were injected intraperitoneally with LiCl (3 mmol/ml/kg; # L-0505, Sigma Chemical Co., St. Louis, MO, USA) 24 h prior to intraperitoneal injection pilocarpine (40 mg/ml/kg; # P-6503, Sigma Chemical Co., St. Louis, MO, USA) (Hirsch et al., 1992). The appearance of clonic motor seizures was considered to be the beginning of SE. To decrease mortality, a single dose of paraldehyde (0.07 ml/kg for P12 rats and 0.3 ml/kg for P25 animals; # 76260, Fluka Chemie AG, Buchs, Switzerland) was injected intraperitoneally 1.5 h after the onset of SE (for details Kubová et al., 2005). Control animals in both age groups were treated with equal doses of LiCl and paraldehyde, but the pilocarpine solution was replaced with saline.

Severity of motor SE was assessed using the following scoring system:

0 – Normal behavior.
1 – Stereotypic behavior (face washing, scratching), isolated myoclonic jerks.
2 – Head bobbing, pivoting, swimming movements.
3 – Clonic seizures with preserved righting reflex.
4 – Repeated episodes of wild running.
5 – Generalized tonic clonic seizures with loss of righting reflex.

Animals were assigned a score for the most severe behavior observed. Latency to the onset of motor seizures was recorded. Mortality was recorded throughout the entire experimental period. Only rats that exhibited behavioral manifestations of seizures progressing to forelimbs clonus (i.e., score 3) for at least 1 h and without periods of wild running and generalized tonic clonic seizures (score 4–5) were used for further studies.

During the entire period of separation from their mothers, P12 animals were maintained at +34 °C with a Physiological–Biological Temperature Controller (TMP-5b; Supertech; Hungary) to compensate for the immature thermoregulation at this age (Conklin and Heggeness, 1971). Approximately 3 h after pilocarpine injection, the pups were injected subcutaneously with 0.9% NaCl (up to 3% of the body weight divided into 2–3 doses) to restore the volume loss, and then returned to their mothers (the duration of isolation from mothers in the control and SE groups was the same). If animals with SE at P25 were not able to ingest normal animal food, they were fed with a paste made of grounded animal food (Bergman; Czech Republic) and 1% glucose solution for up to 3 d after SE. The health status of animals was monitored daily.

On the day of LiCl injection (i.e., P11 or P24), each animal was assigned a code that was kept until completion of the entire study. This system allowed for the individual history of each animal to be recorded for the study duration. Testing was started 3 d (in P12) or 6 d (in P25) after SE and the detailed experimental schedule is summarized in Fig. 1. For technical reasons, data collection for some animals was incomplete (partial tissue destruction prevented morphologic analysis of some brain areas, technical difficulties disrupted EEG recording in some animals, etc.).

Effects of SE on development

Body weight was checked daily for 1 week after SE and then before each open field exposure. To minimize the effects of variability in individual groups, the data were used to calculate relative body weight (body weight at P11 or P24 was taken as 100%). The difference in the relative body weights between two consecutive days was used as a measure of weight gain.

Psychomotor development tests were performed only in the P12 group, because locomotion develops most rapidly during the second week of life and improvement of some skills can be observed from day to day. At around P21, motor skills already have adult features in most parameters (Altman and Sudarshan, 1975). To assess whether SE leads to developmental delay or retardation of sensorimotor abilities, P12 animals were exposed to the following tests: