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Research article

Protective effect of carnosine on febrile seizures in immature mice

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HIGHLIGHTS

- Carnosine attenuated FSs through increasing the latency and decreasing the duration.
- The inhibitory effects of carnosine were through its conversion to histamine.
- Carnosine caused a decrease of glutamate but not GABA levels.

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ABSTRACT

Febrile seizures (FSs) are the most common type of convulsions in childhood and complex FSs represent an increased risk for development of temporal lobe epilepsy. The aim of this study was to analyze the anticonvulsant effects of carnosine, an endogenous dipeptide composed of alanine and histidine, on hyperthermia induced seizure in immature mice. Injection of carnosine significantly increased the latency and decreased the duration of FSs in a dose-dependent manner. In addition, histidine had similar effects on FSs as carnosine. The protective effect of carnosine or histidine was completely abolished by α-fluoromethylhistidine (α-FMH), a selective and irreversible histidine decarboxylase inhibitor, or in histidine decarboxylase deficient (HDC-KO) mice. Peripheral carnosine administration increased the level of carnosine, histidine and histamine in the cortex and hippocampus of mice pups, but decreased glutamate contents in the cortex and hippocampus. These results indicate that carnosine can protect against FSs in mice pups through its conversion to histamine, suggesting that it may serve as an efficient anti-FSs drug in the future.

1. Introduction

Febrile seizures (FSs) are the most common type of convulsions in humans between the ages of 5 months and 5 years, with an incidence varying from 3% to 5% worldwide [11]. Although most FSs are apparently benign, clinical retrospective studies have demonstrated a relationship between a history of prolonged febrile seizures (FSs) during early childhood and mesial temporal sclerosis [3], moreover, prospective studies on children with febrile seizures and complex febrile seizures showing increased risk for TLE [8]. In addition, for experimental animal models, prolonged or repetitive FSs in infant have been closely linked to the development of memory defect and enhanced hippocampal excitability in adult [4,6]. Therefore, it is imperative to prevent the generation of this hyperthermia induced seizures in infant.

Our previous study reported that the central histaminergic system showed an important role in inhibiting the convulsions in mammals [2,13], so brain histamine acts as an endogenous anticonvulsant substance [20,28]. Decreased histamine levels reduce seizure threshold and elevate the severity and duration of seizures [27], whereas increased histamine levels have the opposite effects [26]. However, since histamine is involved in inflammation and

Abbreviations: α-FMH, α-fluoromethylhistidine; FSs, Febrile seizures; HDC-KO, histidine decarboxylase deficient; BBB, blood–brain barrier; HPLC, high-performance liquid chromatography; WT, wild type.

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cannot penetrate the blood-brain barrier (BBB), it cannot be used to directly treat infant that suffered with FSs [21].

Carnosine is an endogenous dipeptide composed of alanine and histidine, expressing in many tissues of the body including the central nervous system, and it can easily cross the BBB [5]. Furthermore, as carnosine is a reservoir for histidine, which is a precursor of histamine [9,22], it has been proposed to serve as a new histaminergic drug and be used in clinical therapeutics instead of histamine. In addition, we previously found that carnosine ameliorated pentylenetetrazole-induced kindled seizures and amygdaloid-kindled seizures in rats [13,29]. In general, carnosine is supposed to be an attractive therapeutic candidate for seizures.

Therefore, in this study we investigated the effect of carnosine on FSs in mice pups and further explored the underlying mechanism.

2. Material and methods

2.1. Animals

All experiments were carried out in accordance with the ethical guidelines of the Zhejiang University Animal Experimentation Committee and in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Furthermore, attempts were made to minimize the number of animals used in the study and their suffering. The animals used in this study were male C57BL/6J mouse pups and histidine decarboxylase deficient (HDC-KO) mouse pups (Experimental Animal Center, Zhejiang University, China), maintained in individual cages with a 12-h light/dark cycle (lights on from 08:00–20:00 h). Water and food were given ad libitum. Experiments were carried out each day between 10:00–17:00 h.

2.2. Generation of experimental febrile seizures

The experimental FSs paradigm was modified from previous methods [7]. In brief, on postnatal days 10–11, mice were placed in a chamber with an ambient temperature of $42 \pm 1\,^{\circ}C$. The body temperature during heating was increased from $33 \pm 0.2\,^{\circ}C$ (basal body temperature) to $40 \pm 0.4\,^{\circ}C$ (threshold body temperature). The interval between mice being put into a hyperthermic chamber and the onset of FSs was regarded as seizure latency. The pups were moved to cool surfaces once FSs was evoked. After that, seizures stop spontaneously and the body temperature decreases at the same time, which reduces to $32.4 \pm 0.5\,^{\circ}C$ 30 min after FSs. The interval between the onset of FSs and its termination was regarded as seizure duration. Seizure latency and duration measurement were based on behavioral observation together with electroencephalogram (EEG) recordings. The behavioral FSs in mice are characterized by forelimb clonus and tonic flexion of the body, often associated with a loss of postural control, which correlates with the epileptiform discharges.

2.3. Drugs

Carnosine (Sigma), histidine (Sigma) and α-fluoromethylhistidine (α-FMH, Merck Sharp & Dohme Research Laboratory, Rahway, NJ, USA) were used in this study. α-FMH was dissolved in sterilized saline and injected i.p. at a dose of 20 mg/kg 4 h before hyperthermia. Carnosine and histidine were injected i.p. 1 h before hyperthermia at indicated dose. There were no obviously behavioral changes after carnosine, histidine and α-FMH injections at all doses used in the present study.

2.4. Electrophysiological recording in vivo

To study the concordance of behavioral and electrophysiological seizures provoked by hyperthermia, we implanted bipolar electrodes unilaterally in the dorsal hippocampus [15]. In detail, P9-P10 mice pups were implanted bilaterally with monopolar steel electrodes under isoflurane anesthesia with stereotactic guidance. Electrodes were implanted 0.5 mm below the dura in the ventral hippocampus at the following coordinates: 2.0 mm lateral to the midline and 2.0 mm rostral to lambda. Ground wires were implanted subcutaneously and held in place next to the electrode wires via a cement head cap. Animals were allowed to recover on a heating pad. Baseline hippocampal electroencephalograms were recorded 24 h later to allow animals to recover from isoflurane anesthesia, thus avoiding affecting seizure susceptibility [14]. Subsequently, animals were gently placed in the hyperthermic chamber to induce febrile seizures. Hippocampal electroencephalograms were obtained in freely moving mice through long, flexible wires. The electrode and cannula positions were verified in all animals.

2.5. Measurements of brain carnosine, histidine, histamine, glutamate and GABA contents

Sample preparation. The mice were killed by decapitation. The brain was quickly removed and placed on an ice-cold stainless steel plate. The brain regions were subsequently dissected into the cortex and hippocampus according to the previously methods [10]. The brain tissue was homogenized in 0.4 M perchloric acid. The homogenate was centrifuged at 12,000 g for 30 min at $4\,^{\circ}C$. Then the supernatant was removed and filtrated with a 0.22 μm polyvinylidene difluoride membrane.

Chromatographic conditions. The tissue samples were analyzed by high-performance liquid chromatography (HPLC) combined with electrochemical detector using the technique developed in our laboratory for the simultaneous and sensitive analysis of carnosine, histidine, histamine, glutamate and GABA [13,22]. The HPLC was controlled and the data acquired and analyzed using CoulArray® software. All of the above equipments were from ESA (Chelmsford, MA, USA). After reacting with the derivative o-phthalaldehyde, analytes were separated on a 3 μm, 3 × 50 mm Capcell Pak MGC18 column from Shiseido (Tokyo, Japan). A two-component gradient elution system was used, with component A of the mobile phase being 100 mM Na2HPO4, 14.4% acetonitrile, and 25.6% methanol, pH 6.8, and component B being similar to A except with 1.8% acetonitrile and 3.2% methanol. A gradient elution profile was used as follows: 0–3.5 min, isocratic 100% B; 3.5–8 min, linear ramp to 5% B; 8–22 min, isocratic 0% B; 22–23 min, linear ramp to 100% B; 23–30 min, isocratic 100% B. The flow rate was set to 0.75 mL/min. The temperature of the column was maintained at $38\,^{\circ}C$. All standards were obtained from Sigma (St. Louis, MO, USA). Under these conditions, the retention time of glutamate, histidine, carnosine, GABA and histamine is 3.17, 4.39, 5.28, 6.03 and 10.96 min, respectively.

2.6. Statistical analyses

Data were presented as means ± SEM. Differences between means were determined using the one-way ANOVA with Tukey's *t*-test or nonparametric Mann-Whitney *T* test. Differences were considered statistically significant when $p < 0.05$.