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Dysfunction of thermoregulation contributes to the generation of hyperthermia-induced seizures

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HIGHLIGHTS

- Over-regulation of body temperature occurred during hyperthermia-induced seizures.
- Pharmacological abolishment of thermoregulation blocked seizure onset.
- Lesion of the PO/AH blocked hyperthermia-induced seizures.

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ABSTRACT

Febrile seizures (FS) are generally defined as seizures taking place during fever. Long-term prognosis, including development of epilepsy and malformation of cognitive function, has been demonstrated after infantile FS. However, the mechanism that triggers seizures in hyperthermic environment is still unclear. We here found that the body temperature of rat pups that experienced experimental FS was markedly decreased (~28 °C) after they were removed from the hyperthermic environment. Both the seizure generation and the temperature drop after seizure attack were abolished by either pre-treatment with chlorpromazine (CPZ), which impairs the thermoregulation, or by an electrolytic lesion of the preoptic area and anterior hypothalamus (PO/AH). However, the non-steroidal anti-inflammatory drug celecoxib did not affect the seizure incidence and the decrease in body temperature after seizure attack. In addition, pentobarbital prevented the generation of seizures, but did not reverse the decrease of body temperature after FS. Therefore, our work indicates that an over-regulation of body temperature occurs during hyperthermic environment, and that the dysfunction of thermoregulation in the PO/AH following hyperthermia contributes to the generation of FS.

1. Introduction

Hyperthermia-induced seizures, also called "febrile" seizures (FS) are the most common form of infantile seizures [25,26]. Although most of these seizures are apparently benign, children with complex ones have higher risks of hippocampal injury, cognitive impairment and epilepsy in later life [10,17,33]. In addition, a high prevalence of patients with intractable temporal lobe epilepsy had an episode of childhood convulsive status epilepticus and hyperthermia-induced seizures [11], and these patients have more severe mesial temporal sclerosis than others [6,14]. Thus, it is necessary to understand the mechanisms underlying FS for searching approaches to preventing fever-related epileptic syndrome.

Previous studies have found that inflammation, respiratory alkalosis and genetic factors contribute to the generation of FS. However, it is still controversial regarding how they generate. For example, although IL-1β lowers the threshold of hyperthermia-induced seizures, mice lacking IL-1R1 still exhibit seizures in hyperthermic environment [10]. Kaila et al. [22] reported that respiratory alkalosis following hyperthermia triggers convulsion, while Baram's group debated that respiratory rate showed little change before the onset of convulsions in a different experimental FS model [9]. In addition, many children develop FS without evidence of additional risk factors or family history of epilepsy [21,25]. Thus, there may be other important mechanisms remaining unknown.

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Body temperature homoeostasis is precisely regulated by the preoptic area and anterior hypothalamus (PO/AH) in mammals, where neurons sensitive to temperature changes are located [12]. Thermal stimulation of the preoptic area in rats induces cortical synchronization [5]. Moreover, some neuromodulators that act on the thermoregulatory neurons in the PO/AH, are increased during fever and plays a role in FS [20]. Therefore, we hypothesized that the disruption of thermoregulation controlled by PO/AH may participate in the generation of seizures under hyperthermia.

In the present study, we found that body temperature fell lower than physiological temperature after hyperthermia-induced seizures, and the abolishment of thermoregulation blocked seizure onset. These results suggest that dysfunction of thermoregulation resulted from hyperthermia contributes to the generation of FS and may serve as a valid therapeutic target.

2. Materials and methods

2.1. Animals

Timed-pregnancy Sprague–Dawley rats were housed in an uncrowded, quiet animal facility room under a 12-h light–dark cycle. Food and water were available *ad libitum*. The day of birth was considered postnatal day 0 (P0). Experiments were carried out between 10:00 and 17:00 in accordance with the ethical guidelines of the Zhejiang University Animal Experimentation Committee and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Efforts were made to minimize the number and suffering of animals.

2.2. Generation of experimental FS

Experimental FS were induced by putting pups (P8–10) to a hyperthermic chamber [3,22]. The behavioral characteristics of seizures including sudden movement arrest followed by facial automatisms (chewing), forelimb clonus, and tonic flexion of the body that was often associated with a loss of postural control. Pups with observed seizures were removed to a cool surface for 2 min and then returned to the hyperthermia chamber. The core temperature was measured before hyperthermia exposure, every 10 min during the hyperthermia exposure and at the first onset of the behavioral seizures. After 55 min hyperthermia exposure, all rats were taken out and the core temperature was measured every 10 min for additional 60 min. The core temperature of littermate controls was maintained within normal range.

2.3. Electroencephalogram (EEG) recording and lesion of PO/AH

Pups (P8) were anaesthetized by inhalation of isoflurane (4% for induction and 2% for maintenance). Twisted-wire bipolar electrodes, with 0.5 mm vertical tip separation [15,29], were implanted into the dorsal hippocampus (AP −1.5, L −1.7, V −3.0) for EEG recording or the PO/AH (AP −0.1, L ±0.8, V −5) for lesion. All coordinates were measured in mm from bregma according to the developing rat brain atlas of Sherwood and Timiras [24] and were further confirmed by toluidine blue staining. A ground electrode was placed in the cerebellum. The assembly was anchored with dental cement and three stainless-steel screws. Electrolytic lesions were made by delivering 1 mA DC for 10 s to the unilateral PO/AH, performing 24 h after the surgery [8]. Sham-operated rats did not receive the DC current. EEG recording and experimental FS were exerted 24 h after surgery or PO/AH lesion. Hippocampal EEG was recorded for 5 min before and throughout the hyperthermia exposure. The power spectral densities of the digitized waveforms were derived using the fast Fourier transform algorithm in the Neuroscan EEG analysis software package [32].

2.4. Drug administration

The pharmacological agents were intraperitoneally administered at the following concentrations: chlorpromazine (1, 2 and 5 mg/kg, Sigma–Aldrich), celecoxib (10 and 20 mg/kg, Pfizer), pentobarbital (30 mg/kg, Abbott.), PTZ (40 mg/kg, Sigma–Aldrich). Drugs were administered 24 h after electrodes implantation (P9).

2.5. Histology

After seizure induction and temperature measurement, pups with electrolytic lesions, were anesthetized and perfused transcardially with 4% paraformaldehyde. The brains were separated and stored in 4% paraformaldehyde at 4 °C for 24 h, and then in 30% sucrose for 5–7 days. The brains were sectioned, stained with toluidine blue and histologically examined for the lesion sites.

2.6. Statistical Analysis

All data are presented as mean ± S.E.M. Statistical analysis was performed with SPSS (v16.0). Comparison between groups was made using *t*-test. One-way ANOVA with Tukey's *post hoc* test and Two-way ANOVA with Bonferroni's *post hoc* test were used for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Rat pups showed a lower body temperature after FS

The body temperature of pups showed no difference among the three groups before hyperthermia exposure (32.49 ± 0.7 °C in control group; 32.8 ± 0.2 °C in 38 °C group and 32.33 ± 0.5 °C in 44–45 °C group, respectively) but dramatically increased after hyperthermia exposure (Fig. 1A and C1, 2). In 38 °C group, pups experienced 55 min of hyperthermia (body temperature: 38.13 ± 0.2 °C), displayed no seizures or epileptic EEG (Fig. 1A, B and C3, 38 °C group). Their body temperature returned to the baseline (Fig. 1A, 38 °C group) 20 min after they were taken out of the hyperthermic chamber, and maintained within the normal range afterward. In 44–45 °C group, the latency to the first onset of seizure was 27.97 ± 0.96 min with epileptiform activities (body temperature: 39.79 ± 0.5 °C, Fig. 1A, B and C3). Sixty minutes after pups were taken out of the chamber, their temperature decreased to 29.74 ± 0.7 °C (Fig. 1A and C4, 44–45 °C group), which was significantly lower than baseline (32.46 ± 0.7 °C).

3.2. Chlorpromazine abolished hyperthermia-induced seizures

The above experiments showed that seizure activity generated when the body temperature increases to an excessive high level. Then we used pharmacological approach to examine the role of thermoregulation in seizure generation. Drugs were administered 30 min prior to hyperthermia exposure. All pups receiving celecoxib (10 and 20 mg/kg), a sulfonamide non-steroidal anti-inflammatory drug, displayed seizures with epileptic EEG (Fig. 2A and B). In groups receiving chlorpromazine (CPZ), all pups treated with 1 mg/kg and 6 out of 8 pups treated with 2 mg/kg CPZ showed behavioral seizures and epileptic EEG, while no seizures or epileptic EEG were observed in 5 mg/kg group (Fig. 2C and D).

In addition, celecoxib at either dose did not affect the temperature drop after seizure attack (Fig. 3A and B). While CPZ at dose of 5 mg/kg, but not 1 or 2 mg/kg, abolished the temperature drop (Fig. 3C–F). By contrast, CPZ decreased basal body temperature under normothermic environment (Fig. 3G).