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ScienceDirect
Neuroscience Letters 416 (2007) 211–216
Neuroscience Letters
www.elsevier.com/locate/neulet

Carnosine inhibits pentylenetetrazol-induced seizures by histaminergic mechanisms in histidine decarboxylase knock-out mice

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Received 22 September 2006; received in revised form 8 December 2006; accepted 5 January 2007

Abstract

In the present study, we used both histidine decarboxylase-deficient (HDC-KO) mice and wild-type (WT) mice to elucidate the possible role of carnosine in pentylenetetrazol (PTZ)-induced seizures. In the acute PTZ challenge study, PTZ (75 mg/kg) was injected intraperitoneally (i.p.) to induce seizures. Carnosine (200, 500 or 1000 mg/kg, i.p.) significantly decreased seizure stage, and prolonged the latency for myoclonic jerks in WT mice in a dose-dependent manner. The effects of carnosine (500 mg/kg) were time-dependent and reached a peak at 1 h. However, it had no significant effect on HDC-KO mice. Carnosine (500 mg/kg) also significantly elevated the thresholds in WT mice but not HDC-KO mice following intravenous (tail vein) administration of PTZ. We also found that α-fluoromethylhistidine substantially reversed the protective effects of carnosine in WT mice. In addition, carnosine pretreatment reduced the cortical EEG activity induced by PTZ (75 mg/kg, i.p.). These results indicate that carnosine can protect against PTZ-induced seizures and its action is mainly through the carnosine–histidine–histamine metabolic pathway. This suggests that carnosine may be an endogenous anticonvulsant factor in the brain and may be used as a new antiepileptic drug in the future.

Keywords: Carnosine; Pentylenetetrazol; Seizure; Histamine; Histidine decarboxylase

Epilepsy is a common chronic neurological disorder, afflicting more than 1% of the population throughout the world. Despite regulatory approval of many antiepileptic drugs (AEDs) over the last decade, a large number of patients remain refractory to currently available AEDs [18]. Due to the limited efficacy and side-effects of current AEDs, it is critical to search for more effective and safer therapeutic agents.

It is generally known that the central histaminergic system plays an important role in the inhibition of convulsions in mammals [22,25]. Activation of this system increases the threshold for convulsions and decreases susceptibility to electrically and chemically induced seizures [13,14,30]. In addition, high doses of several centrally acting H_1 receptor antagonists such as diphenhydramine and chlorpheniramine, which are used as anti-allergic drugs, occasionally induce convulsions in epileptic patients, healthy children and rodents [15,29]. We also previously reported that the seizure development induced by pentylenetetrazol (PTZ) is facilitated in histidine decarboxylase-deficient (HDC-KO) mice compared with that in wild-type (WT) mice [3]. Therefore, it is likely that certain histaminergic substances act as clinical anticonvulsants. However, histamine cannot cross the blood brain barrier and may be involved in brain inflammation [23].

Carnosine (β-alanyl-L-histidine) is a naturally occurring dipeptide, widely distributed in tissues including the animal and human brain, and can easily enter the central nervous system from the periphery [4]. Carnosine serves as a reservoir for histidine, which is a precursor of histamine [7]. It has many putative roles such as anti-inflammatory agent, free radical scavenger [1], and protein glycosylation inhibitor [10], and may serve as a neurotransmitter in the olfactory bulb [19]. However, little is known about the role of carnosine in the brain. Recent studies have showed that it can significantly inhibit amygdaloid kindled seizures in rats, and it simultaneously induces a significant increase in carnosine, histidine and histamine levels in the amygdala [12]. The protective effect of

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doi:10.1016/j.neulet.2007.01.075

carnosine may be partially mediated by the metabolic pathway carnosine–histidine–histamine in the brain. In the present study, we used both HDC-KO and WT mice to further elucidate the possible role of carnosine in PTZ-induced seizures, which is an animal model of human absence epilepsy and myoclonic, generalized tonic–clonic seizures.

HDC-KO and its WT (C57BL/6 strain) male mice were housed in individual cages in an air-conditioned room with controlled temperature (23 ± 31 °C) and humidity (40–70%) under a 12-h light/12-h dark cycle (lights on from 06:00 to 18:00). Water and food were available *ad libitum*. Behavioral experiments were carried out each day between 10:00 and 17:00. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

For recording the cortical EEG, all mice were anaesthetized with chloral hydrate (4%, w/v, at a dose of 10 ml/kg body weight) and mounted in a stereotaxic apparatus (WPI, 502600, Sarasota, FL, USA). Two screw electrodes were placed supradurally over the frontal cortex on each side (AP: −1 mm, L: ±1 mm), generating one cortical EEG bio-potential. The ground electrode was placed over the right occipital lobe. Electrodes were connected to a miniature receptacle, which was attached to the skull with dental cement, as previously described [12]. Animals were allowed to recover for at least a week in their home cages. The EEGs were recorded with the Powerlab system (AD instruments, NSW, Australia) 10 min before and 30 min after PTZ treatment.

PTZ was injected intraperitoneally (i.p.) at a dosage of 75 mg/kg, a dose which produced clonic seizures in all the control animals. Immediately after PTZ administration, the mice were placed in a clear plastic cage and observed for 30 min. Behavior was noted according to the following scale: Stage 0, no response; Stage 1, ear and facial twitching; Stage 2, myoclonic body jerks; Stage 3, clonic forelimb convulsions; Stage 4, generalized clonic convulsions and turning onto the side; Stage 5, generalized clonic–tonic convulsions; Stage 6, death within 30 min. Between subjects, the cage was thoroughly cleaned with wet/dry cloths and 70% ethanol to remove any olfactory cues.

Seizure thresholds were determined using the PTZ infusion method previously described [27] with minor modification. The animals were first lightly restrained in a transparent box with a hole, and a 30 gauge dental needle was inserted into the lateral tail vein. The needle was then secured to the tail with a narrow piece of adhesive tape. With the mouse moving freely, the PTZ solution (15 mg/kg) was infused into the tail vein at a constant rate of 0.3 ml/min using a Hamilton microsyringe (WPI, UMC4), which was connected to the dental needle by polyethylene tubing. Minimal doses of PTZ (mg/kg of mouse weight) needed to induce myoclonic jerks and clonic convulsive seizures were measured as the thresholds ($15 \times U/4000 \times W$, where U is the infusion unit recorded by the Hamilton microsyringe, and W is the mouse body weight) for myoclonic jerks and clonic convulsive seizures. The examiner was blind as to groups and treatments.

The drugs used were PTZ (Sigma, St. Louis, MO, USA), carnosine (Sigma), and α-fluoromethylhistidine (α-FMH, Merck Sharp & Dohme Research Laboratory, Rahway, NJ, USA). Drugs were all dissolved in saline and were administered i.p. in a volume not exceeding 10 ml/kg. Carnosine and α-FMH were injected i.p. 1 and 4 h before PTZ injection, respectively. There were no perceptible behavioral changes after carnosine injection at all doses used in the present study.

Statistical differences of thresholds and the EEG characteristics were evaluated using Student's *t*-test. Other analyses used one-way analysis of variance followed by Dunnett's *t*-test. Significance was set at $p < 0.05$. All values are given as mean ± S.E.M.

Carnosine decreased seizure stage and prolonged latency for onset of myoclonic jerks to PTZ-induced (75 mg/kg) seizures in a dose- and time-related manner in WT mice (Fig. 1). At a dose of 100 mg/kg, carnosine slightly inhibited the PTZ -induced seizures, while at doses of 200–1000 mg/kg it significantly decreased seizure stage and prolonged the latency for myoclonic jerks ($p < 0.05$, Fig. 1A and B). However, no significant protection by carnosine was found in HDC-KO mice at all doses used. The time-course of the protective effects of carnosine (500 mg/kg) on PTZ-induced seizures in WT mice reached a peak at 1 h (Fig. 1C and D). In addition, histidine (500 mg/kg, i.p.) significantly inhibited PTZ-induced seizures in WT mice but not in HDC-KO mice (data not shown).

In cortical EEG recordings, the earliest evidence of PTZ-induced epileptiform activity was elicitation of the first spike (myoclonic jerks), and the following pattern of activation included an early short period of multiple spikes (clonic), followed by high-frequency spikes (tonic), and then the EEG flattening at death [17]. We found that PTZ (75 mg/kg) induced epileptiform activity in all the experimental animals (Fig. 2A). Compared with the control group, carnosine (500 mg/kg) significantly delayed the latency for onset of the first spike (85 ± 10 s *versus* 58 ± 7 s; Fig. 2B). All types of epileptiform activity appeared synchronously in the control animals, while in most carnosine-treated mice, the high-frequency spikes (tonic) were not present (3.8 ± 0.4 *versus* 0.8 ± 0.4; Fig. 2C).

In addition, thresholds of myoclonic jerks and clonic convulsive seizures in HDC-KO mice were significantly lower than those in WT mice (Fig. 3). Carnosine (500 mg/kg) significantly elevated the thresholds of myoclonic jerks and clonic convulsive seizures in WT mice, but had no significant effect upon those in HDC-KO mice.

α-FMH, a selective and irreversible HDC inhibitor, significantly reversed the carnosine-induced anticonvulsive effect on PTZ-induced (75 mg/kg) seizures in WT mice (Fig. 4). At a dose of 10 mg/kg, it slightly increased seizure stage, but significantly shortened the latency for myoclonic jerks, while at a dose of 20 mg/kg, it significantly increased seizure stage and shortened the latency for myoclonic jerks. In addition, α-FMH alone had no significant influence on PTZ-induced (75 mg/kg) seizures in WT mice at the doses used in this study.

It has been demonstrated that carnosine can be metabolically transformed into histamine by carnosinase and HDC enzymes [6,16]. Recent data showed that carnosine protects against NMDA-induced necrosis in differentiated PC12 cells. The mechanism of this protection may involve the carnosine–histidine–histamine pathway [24]. Recently, we