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HYPERTHERMIA AGGRAVATES STATUS EPILEPTICUS-INDUCED EPILEPTOGENESIS AND NEURONAL LOSS IN IMMATURE RATS

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Abstract—This study tightly controlled seizure duration and severity during status epilepticus (SE) in postnatal day 10 (P10) rats, in order to isolate hyperthermia as the main variable and to study its consequences. Body temperature was maintained at 39 ± 1 °C in hyperthermic SE rats (HT + SE) or at 35 ± 1 °C in normothermic SE animals (NT + SE) during 30 min of SE, which was induced by lithium–pilocarpine (3 mEq/kg, 60 mg/kg) and terminated by diazepam and cooling to NT. All video/EEG measures of SE severity were similar between HT + SE and NT + SE pups. At 24 h, neuronal injury was present in the amygdala in the HT + SE group only, and was far more severe in the hippocampus in HT + SE than NT + SE pups. Separate groups of animals were monitored four months later for spontaneous recurrent seizures (SRS). Only HT + SE animals developed convulsive SRS. Both HT + SE and NT + SE animals developed electrographic SRS (83% vs. 55%), but SRS frequency and severity were higher in hyperthermic animals (12.5 ± 3.5 vs. 4.2 ± 2.0 SRS/day). The density of hilar neurons was lower, thickness of the amygdala and perirhinal cortex was reduced, and lateral ventricles were enlarged in HT + SE over NT + SE littermates and HT/NT controls. In this model, hyperthermia greatly increased the epileptogenicity of SE and its neuropathological sequelae. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: neuronal injury, temperature, immature brain, lithium/pilocarpine model, EEG monitoring.

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Abbreviations: CB, cacodylate buffer; DZP, diazepam; EM, electron microscopy; HT, hyperthermia; NT, normothermic; SE, status epilepticus; SRS, spontaneous recurrent seizure; TLE, temporal lobe epilepsy.

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INTRODUCTION

The impact of hyperthermia (HT) on the long-term consequences of status epilepticus (SE) is poorly understood. HT is thought to confer an immunological advantage over invading microorganisms, probably by enhancing an inflammatory response, and a number of studies have shown increased survival or recovery in HT compared to therapeutically induced normothermia during infections (Stanley et al., 1975; Doran et al., 1989; Graham et al., 1990; Ahkee et al., 1997; Kuikka et al., 1997; Kuikka and Valtonen, 1998; Jiang et al., 2000, 2002; Gozzoli et al., 2001). On the other hand, many investigations have shown deleterious effects of inflammation on seizure-associated brain injury (Vezzani and Granata, 2005; Fabene et al., 2008), while some studies suggest that antiinflammatory treatments worsen outcome (Holtman et al., 2010). HT has been reported to have no effect on FSE-associated neuronal injury in rat pups (Sarkisian et al., 1999), although it aggravates the brain damage produced by SE in adult rats (Liu et al., 1993; Lundgren et al., 1994). It also worsens outcome after cardiac arrest (Zeiner et al., 2001) and other hypoxic–ischemic insults at any age (Inder et al., 2004; Eicher et al., 2005a,b; Gluckman et al., 2005; Shankaran et al., 2005), and after traumatic brain injury (Dietrich et al., 1996), and enhances the inflammatory reaction associated with ischemic neuronal injury (Ginsberg and Busto, 1998; Aiyagari and Diringer, 2007).

We wanted first to examine whether SE-associated neuronal injury in immature rats truly differs from all other types of neuronal injury in its response to HT (Sarkisian et al., 1999), and second to investigate the role of HT in adverse consequences of SE, such as epileptogenicity, which were not included in previous studies. Current models of febrile SE cannot dissociate the role of HT from that of seizures, since HT is the trigger for seizure induction (Toth et al., 1998; Dube et al., 2000b; Bender et al., 2003). We developed a model of SE in P10 rat pups, in which seizures of similar intensity can be maintained for 30 min in normothermic or hyperthermic pups. Our results suggest that the presence of HT may act as a ''second hit'' which strongly potentiates seizure-induced neuronal loss and epileptogenesis.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar albino rats (Simonsen Lab, Gilroy, CA, USA), 10 days old (P10), were used. The day of birth was

considered as day 0. Pups were weaned at P21. All animals were housed in a temperature- and humidity-controlled room with 12-h light-dark cycles (light cycle starts at 7 am) and had free access to food and water. All experiments were conducted with the approval of and in accordance with the regulations of the Institutional Animal Care and Use Committee of West Los Angeles VA Medical Center.

Induction of SE and HT

Lithium chloride (3 mEq/kg) was administered intraperitoneally at P9 (postnatal day nine) and the next day, SE was induced with subcutaneous pilocarpine hydrochloride (60 mg/kg). To induce HT, the pups were placed immediately after pilocarpine injection into a specially constructed warming chamber floating on a water bath maintained at 40 °C (HT + SE group). The body temperature was monitored repeatedly throughout the experiment using digital thermometer with rectal probe (Tegam 872A, Geneva, Ohio, USA). After the rectal temperature reached 39 °C, the pups were kept in the chamber for 30 min. Then they were removed, injected with diazepam (1 mg/kg, i.p.) which effectively terminated seizures, and placed on a cool surface (room temperature) until their core temperature returned to 35 °C (Fig. 1A). For the recovery period, they were kept on a surface heated to 35 °C until they completely recovered (i.e. 6 h) and returned to their mother. The rectal temperature was measured at baseline and at 10-min intervals until 20 min after diazepam (DZP) administration and then four times in 30-min intervals. The normothermic pups (NT + SE group) were treated the same way except that they were kept at 35 °C throughout the experiment. Rat pups are poikilotherms, and their temperature in the nest is around 34 °C (Conklin and Heggeness, 1971). The control groups were exposed to HT only , to lithium and hyperthermia (HT + Li), to lithium alone (NT + Li), to DZP alone or to pilocarpine alone with or without hyperthermia (HT + lowPilo, NT + lowPilo). All animals were rehydrated with saline approximately 5 h after SE (10% of body weight, s.c.).

Measuring brain temperature

To record brain temperature during SE under both hyperthermic and normothermic conditions, separate group of animals (six per group) were used. The temperature probe (transmitter XM-FH-BP-06, Mini-Mitter Co., Bend, OR, USA) was implanted in the left cerebral cortex 24 h before SE under 1.5% isoflurane anesthesia, 2 mm left of midline, midway between the bregma and lambda, and 2.5 mm below the skull surface. To minimize animal discomfort, skin at the implant site was infiltrated with xylocaine (2%) prior to surgery. The probe was fixed to the skull using cyanoacrylate tissue adhesive.

SE was induced as described before. The brain temperature was measured using radio telemetry (receiver PhysioTel, Data Sciences, St. Paul, MN, USA). Data were recorded every minute as frequencies generated by the transmitter and were converted to temperature by use of a calibration curve provided by the manufacturer. In the same animal, rectal temperature was measured every 4 min using a rapid reading thermocouple probe and reader which provided temperature directly.

Acute video-EEG recording

In order to characterize the effect of HT on acute SE, additional animals belonging both NT + SE (n = 10) and HT + SE (n = 10) groups were prepared for video-EEG recording. Under isoflurane (1.5%) anesthesia, registration screw electrodes were implanted over the right and left occipital cortex (coordinates AP = −3.5 mm, L = 3 mm) one day before SE (P9). The ground electrode was implanted into the skull over the cerebellum and anchored with dental cement. After surgery, animals were kept on a 37 °C pad until they recovered and then returned to their dams. Lithium was administered on the day of the surgery.

One day later (P10), EEG electrodes were connected to tethered cables with swivel mounts (Plastic One, Roanoke, VA, USA), which fed the signal to a monitoring and recording system (Harmonie Software, Stellate System, Montreal, Quebec, Canada). After 8 h of continuous monitoring, the animals were returned to their mother for 2 h (feeding). Then they were monitored for additional 12 h. EEG recording started 15 min prior to pilocarpine administration (baseline). The severity of SE was assessed by measuring the following parameters: latency to onset of SE (time from pilocarpine injection to onset of the first behavioral seizure), pre-treatment seizure time (time spent in seizures before diazepam injection), post-treatment seizure time (cumulative time spent seizing, timed from the diazepam injection, subtracting interictal time), duration of SE (time from onset of SE to the end of the last seizure, including interictal time), total seizure time (total time spent in seizures, timed from seizure onset to the end of the last seizure, subtracting interictal time), behavioral seizure score (highest behavioral seizure score attained during SE) and spike frequency (spikes per hour), using Harmonie Software settings: Seizures are defined by the software as a discharge lasting at least 3 s, with a mean frequency higher than 3 Hz, coefficient of variation $\geqslant$65, and amplitude 2.7 times higher than baseline. For spike detection, the amplitude threshold was set at four times baseline.

Severity of behavioral seizures was evaluated using a modified Racine scale (Haas et al., 1990): 0- behavioral arrest, 1- facial clonus, 2- head nodding, 3- forelimb clonus, 4- forelimb clonus and rearing, 5- clonus with rearing and falling, 6- wild running and jumping with vocalization. The highest behavioral score observed was used for every animal.

Histology of acute neurodegeneration

Additional groups of HT + SE (n = 8) and NT + SE (n = 8) pups were prepared to characterize the effects

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