



Carnosine, a precursor of histidine, ameliorates pentylenetetrazole-induced kindled seizures in rat

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Abstract

Carnosine (β -alanyl-L-histidine) has been characterized as a putative neurotransmitter. However, so far, understanding of the role of carnosine in the brain is very limited. The objective of this study was to examine the effects of carnosine on the development of pentylenetetrazol (PTZ) kindling seizures and protection against the PTZ kindled seizures in rats. Chemical kindling was elicited by repeated intraperitoneal injection of PTZ (35 mg/kg) once every 48 h until the occurrence of Stage 4–5 seizures, and the seizure activity of kindling was recorded for 30 min. In an acute PTZ challenge study, 60 mg/kg PTZ was used to induce kindled seizure. Injection of carnosine (200, 500 mg/kg, i.p.) significantly decreased seizure stage, and prolonged the latencies for myoclonic jerks, in a dose- and time-dependent manner. In the seizure development process, 500 mg/kg carnosine also significantly delayed the onset of PTZ kindled seizures. In addition, carnosine significantly reversed decreased histamine levels induced by PTZ kindled seizure in the hippocampus. These results indicate that carnosine can protect against PTZ-induced seizures in both the development of kindling and the challenge process in rats. The results suggest that carnosine might be an endogenous anticonvulsant factor in the brain and can be used as a new antiepileptic drug in future.

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The role of brain histamine in regulating seizure susceptibility has recently been documented, and an anticonvulsant action of endogenous histamine has been postulated [5,14,19,23,28]. Both histamine and histidine increase the threshold for amygdaloid kindling and pentylenetetrazol (PTZ)-induced seizures [4,14,27]. Several H₁ receptor antagonists, such as diphenhydramine and chlorpheniramine, occasionally induce convulsions in epileptic patients, healthy children and rodents [13,15,26]. We also reported that the seizure development induced by PTZ is facilitated in H₁ receptor knockout mice and histidine decarboxylase-deficient mice compared with wild-type mice [5].

However, histamine cannot cross the blood–brain barrier (BBB) and may be involved in brain inflammation [21]. The

infusion of histamine into rat substantia nigra results in an acute inflammatory response manifested by a loss of glial fibrillary acidic protein-immunolabeled astrocytes [24]. On the other hand, carnosine has been characterized as a putative neurotransmitter in olfactory receptor neurons [3]. Carnosine may be a potent protective agent in the CNS [2,11,22] and may play a role in neuron–glia interactions [16]. However, little is known about the role of carnosine in the brain. Carnosine serves as a reservoir for histidine, which is a precursor of histamine [8]. Given the relationship between carnosine and histamine, it is proposed that carnosine may be a new histaminergic drug and could be used clinically instead of histamine. We have recently reported that carnosine can prevent amygdaloid kindling seizures in rats [13]. Yet, there is limited information about the effects of carnosine on PTZ-induced kindling seizures, which is an animal model of human absence epilepsy and myoclonic, generalized tonic-clonic seizures. Therefore, our investigations were designed to elucidate the pharmacological mechanisms of the effects of carnosine on both the development of kindling seizures and the

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kindled seizures themselves following PTZ administration in rats.

All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals used in this study were male Sprague–Dawley rats (220–300 g, Grade II, Certificate No. 22-9601018, Experimental Animal Center, Zhejiang University), maintained in individual cages with a 12-h light–dark cycle (lights on from 8:00 to 20:00). Food and water were given ad libitum. Experiments were carried out each day between 12:00 and 17:00.

To induce kindling, a 35 mg/kg dose of PTZ (Sigma, St. Louis, MO, USA) was injected i.p. every 48 h [4,9,27]. After each PTZ treatment, rats were placed separately under glass funnels, the appearance of clonic and tonic seizures were recorded during individual observation for 30 min. Seizure intensities were classified as follows: 0: no response; Stage 1: ear and facial twitching; Stage 2: convulsive waves throughout the body; Stage 3: myoclonic jerks, rearing; Stage 4: turning over onto one side; Stage 5: turning over onto the back, generalized tonic-clonic seizures. In addition, the latency to the onset of myoclonic jerks was measured and analyzed. In an acute PTZ challenge study, 60 mg/kg PTZ was used to elicit kindled seizure. Each rat was treated by one dose of PTZ, and also measures for 30 min.

Carnosine was injected i.p. 2 h before PTZ treatment. Thirty min after PTZ acute treatment, rats were quickly sacrificed by decapitation. The brain was removed and placed on an ice-cold stainless steel plate, and subsequently dissected into cortex and hippocampus. The brain tissues were stored at -80°C until assayed. The brain tissue was homogenized (Polytron homogenizer, Kinematica, Lucern, Switzerland) in 3% perchloric acid containing 5 mM disodium EDTA and 5-hydro- N^{ω} -methyltryptamine and then centrifuged at $15,000 \times g$ for 20 min at 4°C . The tissue samples were analyzed by high-performance liquid chromatography (HPLC) combined with electrochemical detection using a technique developed in our laboratory [13]. The system consisted of a model 582 pump, a model 540 autosampler and a 4-channel CoulArray electrochemical detector (ESA, Chelmsford, MA, USA). The HPLC was controlled and the data acquired and analyzed using CoulArray[®] software. After reacting with the derivatative *o*-phthalaldehyde, analytes were separated on a $3 \mu\text{m}$, $3 \text{ mm} \times 50 \text{ mm}$ Capcell Pak MG C18 column (Shiseido, Tokyo, Japan). A two-component gradient elution system was used, with component A of the mobile phase being 100 mM Na_2HPO_4 , 13% acetonitrile, and 22% methanol, pH 6.8, and component B being similar to A except with 5.6% acetonitrile and 9.4% methanol. A gradient elution profile was used as follows: 0–3.5 min, isocratic 100% B; 3.5–20 min, linear ramp to 0% B; 20–22 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°C . The first cell was set at +250 mV, the second at +350 mV. The detection limit (signal/noise ≥ 3) was 1 ng for histamine.

All results are expressed as the mean \pm S.E.M. Statistical analyses used SPSS 11.5 for Windows. One-way analysis of variance (ANOVA) with Dunnett's test was used to calculate the significance. In the case of kindling seizure development, the

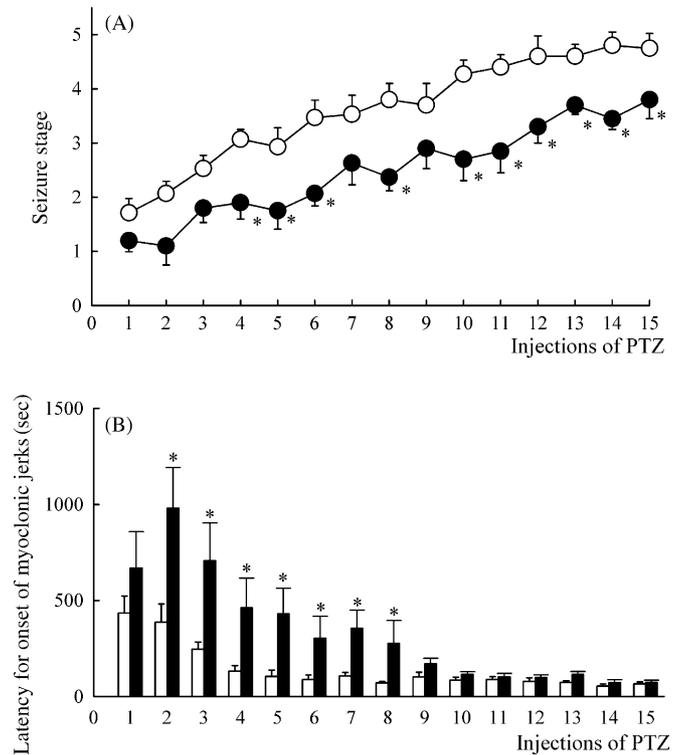


Fig. 1. Effect of carnosine on seizure development of PTZ (35 mg/kg) kindling in rats. Carnosine was injected i.p. 2 h before PTZ treatment. (A) Effect on seizure stage. Open circles indicate saline-treated group; closed circles indicate carnosine (500 mg/kg) treated group. (B) Effect on latency to onset of myoclonic jerks. Open bars: saline-treated group. Filled bars: carnosine-treated group. Each value represents the mean \pm S.E.M. from 11 to 14 rats. * $p < 0.05$ compared to control group.

Kruskal–Wallis test followed by multiple comparison (Bonferroni correction) was used to calculate significance.

Carnosine inhibited PTZ-induced seizure development ($p < 0.05$, Fig. 1). At a dose of 500 mg/kg it decreased PTZ-induced seizure stage, and prolonged the latency for myoclonic jerks ($p < 0.05$). In the acute PTZ challenge process, as shown in Fig. 2, carnosine also decreased the stage of PTZ kindled seizures dose- and time-dependently. At 100 mg/kg, it slightly inhibited the PTZ kindled seizures, but the effect did not reach significance. Carnosine at 200 mg/kg decreased seizure stage and prolonged the latencies for myoclonic jerks ($p < 0.05$) at 2 h after injection. At 500 mg/kg it inhibited the PTZ kindled seizures at 1 and 2 h after injection ($p < 0.05$). At 1000 mg/kg, significant effects on PTZ-induced seizures were not observed, resulting in a bell-shaped dose response curve. In addition, as shown in the Fig. 3, PTZ kindled seizure significantly decreased histamine levels both in the cortex and hippocampus. Carnosine (500 mg/kg) slightly increased the histamine level in the cortex and significantly increased histamine levels in the hippocampus ($p < 0.05$), at 1000 mg/kg, it significantly increased histamine levels both in the cortex and hippocampus ($p < 0.05$, Fig. 3).

In the present study, we provide the first evidence that carnosine significantly protected against both the development and process of PTZ-induced kindled seizures. Previous data showed that long-term treatment with a low histamine diet

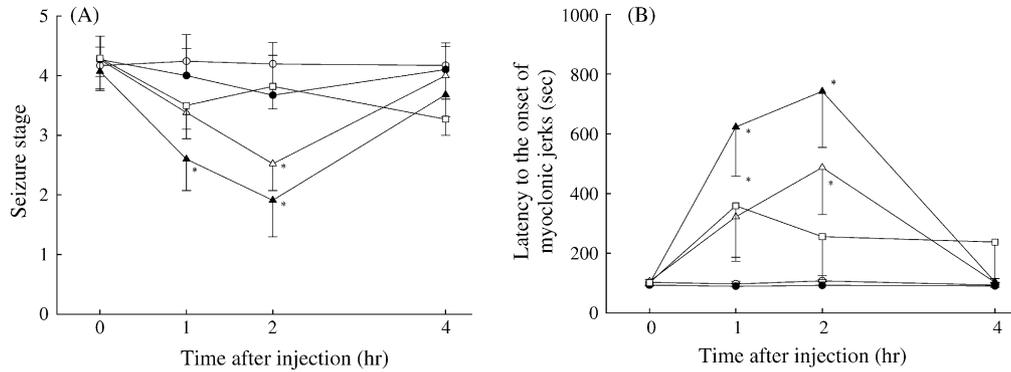


Fig. 2. Dose- and time-dependent effects of carnosine on PTZ (60 mg/kg) kindled seizures in rats. Carnosine was injected i.p. 2 h before PTZ acute treatment. (A) Effects on seizure stage. (B) Effects on latency to onset of myoclonic jerks. The carnosine-treated groups were given doses of 100 (closed circles), 200 (open triangles) or 500 mg/kg (closed triangles). Controls were given saline (open circles). Each value represents the mean \pm S.E.M. from 11 to 14 rats. * $p < 0.05$ compared to control group.

enhances seizure development induced by PTZ kindling [12], while this effect can be reversed by simultaneous treatment with a high carnosine diet (data not shown). Because carnosine can easily cross the BBB and has few side effects [10], it may be a new potential anticonvulsant drug for clinical therapy of human absence epilepsy and myoclonic, generalized tonic-clonic seizures.

Our previous data showed that carnosine at doses of 500, 1000 and 1500 mg/kg significantly decreases seizure stage and afterdischarge duration in amygdaloid kindled seizures, in a dose-dependent and time-related manner (peaking at 1 h), and increases hippocampal histamine levels, which also peak at 1 h [13]. However, in the present study, carnosine at the lower doses of 200 and 500 mg/kg protected against PTZ kindled seizures with a peak at 2 h. The inhibition showed a bell-shaped curve, decreasing at the higher doses of 1000 mg/kg at which carnosine markedly increased histamine levels both in the cortex and hippocampus. It is hard to explain why it produced a bell-shaped effect. Wada et al. also reported histidine can suppress kindled seizure generalization, whereas it accelerates the development of kindling, suggesting a biphasic action of histamine in the kindling model [25]. This may be due to different mechanisms underlying the different seizures. Further studies are needed, therefore, to examine the bell-shaped curve of carnosine effects in the kindling model. These findings at least suggest that dif-

ferent doses of carnosine may contribute to the treatment of different types of epilepsy.

It was interesting to find that an acute PTZ treatment significantly decreased histamine levels both in the cortex (33.5%) and hippocampus (29.4%), and in contrast that carnosine (500 mg/kg, a dose that inhibited PTZ kindled seizures) simultaneously caused a marked increase of histamine levels in the hippocampus. However, cortical levels of histamine did not significantly change. Previous data also showed that carnosine only causes a marked increase of histamine levels in the hippocampus and amygdala but not cortex [13]. This finding suggests that the carnosine-induced antiseizure action is dependent on an increase in histamine levels in the hippocampus, although it is well known that the cortex plays an important role in electrically kindled seizures. For example, we have also reported that once fully kindled by PTZ, this type of seizure is sensitive to histamine levels in the cortex [13]. We have no further data to explain these phenomena. In addition, it is reported that carnosine inhibits the activity of histidine decarboxylase partially purified from whole bodies of fetal rats [18], which suggest that carnosine may decrease histamine level. However, carnosine increased histamine level in the hippocampus after PTZ acute treatment. It may be that the activity of histidine decarboxylase influenced by carnosine may be brain region-dependent; secondary that, the stress of PTZ acute treatment may change the enzyme sen-

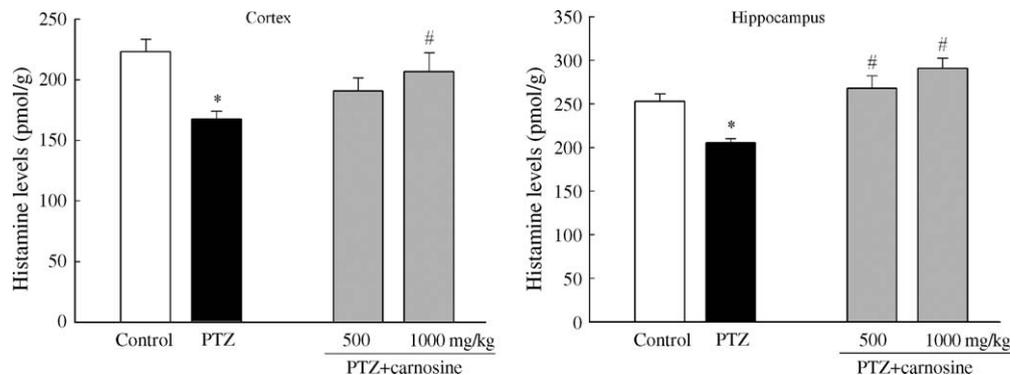


Fig. 3. Effects of carnosine on histamine levels in cortex and hippocampus of rats. Carnosine was injected i.p. 2 h before PTZ treatment. Thirty minutes after PTZ acute treatment, rats were quickly sacrificed by decapitation. Each value represents the mean \pm S.E.M. of 8–10 rats. * $p < 0.05$ compared to control group. # $p < 0.05$ compared to PTZ group.

sitivity to carnosine. Therefore, the observed effect of carnosine is, at least in part, due to increased histamine synthesis in the hippocampus. Thus, these results provide additional evidences to support the idea that carnosine can be metabolically transformed into histamine in the CNS. Further biochemical studies are needed to investigate the mechanism.

To date, carnosine has been proposed to provide a non-mast cell source of histidine in many histamine-rich tissues, available for histamine synthesis during periods of physiological stress [6]. Carnosine (via histidine) may be metabolically transformed into histamine [7,10]. Evidence of inflammatory processes in the clinical manifestations and neuropathological sequelae of epilepsy have accumulated in the last decade [17]. So, it may be necessary to simultaneously provide anti-inflammatory treatment when carrying out anticonvulsant therapy. Carnosine is a powerful anti-inflammatory, presumably due to its anti-oxidant and anti-glycation properties [1,20]. We also found that chronic treatment with carnosine can decrease TNF- α in the hippocampus (data not shown). Therefore, the remarkable anti-inflammatory and anticonvulsant effects of carnosine reflect the high potential therapeutic value of this compound as an antiepileptic drug.

In summary, our experiments provide preliminary evidence that carnosine has a significant anticonvulsant effect on PTZ-kindled seizures in rats. The present study indicates that carnosine may be an endogenous anticonvulsant factor in the brain and calls for its further study as a potentially efficient antiepileptic drug.

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References

- [1] A. Boldyrev, S. Severin, The histidine-containing dipeptides, carnosine and anserine: distribution, properties and biological significance, *Adv. Enzyme Regul.* 30 (1990) 175–194.
- [2] A. Boldyrev, R. Song, D. Lawrence, D.O. Carpenter, Carnosine protects against excitotoxic cell death independently of effects on reactive oxygen species, *Neuroscience* 94 (1999) 571–577.
- [3] L. Bonfanti, P. Peretto, S. De Marchis, A. Fasolo, Carnosine-related dipeptides in the mammalian brain, *Prog. Neurobiol.* 59 (1999) 333–353.
- [4] Z. Chen, W.D. Li, L.J. Zhu, Y.J. Shen, E.Q. Wei, Effects of histidine, a precursor of histamine, on pentylenetetrazole-induced seizures in rats, *Acta Pharmacol. Sin.* 23 (2002) 361–366.
- [5] Z. Chen, Z.Y. Li, E. Sakurai, J.I. Mobarakeh, H. Ohtsu, T. Watanabe, T. Watanabe, K. Iinuma, K. Yanai, Chemical kindling induced by pentylenetetrazol in histamine H1 receptor gene knockout mice (H1KO), histidine decarboxylase-deficient mice (HDC^{-/-}) and mast cell-deficient W/W^v mice, *Brain Res.* 968 (2003) 162–166.
- [6] D.W. Fitzpatrick, H. Fisher, Histamine synthesis, imidazole dipeptides, and wound healing, *Surgery* 91 (1982) 430–434.
- [7] J.C. Fitzpatrick, H. Fisher, L. Flanbaum, Mobilization of renal carnosine and histidine to histamine during compound-48/80-induced shock, *Nephron* 59 (1991) 299–303.
- [8] L. Flanbaum, J.C. Fitzpatrick, D.N. Brotman, A.M. Marcoux, E. Kasziba, H. Fisher, The presence and significance of carnosine in histamine-containing tissues of several mammalian species, *Agents Actions* 31 (1990) 190–196.
- [9] D. Getova, W. Froestl, N.G. Bowery, Effects of GABAB receptor antagonism on the development of pentylenetetrazol-induced kindling in mice, *Brain Res.* 809 (1998) 182–188.
- [10] S.M. Greene, F.L. Margolis, M. Grillo, H. Fisher, Enhanced carnosine (beta-alanyl-L-histidine) breakdown and histamine metabolism following treatment with compound 48/80, *Eur. J. Pharmacol.* 99 (1984) 79–84.
- [11] A.R. Hipkiss, Glycation, ageing and carnosine: are carnivorous diets beneficial? *Mech. Ageing Dev.* 126 (2005) 1034–1039.
- [12] C.L. Jin, E. Sakurai, Y. Kiso, J.H. Luo, K. Yanai, Z. Chen, Influence of low dietary histamine on seizure development of chemical kindling induced by pentylenetetrazol in rats, *Acta Pharmacol. Sin.* 26 (2005) 423–427.
- [13] C.L. Jin, L.X. Yang, X.H. Wu, Q. Li, M.P. Ding, Y.Y. Fan, W.P. Zhang, J.H. Luo, Z. Chen, Effects of carnosine on amygdaloid kindled seizures in Sprague-Dawley rats, *Neuroscience* 135 (2005) 939–947.
- [14] C. Kamei, K. Ishizawa, H. Kakinoki, M. Fukunaga, Histaminergic mechanisms in amygdaloid-kindled seizures in rats, *Epilepsy Res.* 30 (1998) 187–194.
- [15] C. Kamei, M. Ohuchi, Y. Sugimoto, C. Okuma, Mechanism responsible for epileptogenic activity by first-generation H1-antagonists in rats, *Brain Res.* 887 (2000) 183–186.
- [16] S.D. Marchis, C. Modena, P. Peretto, A. Migheli, F.L. Margolis, A. Fasolo, Carnosine-related dipeptides in neurons and glia, *Biochemistry (Mosc)* 65 (2000) 824–833.
- [17] M. Oprica, C. Eriksson, M. Schultzberg, Inflammatory mechanisms associated with brain damage induced by kainic acid with special reference to the interleukin-1 system, *J. Cell Mol. Med.* 7 (2003) 127–140.
- [18] Y. Sakamoto, T. Watanabe, H. Hayashi, Y. Taguchi, H. Wada, Effects of various compounds on histidine decarboxylase activity: active site mapping, *Agents Actions* (1985) 32–37.
- [19] R. Scherkl, A. Hashem, H.H. Frey, Histamine in brain-its role in regulation of seizure susceptibility, *Epilepsy Res.* 10 (1991) 111–118.
- [20] N.W. Seidler, G.S. Yeagans, Effects of thermal denaturation on protein glycation, *Life Sci.* 70 (2002) 1789–1799.
- [21] A.J. Silverman, A.K. Sutherland, M. Wilhelm, R. Silver, Mast cells migrate from blood to brain, *J. Neurosci.* 20 (2000) 401–408.
- [22] R. Tabakman, P. Lazarovici, R. Kohen, Neuroprotective effects of carnosine and homocarnosine on pheochromocytoma PC12 cells exposed to ischemia, *J. Neurosci. Res.* 68 (2002) 463–469.
- [23] L. Tuomisto, U. Tacke, Is histamine an anticonvulsive inhibitory transmitter? *Neuropharmacology* 25 (1986) 955–958.
- [24] M.L. Vizuete, M. Merino, J.L. Venero, M. Santiago, J. Cano, A. Machado, Histamine infusion induces a selective dopaminergic neuronal death along with an inflammatory reaction in rat substantia nigra, *J. Neurochem.* 75 (2000) 540–552.
- [25] Y. Wada, J. Shiraishi, M. Nakamura, Y. Koshino, Biphasic action of the histamine precursor L-histidine in the rat kindling model of epilepsy, *Neurosci. Lett.* 204 (1996) 205–208.
- [26] H. Yokoyama, S. Mitsumoto, S. Iinuma, K. Onodera, T. Watanabe, Central acting histamine H1 antagonists promote the development of amygdala kindling in rats, *Neurosci. Lett.* 217 (1996) 194–196.
- [27] L. Zhang, Z. Chen, Y. Huang, W. Hu, E. Wei, K. Yanai, Effects of endogenous histamine on seizure development of pentylenetetrazole-induced kindling in rats, *Pharmacology* 69 (2003) 27–32.
- [28] L. Zhang, Z. Chen, K. Ren, R. Leurs, J. Chen, W. Zhang, B. Ye, E. Wei, H. Timmerman, Effects of clobenpropit on pentylenetetrazole-kindled seizures in rats, *Eur. J. Pharmacol.* 482 (2003) 169–175.