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Harmful effect of kainic acid on brain ischemic damage is not related to duration of status epilepticus

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Abstract

Status epilepticus is common in infants and may have long-term consequences on the brain persisting into adulthood. Vascular ischemia is a common cause of stroke in adulthood. The extent of stroke in 15-day-old rats is larger when previously exposed to kainic acid-induced status epilepticus. In this paper, we assess whether shortening the duration of seizures modifies subsequent susceptibility to middle cerebral artery occlusion. We administered pentobarbital 50 mg/kg to abort seizures after 1 h. Although administration of pentobarbital aborted seizures, it had no effect on volume of infarction following ischemia. This study indicates that there is dissociation between stopping status epilepticus and modifying its long-term consequences.

Keywords

Kainic acid; Rat; Development; Status epilepticus; Stroke; Pentobarbital

Introduction

Status epilepticus (SE) early in life may alter the susceptibility to seizure-induced damage in adulthood [1]. SE occurs most commonly in pediatric patients [2] and in the neonatal period seizures often are prolonged, sometimes lasting hours [3]. Stroke is the most common neurological insult in the elderly and is the third most common cause of death [4]. The available data suggest that the consequences of SE are age and etiology dependent [5]. In a previous

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study [6] we showed that SE, induced in post-natal age 15 days (P15) rats, affected the extent of stroke, induced by 1-h middle cerebral artery occlusion (MCAo) 5 weeks later (as the rat begins adulthood). The observed effect was dependent on the cause of SE. Rats with kainic acid (KA)-induced SE had larger areas of infarction than rats with flurothyl (FE)-induced SE. SE caused by KA lasted up to 24 h while the FE SE only lasted 1 h. Among the possible causes for this observation that were mentioned in that report, it was proposed that the duration of SE may cause the observed difference in stroke susceptibility.

We recently reported that in P15 rats, KA-SE can be stopped after 1 h, using pentobarbital (PTB) 50 mg/kg [7]. In that study, treatment with this dose of PTB resulted in behavioral cessation of seizures which was correlated with EEG cessation of seizures as well. The difference in susceptibility to ischemic damage between the two groups may be related to the duration of SE. Therefore, in the present study we administered PTB after 1 h of ongoing SE and compared the stroke outcome in these animals to the outcome in the animals with prolonged SE. We also administered PTB to a group of control rats that did not receive KA.

Methods

Animals

We used male Sprague–Dawley rats (Taconic Farms, New York, NY, USA) housed under standard conditions in our animal facility, accredited by the American Association for Accreditation of Laboratory Animal Care. Animals were initially housed with their dam (10 pups per mother per cage), and at P15 were exposed to KA. Afterward, rats from the different groups were equally distributed among the mothers to avoid any litter effect. Animals were weaned at P21 and randomly distributed, three rats per cage, with free access to water and food until the day they were killed. Food but not water was withheld the night before surgery in animals submitted to experimental ischemia. All animal procedures were in accord with Guidelines for the Care and Treatment of Laboratory Animals of the National Institutes of Health.

Detailed procedures were published previously [6]. Briefly, rats were randomly selected and placed into groups: one group received KA 3.5 mg/kg, i.p. (Ocean Produce International, Shelburne, NS, Canada). In this group some rats progressed to status epilepticus ($n = 26$) while others exhibited only scratching behaviors ($n = 18$) shown prior to not be associated with electrographic seizure patterns [6]. The rats that had seizures were split into two groups, one group being left untreated ($n = 13$) while the other group was treated after 1 h of SE with PTB 50 mg/kg to stop the SE ($n = 13$). A control group of rats was not exposed to KA but was only given PTB ($n = 10$). The rats were monitored continuously for behavioral seizures during the first 90 min from injection. After treatment with PTB, the rats were observed for 60 more minutes and then returned to their dam.

Five weeks later they were subjected to transient focal ischemia for 1 h by MCAo as described previously [6]. Rat brains delivered after sacrifice were stained using TTC (a saline solution containing 2.0% 2,3,5-triphenyltetrazolium chloride) within 3 min of death. The stained sections were scanned on a flatbed scanner and the infarct volume calculated after edema correction. The unstained area in each image was delineated and quantified from a video image-analyzing system (NIH Scion Image, version 1.65) and volume of infarct was calculated by subtracting the noninfarcted area of the infarcted hemisphere from the normal contralateral hemisphere. The analysis was made by an investigator (S.M.) blinded to the experimental treatment.

Data are expressed as the mean $\pm$ SEM. Statistically significant differences between the data were evaluated with the ANOVA followed by the Fisher's post hoc test.

Results

There were significant differences in the extent of the stroke depending on the treatment ($F = 3.432$, $P = 0.0238$). Fisher's post hoc analysis was therefore done to analyze specific differences between the groups. Rats that experienced SE following KA injection had larger volumes of infarctions compared to those without seizures ($P = 0.02$) and those that received PTB alone ($P = 0.03$). Treatment of SE after 1 h with PTB 50 mg/kg was effective in stopping the seizures but did not alter stroke susceptibility to MCAo compared to rats with SE that were not treated. These rats still exhibited a significant increase in stroke size when compared to KA rats without seizures ($P = 0.02$) and rats that received PTB alone ($P = 0.03$). There was no significant difference between the rats that received KA without having seizures and the rats that received PTB alone (Fig. 1).

Discussion

In a previous study [6] SE was induced in postnatal age 15 days (P15) rats with KA or FE. In the rat, the second and third postnatal week roughly correspond, in terms of stage of brain development, to that of the infant and very young human child [8]. Five weeks later, these rats were subjected to a transient focal ischemia by MCAo. The results showed that FE was neuroprotective long term, and that the animals with FE SE had a smaller stroke than the control group of rats with FE that did not experience any seizures. However, rats with KA-induced SE had a larger stroke than their age-matched controls. It was postulated that one of the possibilities for the increased susceptibility to ischemic damage was secondary to the prolonged SE. The goal of this study was to determine whether the duration of SE is the cause of the increased susceptibility to subsequent stroke or whether it is the KA-induced SE regardless of duration.

Stopping KA-induced SE at 1 h following onset of SE, did not alter the long-term changes in the brain that are seen weeks later, as a susceptibility to infarction following MCAo. Although SE was stopped, no differences were found 5 weeks later in stroke volume. These data indicate that KA-SE causes long-term changes to the brain, making it more vulnerable to ischemic damage. Treating the SE after 1 h does not alter the long-term dysfunction, despite controlling the seizures at time of SE. As the changes in the brain are not due to the duration of seizures, further research is needed to determine the cause of the changes. It is important to determine the extent of the acute and chronic effects of SE on the brain and whether these changes can be modified with treatment as the current treatments may be reducing the duration of seizures without effecting the long-term changes that occur in the brain.

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Fig. 1.

Average volume of infarction with standard error bars. There is no difference in volume of infarction following MCAo between rats with KA-induced SE and rats with KA-induced SE treated with PTB. There is a significantly larger area of infarction in these groups compared to rats that did not experience KA-induced SE or received PTB only