Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Effects of carnosine on the evoked potentials in hippocampal CA1 region*

Zhou-yan FENG†, Xiao-jing ZHENG, Jing WANG

(*Key Lab of Biomedical Engineering of Ministry of Education, College of Biomedical Engineering and Instrument Science, Zhejiang University, Hangzhou 310003, China*)

†E-mail: hnfzy@yahoo.com.cn

Received Nov. 22, 2008; Revision accepted Mar. 25, 2009; Crosschecked Apr. 28, 2009

Abstract: Objective: To directly examine the effects of carnosine on neuronal excitation and inhibition in rat hippocampus in vivo. Methods: Artificial cerebrospinal fluid with carnosine was directly administrated over the exposed rat hippocampus. The changes of neuron activity in the CA1 region of hippocampus were evaluated by orthodromically- and antidromically-evoked potentials, as well as paired-pulse stimulation paradigm. Results: In both orthodromic and antidromic response potentials, carnosine transformed population spikes (PSs) with single spike into epileptiform multiple spikes. In addition, similar to the effect of γ-aminobutyric $acid_A$ ($GABA_A$) antagonist picrotoxin, carnosine decreased paired-pulse stimulating depression significantly. However, no significant change was observed in the spontaneous field potentials during the application of carnosine. Conclusion: The results indicate a disinhibition-induced excitation effect of carnosine on the CA1 pyramidal neurons. It provides important information against the application of carnosine as a potential anticonvulsant in clinical treatment.

Key words: Carnosine, Hippocampus, Evoked potential, Epileptic seizure
doi:10.1631/jzus.B0820370 **Document code:** A **CLC number:** R742

INTRODUCTION

Carnosine (β-alanyl-L-histidine), a dipeptide, is rich in the brains of mammals (Bonfanti *et al*., 1999). It was even regarded as a putative neurotransmitter in the olfactory receptor neurons (Margolis, 1974; Nicoll *et al*., 1980). Because it can penetrate the blood-brain barrier (BBB) easily and has few side effects, the therapeutic potentials of carnosine to some brain disorders have attracted great attention. Recent investigations have shown that carnosine has inhibitory effects on both electric stimulation-kindled seizures and chemically-induced seizures (Jin *et al*., 2005; Wu *et al*., 2006; Zhu *et al*., 2007). It suggests that carnosine may be used as a new anticonvulsant to treat epilepsy.

Certainly, the inhibitory effects of carnosine in the brain have been observed. For example, carnosine can significantly decrease the glutamate release in some brain regions, such as the amygdala and hippocampus (Jin *et al*., 2005; Shen *et al*., 2007). It can also decrease the seizure duration and prolong the seizure latency (Jin *et al*., 2005; Wu *et al*., 2006; Zhu *et al*., 2007). However, other literatures have reported excitatory effects of carnosine on neuron activity. For instance, whole-cell voltage-clamp experiments showed that the use of carnosine caused excitatory inward current responses in the olfactory bulb neurons of cultured slices (Kanaki *et al*., 1997). In addition, carnosine induced sustained excitatory depolarization in olfactory bulb cells and caused an excitatory oscillation in the evoked potentials in vivo (Gonzalez-Estrada and Freeman, 1980). Intracerebroventricular injection of carnosine also induced hyperactivity and increased vocalization in chicks

* Project (Nos. 30570585 and 30770548) supported by the National Natural Science Foundation of China

(Tomonaga *et al*., 2004; 2005). Therefore, it is not yet clear whether carnosine is excitatory or inhibitory to neurons.

In order to further directly investigate the effects of carnosine on the neuronal excitation and inhibition, in the present study artificial cerebrospinal fluid (ACSF) with carnosine was directly administrated over the exposed rat hippocampus. The changes of neuron activity in the CA1 region of the hippocampus were evaluated by orthodromically- and antidromically-evoked potentials, as well as paired-pulse stimulation paradigm. Orthodromically-evoked response reflects both the neuronal excitability and the excitatory synaptic transmission, whereas antidromically-evoked response reflects mainly the neuronal excitability (Andersen *et al*., 1971; 2000). In addition, the ratio of the paired-pulse response amplitudes can reflect the GABAergic inhibition activity from inhibitory inter-neurons in the CA1 region (Davies *et al*., 1990). Therefore, the results of this study could reveal additional information of the effects of carnosine on neuron activity, which is important to the development of new anticonvulsants.

MATERIALS AND METHODS

Surgical procedures and experimental protocol

All procedures used in this study were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Health, China). An in vivo solution administration method was used to take the advantage of intact neuronal circuits in rat hippocampus (Feng and Durand, 2003). Briefly, adult Sprague Dawley rats (200~350 g; n=24) were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic apparatus (Stoelting Co., Wood Dale, Illinois, USA). Body temperature was maintained at ~37 °C. The skull over the left cortex was opened, and the neocortex overlying the left dorsal hippocampus was removed. Immediately after the exposure of the hippocampus, control ACSF was placed over the surface of the exposed dorsal hippocampus. After electrode placements and baseline recordings, ACSF with carnosine or picrotoxin was perfused over the hippocampus to examine the effects of these drugs for 30 min. Finally, the perfusion solution was switched back to a control ACSF.

Solutions and drugs

Control ACSF consisted of (in mmol/L): 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1.5 $MgSO_4$, 26 $NaHCO_3$, and 2 g/L D-glucose. Carnosine or γ-aminobutyric $acid_A$ ($GABA_A$) antagonist picrotoxin (PTX) was added into control ACSF respectively to make carnosine solutions (25, 50, and 100 mmol/L) or PTX solution (0.1 mmol/L). Carnosine and PTX were obtained from Sigma (USA), and other chemicals from China National Pharmaceutical Group.

Electric field potential recording in the hippocampal CA1 region

The field potential recording protocol was similar to previous preparations (Feng and Durand, 2006). Briefly, silicon electrode arrays ordered from NeuroNexus Technologies, Ann Arbor, Michigan, USA were used for extracellular recording of the evoked and spontaneous field potentials in the CA1 region of the hippocampus. Bipolar stimulating electrodes were made from pairs of insulated nichrome wires (a diameter of 80 μm) with a ~0.5 mm vertical tip separation. The recording electrode (RE) was positioned in the exposed left hippocampal CA1 area (AP, –3.0; ML, 2.6). One stimulating electrode (SE1) was inserted into the Schaffer collaterals of the same side (AP, –2.0; ML, 2.3) for orthodromically stimulating the CA1 pyramidal neurons. Another stimulating electrode (SE2) was put on the alveus of the exposed hippocampus (AP, –4.0; ML, 2.5) for antidromically stimulating the CA1 neurons. Two separate stainless steel screws were fixed in the bone of nose and served as reference electrode and ground electrode, respectively. Patterns of the evoked potentials guided vertical positioning of the recording probe and the stimulating electrodes (Kloosterman *et al*., 2001).

Single stimulus pulse with 0.1-ms duration and 0.30 to 0.40-mA amplitude delivered by 2100 isolated pulse stimulator (A-M System Inc., Carlsborg, WA, USA) was used to orthodromic and antidromic stimulations in the CA1 region. The paired-pulse depression (PPD) was measured by paired-pulse stimuli with a 50-ms interval to evaluate the effects of GABAergic recurrent inhibition in the CA1.

Four-channel amplifiers (Model 1700, A-M System Inc.) were used to amplify the field potential signals. The filter frequency ranges were set at 0.1 to

5 kHz for both spontaneous and evoked potential recordings. The analog signals were then sampled at a rate of 20 kHz by using a data-acquisition system (PowerLab ML795, AD Instruments Inc., Castle Hill, NSW, Australia) and stored in hard disks for offline analysis.

Data analysis

Both orthodromically- and antidromically-evoked population spikes (PSs) that recorded in the CA1 pyramidal layer were used to evaluate the effects of chemicals. During baseline recordings in a control ACSF, PSs were always characterized by a single large negative peak. Thus, the amplitude of orthodromic PS was measured as the average of the potential differences of the negative PS peak from the preceding and following positive peaks, while the amplitude of antidromic PS was measured by the potential difference between the onset of the spike and the negative PS peak. During the periods of drug applications, evoked PSs could have multiple spike peaks. In this case, both the amplitude of the "first spike" and the amplitude of the "other spikes" (i.e., the sum amplitude of the second and third spikes, and so on) in the evoked PSs were calculated. The amplitude of the "total spikes" in the PS was the sum of the amplitudes of the "first spike" and the "other spikes." Paired-pulse ratio (PPR), defined as the ratio of the amplitude of the second PS to that of the first PS, was calculated to evaluate PPD.

To eliminate the influences of individual differences of animals, normalized amplitudes of the evoked PS were calculated by dividing every absolute amplitude value by the amplitude of the corresponding baseline recordings within the same experimental preparation.

Data are expressed as the mean±standard deviation. One-way analysis of variance (ANOVA) and Bonferroni's post-hoc test, as well as Student's *t*-test, were applied for statistical comparisons among different experimental groups.

RESULTS

Effect of carnosine on the evoked potentials in hippocampal CA1 region

As shown in Fig.1, following the baseline recording of evoked potentials, the control ACSF on the exposed dorsal hippocampus was replaced by an ACSF with 100 mmol/L carnosine. During the 30 min period of the application of 100 mmol/L carnosine, both the orthodromically- and antidromically-evoked PSs changed rapidly from initial single spike potentials into multiple spike potentials. The average numbers of spikes in the orthodromic and antidromic responses were 3.17±1.83 and 2.67±0.52, respectively. Both of them were statistically different

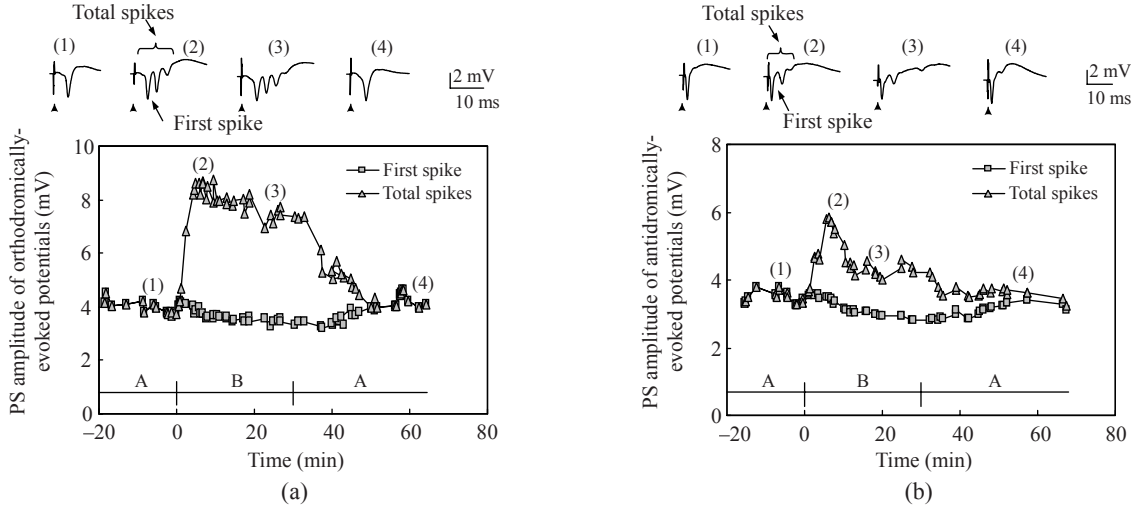

Fig.1 Changes of the orthodromically- and antidromically-evoked PSs during the application of 100 mmol/L carnosine
The bars in the bottom of each plot indicate the period of the carnosine application (A: normal ACSF; B: ACSF with carnosine 100 mmol/L). Examples of the evoked potentials collected during different periods are shown on the top, within which arrow heads indicate stimulation artifacts. (a) The addition of carnosine in ACSF rapidly transformed single spike orthodromic PS potentials into multiple spikes. The amplitude of total spikes increased significantly. However, the amplitude of the first spike in the evoked potentials decreased slightly; (b) Similar changes were observed in the antidromically-evoked PS potentials

from the single spike responses in the baseline recording (*t*-test, $P<0.01$, $n=6$). The amplitude of the "total spikes" in the evoked PSs increased rapidly during the early period, while the amplitude of the "first spike" decreased slowly. When the solution exchanged back to control ACSF, the orthodromic and antidromic PSs both gradually returned to single spike potentials similar to the baseline recordings.

The effects of different doses of carnosine at 25, 50, and 100 mmol/L on both the orthodromically- and antidromically-evoked PS potentials were investigated by using the same experimental protocol (Fig.2). To identify the relatively quick effects and slow effects separately, the PS responses during the first 5 min and late 20~30 min periods following the application of carnosine were evaluated.

For the orthodromic PSs (Fig.2a), during both the first 5 min and 20~30 min, there were significant dose-dependent increases in the amplitudes of the "total spikes" (ANOVA, $F>8.9$, $P<0.003$, $n=6$). These significant changes were mainly resulted from the increases of the amplitudes of "other spikes." In addition, for each of the doses, the amplitudes of the "other spikes" in the 20~30 min period were all significantly larger than those in the first 5 min period (*t*-test, $P<0.05$, $n=6$). The "first spike" did not have significant differences among different doses. However, comparisons between the data in the two periods show that for the 100 mmol/L dose, the amplitude of the "first spike" in the 20~30 min period was significantly smaller than that in the first 5 min period (*t*-test, $P<0.01$, $n=6$).

For the antidromic PSs (Fig.2b), there were significant dose-dependent decreases in the amplitudes of "first spike" during the 20~30 min period (ANOVA, $F=5.0$, $P=0.02$, $n=6$). However, the amplitudes of the "other spikes" in both the first 5 min and 20~30 min periods showed significant dose-dependent increases (ANOVA, $F>4.8$, $P<0.03$, $n=6$). The decrease in the "first spike" together with the increase in the "other spikes" resulted in the non-monotone amplitude changes of the "total spikes" in both the first 5 min and 20~30 min periods. Comparisons between the data in the two periods show that, for each of the doses, the amplitudes of the "first spike" in the 20~30 min period were all significantly smaller than those in the first 5 min period (*t*-test, $P<0.03$, $n=6$).

These results show that carnosine induced epileptic multiple spike activity in the evoked PSs, which suggests its excitation and/or disinhibition effect on the CA1 neurons. To further investigate the effect of carnosine, a paired-pulse stimulation paradigm was used.

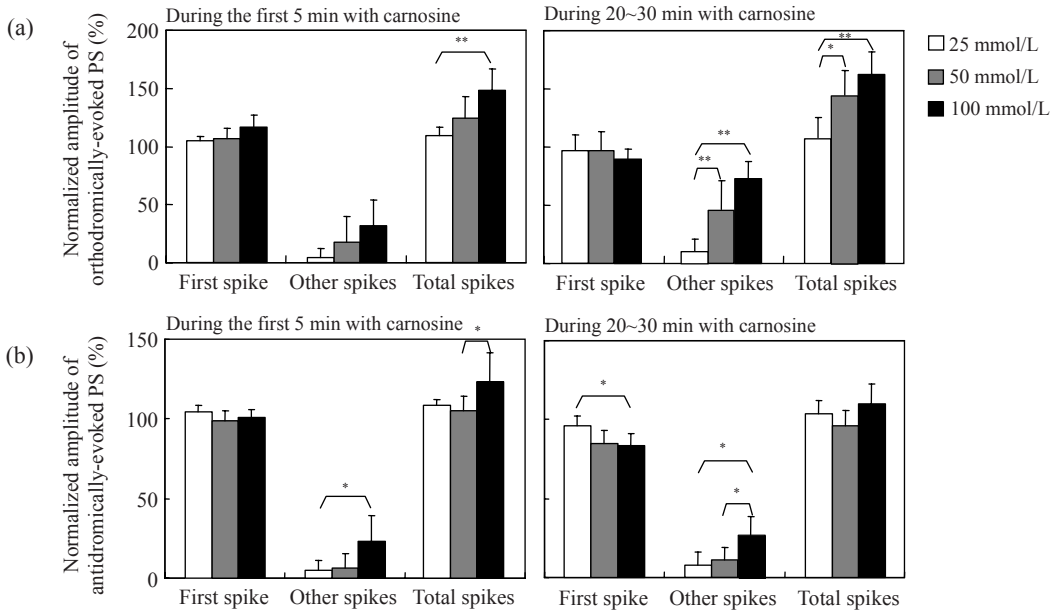

Fig.2 Changes of the evoked PS potentials by different doses of carnosine

(a) Orthodromically- and (b) antidromically-evoked PSs during the first 5 min and 20~30 min following the applications of 25, 50, and 100 mmol/L carnosine. Each panel shows the normalized amplitudes of the first spike, the other spikes and the total spikes. * $P<0.05$, ** $P<0.01$ (ANOVA and Bonferroni's post-hoc test, $n=6$)

Effect of carnosine on the paired-pulse depression in the orthodromically-evoked potentials

The appearance of multiple spike PSs following the application of carnosine was similar to the multiple spike activity induced by blocking GABAergic inhibition using $GABA_A$ antagonist, e.g., PTX. Therefore, the effect of carnosine on inter-neuron inhibition activity in the CA1 region was investigated by a paired-pulse paradigm.

Under normal physiological condition, a first orthodromic stimulus in the Schaffer collateral with enough current intensity will evoke a large amplitude PS in the CA1 pyramidal layer. However, due to GABAergic inhibition (Kapur *et al*., 1989; Tuff *et al*., 1983), following the first one and within an inter-pulse interval (IPI) of less than 100 ms, a second same stimulus will induce only a PS response with much smaller amplitude than the first one, and sometimes such a PS response even disappeared. In this study, paired-pulse stimuli with IPI of 50 ms were used to evaluate the effect of high concentration carnosine (100 mmol/L) on PPD. PTX (0.1 mmol/L) was used in other experimental preparations for comparison.

During baseline recordings, PPD was significant in the orthodromically-evoked PS with a single spike, but not in the antidromically-evoked PS (Fig.3a). Following 10-min application of 100 mmol/L carnosine, multiple spikes appeared in the evoked PSs and the second evoked PS increased in the orthodromic paired-pulse responses, indicating a decrease of PPD. No significant change was observed in spontaneous potential recording.

Similar to the carnosine group, the baseline recordings in the PTX group also had significant PPD in the orthodromic PS (Fig.3b). Following 10-min application of 0.1 mmol/L PTX, the PPD decreased significantly. At the same time, an afterdischarge followed the paired-pulse evoked potentials. Short bursts (i.e., interictal activity) also appeared in the spontaneous potential recording.

PPRs for both the "first spike" and "total spikes" amplitudes were calculated to evaluate PPD (Fig.4). Because the baseline evoked PS potentials in control ACSF always had a single spike, the PPR of the "total spikes" was equal to the PPR of the "first spike" for the baseline recordings. For both the carnosine and PTX groups, the PPRs of the "first spike" and the

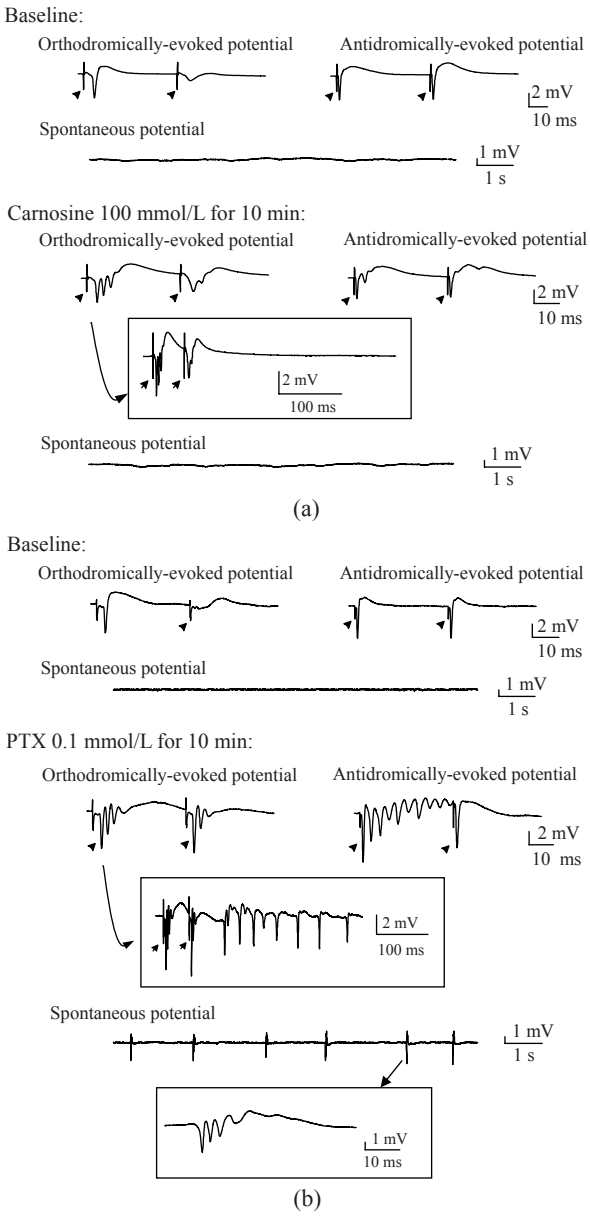

Fig.3 Comparisons between the effects of carnosine and PTX on both the evoked and spontaneous potentials in the CA1 region

(a) The effects of carnosine. Top: baseline paired-pulse orthodromic and antidromic PSs with a 50 ms paired-pulse stimulation interval. Bottom: 10 min following the application of 100 mmol/L carnosine, both orthodromic and antidromic PSs became as multiple spikes. The second response in the orthodromic PS increased, indicating a decrease of PPD. The spontaneous potential remained similar to baseline recording; (b) The effects of PTX. Top: baseline recording similar to (a). Bottom: 10 min following the application of 0.1 mmol/L PTX, evoked PSs also became as multiple spikes and followed by an afterdischarge (inset). The PPD in the orthodromic response decreased significantly. Short bursts appeared in the spontaneous potential (inset)

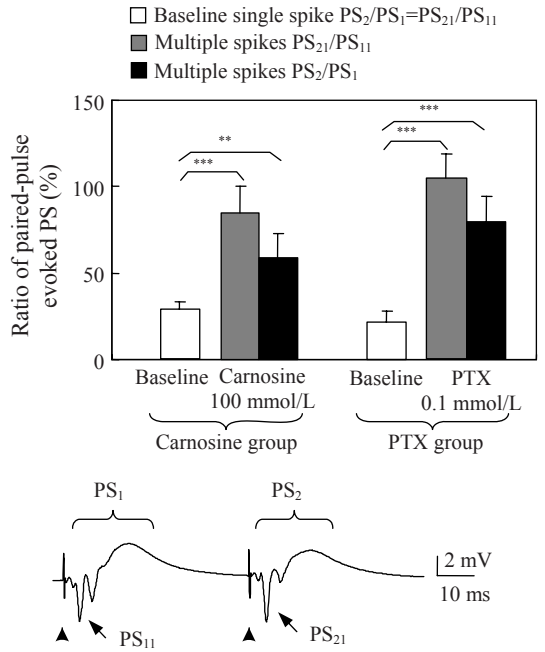

Fig.4 Effects of carnosine and PTX on the paired-pulse depression (PPD) in the orthodromically-evoked PS potentials with 50 ms paired-pulse stimulation intervals

The "first spike" ratio (PS_{21}/PS_{11}) and the "total spikes" ratio (PS_2/PS_1) were calculated for the evoked responses with multiple spikes. Note that for the baseline recording response with only single spike, the "total spikes" ratio is equal to the "first spike" ratio. $^{**}P<0.01$; $^{***}P<0.001$ (*t*-test, $n=6$)

"total spikes" amplitudes were all significantly larger than their corresponding baseline recordings (*t*-test, $P<0.01$, $n=6$). This result indicates that carnosine decreased the inhibition activity in the CA1 region.

DISCUSSION

The novel findings of the study are: (1) Carnosine transformed single spike PSs into multiple spike PSs in both orthodromically- and antidromically-evoked responses in the hippocampal CA1 region. However, with the prolonged carnosine the first spike in the multiple spike PSs decreased rather than increased. (2) Similar to the effect of $GABA_A$ antagonist PTX, carnosine decreased paired-pulse depression significantly. On the other hand, dissimilar to the short bursts induced by PTX in the spontaneous potentials, no significant change was observed in the spontaneous potentials by carnosine.

Both the appearance of epileptiform activity with multiple spikes in the evoked responses and the decrease of PPD in the paired-pulse stimulation paradigm indicated the enhancement of the excitation of the principal neurons in the CA1 region by carnosine. These results are similar to the excitatory effect of extracellular high potassium concentration on the hippocampal neurons (Korn *et al*., 1987; Rutecki *et al*., 1985) and the effect of blockade in inhibitory synaptic input by GABAergic antagonist such as PTX (Muller and Misgeld, 1991). Since the first spike in the multiple spike evoked PSs did not increase, the enhancement of excitation was presumably induced by disinhibition of interneuron activity in the hippocampus. The observations of the direct effect of carnosine on CA1 neurons are consistent with the previously reported excitatory effects of carnosine on neuron activity in the olfactory bulb neurons in vitro (Kanaki *et al*., 1997) and in vivo (Gonzalez-Estrada and Freeman, 1980).

However, different from the appearance of spontaneous epileptiform burst induced by the PTX in this study or by high concentration of potassium (Korn *et al*., 1987; Rutecki *et al*., 1985), carnosine did not induce any spontaneous epileptiform activity in the spontaneous field potential recordings. This may be due to the insufficient strength of disinhibition effect of carnosine; however, it may also have an alternative explanation. According to the decrease rather than increase of the first spike in the evoked response following prolonged application of carnosine, it is presumable that carnosine might have inhibitory effects on both principal pyramidal neurons and interneurons in the hippocampal CA1 region, such as a decrease of glutamate release (Jin *et al*., 2005; Shen *et al*., 2007). The inhibitory action on the pyramidal neurons could decrease the first spike in the evoked response and thereby prevent the occurrence of spontaneous bursts. Meanwhile, the inhibitory action on the interneurons could decrease local GABAergic inhibitory synaptic input to the pyramidal neurons (Andersen *et al*., 1971; Karnup and Stelzer, 1999) and thereby indirectly increase the evoked activity of the pyramidal neurons. The total effects from these two actions could lead to the present observations that the responses were dominated by a disinhibition effect.

In addition, the high concentrations (25~100 mmol/L) of carnosine used in this study are consistent with some previously reported in vivo preparations by

using i.p. doses of 200~1500 mg/kg (Jin *et al*., 2005; Wu *et al*., 2006; Zhu *et al*., 2007). Since carnosine can penetrate blood-brain barrier easily (Crush, 1970), the corresponding concentrations in the brain of these i.p. administrations of carnosine are estimated in a range of 30~200 mmol/L (Zhang and Zhang, 2004).

In conclusion, the direct administration of carnosine on the hippocampus revealed a disinhibition-induced excitatory effect on the CA1 pyramidal neurons. It provides important information against the application of carnosine as a potential anticonvulsant in clinical treatment.

References

Andersen, P., Bliss, T.V.P., Skrede, K.K., 1971. Lamellar organization of hippocampal excitatory pathways. *Exp. Brain. Res*., **13**(2):222-238. [doi:10.1007/BF00234087]

Andersen, P., Soleng, A.F., Raastad, M., 2000. The hippocampal lamella hypothesis revisited. *Brain Res*., **886**(1-2):165-171. [doi:10.1016/S0006-8993(00)02991-7]

Bonfanti, L., Peretto, P., de Marchis, S., Fasolo, A., 1999. Carnosine-related dipeptides in the mammalian brain. *Progr. Neurobiol.*, **59**(4):333-353. [doi:10.1016/S0301-0082(99)00010-6]

Crush, K.G., 1970. Carnosine and related substances in animal tissues. *Comp. Biochem. Physiol.*, **34**(1):3-30. [doi:10.1016/0010-406X(70)90049-6]

Davies, C.H., Davies, S.N., Collingridge, G.L., 1990. Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *J. Physiol*., **424**:513-531.

Feng, Z., Durand, D.M., 2003. Low-calcium epileptiform activity in the hippocampus in vivo. *J. Neurophysiol*., **90**(4):2253-2260. [doi:10.1152/jn.00241.2003]

Feng, Z., Durand, D.M., 2006. Effects of potassium concentration on firing patterns of low-calcium epileptiform activity in anesthetized rat hippocampus: inducing of persistent spike activity. *Epilepsia*, **47**(4):727-736. [doi:10.1111/j.1528-1167.2006.00499.x]

Gonzalez-Estrada, M.T., Freeman, W.J., 1980. Effects of carnosine on olfactory bulb EEG, evoked potentials and DC potentials. *Brain Res.*, **202**(2):373-386. [doi:10.1016/0006-8993(80)90149-3]

Jin, C.L., Yang, L.X., Wu, X.H., Li, Q., Ding, M.P., Fan, Y.Y., Zhang, W.P., Luo, J.H., Chen, Z., 2005. Effects of carnosine on amygdaloid-kindled seizures in Sprague-Dawley rats. *Neuroscience*, **135**(3):939-947. [doi:10.1016/j.neuroscience.2005.06.066]

Kanaki, K., Kawashima, S., Kashiwayanagi, M., Kurihara, K., 1997. Carnosine-induced inward currents in rat olfactory bulb neurons in cultured slices. *Neurosci. Lett*., **231**(3):167-170. [doi:10.1016/S0304-3940(97)00555-7]

Kapur, J., Stringer, J.L., Lothman, E.W., 1989. Evidence that repetitive seizures in the hippocampus cause a lasting reduction of GABAergic inhibition. *J. Neurophysiol*., **61**(22):417-426.

Karnup, S., Stelzer, A., 1999. Temporal overlap of excitatory and inhibitory afferent input in guinea-pig CA1 pyramidal cells. *J. Physiol*., **516**(2):485-504. [doi:10.1111/j.1469-7793.1999.0485v.x]

Kloosterman, F., Peloquin, P., Leung, L.S., 2001. Apical and basal orthodromic population spikes in hippocampal CA1 in vivo show different origins and patterns of propagation. *J. Neurophysiol*., **86**(2):2435-2444.

Korn, S.J., Giacchino, J.L., Chamberlin, N.L., Dingledine, R., 1987. Epileptiform burst activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition. *J. Neurophysiol.*, **57**(1):325-340.

Margolis, F.L., 1974. Carnosine in the primary olfactory pathway. *Science*, **184**(4139):909-911. [doi:10.1126/science.184.4139.909]

Muller, W., Misgeld, U., 1991. Picrotoxin- and 4-aminopyridine-induced activity in hilar neurons in the guinea pig hippocampal slice. *J. Neurophysiol*., **65**(1):141-147.

Nicoll, R.A., Alger, B.E., Jahr, C.E., 1980. Peptides as putative excitatory neurotransmitters: carnosine, enkephalin, substance P and TRH. *Proc. R. Soc. Lond. B. Biol. Sci*., **210**(1178):133-149. [doi:10.1098/rspb.1980.0124]

Rutecki, P.A., Lebeda, F.J., Johnston, D., 1985. Epileptiform activity induced by changes in extracellular potassium in hippocampus. *J. Neurophysiol*., **54**(5):1363-1374.

Shen, Y., Hu, W.W., Fan, Y.Y., Dai, H.B., Fu, Q.L., Wei, E.Q., Luo, J.H., Chen, Z., 2007. Carnosine protects against NMDA-induced neurotoxicity in differentiated rat PC12 cells through carnosine-histidine-histamine pathway and H(1)/H(3) receptors. *Biochem. Pharmacol.*, **73**(5):709-717. [doi:10.1016/j.bcp.2006.11.007]

Tomonaga, S., Tachibana, T., Takagi, T., Saito, E.S., Zhang, R., Denbow, D.M., Furuse, M., 2004. Effect of central administration of carnosine and its constituents on behaviors in chicks. *Brain Res. Bull.*, **63**(1):75-82. [doi:10.1016/j.brainresbull.2004.01.002]

Tomonaga, S., Tachibana, T., Takahashi, H., Sato, M., Denbow, D.M., Furuse, M., 2005. Nitric oxide involves in carnosine-induced hyperactivity in chicks. *Eur. J. Pharmacol*., **524**(1-3):84-88. [doi:10.1016/j.ejphar.2005.09.008]

Tuff, L.P., Racine, R.J., Adamec, R., 1983. The effects of kindling on GABA-mediated inhibition in the dentate gyrus of the rat. I. Paired-pulse depression. *Brain Res.*, **277**(1):79-90. [doi:10.1016/0006-8993(83)90909-5]

Wu, X.H., Ding, M.P., Zhuge, Z.B., Zhu, Y.Y., Jin, C.L., Chen, Z., 2006. Carnosine, a precursor of histidine, ameliorates pentylenetetrazole-induced kindled seizures in rat. *Neurosci. Lett*., **400**(1-2):146-149. [doi:10.1016/j.neulet.2006.02.031]

Zhang, Y., Zhang, H., 2004. A computational method study of brain-blood partitioning. *J. Beijing Normal Universiy (Nature Science)*, **40**(1):80-84.

Zhu, Y.Y., Zhu-ge, Z.B., Wu, D.C., Wang, S., Liu, L.Y., Ohtsu, H., Chen, Z., 2007. Carnosine inhibits pentylenetetrazol-induced seizures by histaminergic mechanisms in histidine decarboxylase knock-out mice. *Neurosci. Lett*., **416**(3):211-216. [doi:10.1016/j.neulet.2007.01.075]