L-Homocarnosine, L-carnosine, and anserine attenuate brain oxidative damage in a pentylenetetrazole-induced epilepsy model of ovariectomized rats

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Abstract

In this study, we investigated the protective effect of L-homocarnosine, L-carnosine, and anserine (HCA) on seizure-induced brain injuries. We evaluated the protective effect of HCA on brain oxidative damage in a pentylenetetrazole (PTZ)-induced epilepsy model using ovariectomized (OVX) rats. The experimental groups were as follows: group I, sham; group II, sham + PTZ; group III, sham + HCA + PTZ; group IV, OVX; group V, OVX + PTZ; and group VI, OVX + HCA + PTZ. We determined the levels of lipid peroxidation, glutathione peroxidase (Gpx), reduced glutathione (GSH), catalase, superoxide dismutase (SOD), and thiol in brain hippocampal and cortical tissue. The biochemical markers were significantly altered in the brain tissue of OVX rats. HCA supplementation significantly reduced lipid peroxidation and increased GSH, Gpx, SOD, catalase, and thiol levels in PTZ-treated OVX rats. Rats with an ovariectomy showed a significant protective effect against PTZ through elevation of the latency of generalized tonic-clonic seizures (GTCS). HCA substantially increased minimal clonic seizure and GTCS latency in the OVX-PTZ and sham-PTZ groups. In summary, our experimental data indicate that combined supplementation of HCA substantially increased anticonvulsant activity. Moreover, combined HCA supplementation reduced oxidative damage by decreasing lipid peroxidation and increasing antioxidant levels in the brain of a PTZ-induced seizure rodent model.

Keywords Anserine · L-Carnosine · L-Homocarnosine · Rats · Seizures

Introduction

L-Homocarnosine, L-carnosine, and anserine (HCA) are bioactive dipeptides found in skeletal muscles and the brain (Zinellu et al. 2011) at levels of 1–20 mM (Veiga-da-Cunha et al. 2014). Specifically, L-homocarnosine has been shown to exist in the brain, and cerebrospinal fluid at levels ranging from 2 to 50 pM (Solis et al. 2015), and L-carnosine is also present in the olfactory bulb and epithelium at levels

of 0.3–5 mM (Fouad et al. 2017). Promyslov and Mirzoian (1976) previously described the active role of L-homocarnosine in brain tissue. Reduced levels of L-homocarnosine were observed in human glial tumors compared to normal brain tissue, whereas elevated levels were found in the brain tissue of an experimental animal model of brain trauma (Promyslov and Mirzoian 1976). Kohen et al. (1988) noted that HCA exerted antioxidant activity in brain tissue, and Tabakman et al. (2002) demonstrated the neuroprotective potential of HCA in ischemia-induced PC12 cells. Moreover, Kang (2010) described the protective effects of L-homocarnosine and L-carnosine against DNA damage.

Epilepsy is a well-known neurological disease caused by altered cellular and biochemical events (Serikawa et al. 2015). To date, the mechanisms and biochemical events underlying epilepsy have not been elucidated. Therefore, it is necessary to study the biochemical and molecular events that occur in seizure-induced brain injuries (Patel 2004). Ashrafi et al. (2007) previously reported that oxidative stress plays a role in the pathogenesis of epilepsy. Moreover, Sudha

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et al. (2001) reported that the increased free radical production and hyperexcitability of neurons are considered primary causative agents in several neurological disorders. Low levels of antioxidants coupled with increased oxidative metabolism can result in free radical damage in the brain. Indeed, Sudha et al. (2001) showed that high levels of oxidative damage to proteins, lipids, and DNA occurred in prolonged seizures, and significant levels of free radicals have been found in the brain during seizures (Rodrigues et al. 2012). Thus, we aimed to determine the effect of HCA on brain oxidative damage in a pentylenetetrazole (PTZ)-induced epilepsy model using ovariectomized (OVX) rats.

Materials and methods

Materials

L-Carnosine (C9625-10MG), L-homocarnosine (H4885), anserine (A1131), PTZ (P6500-25G), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), and other chemicals were purchased from Sigma-Aldrich (Shanghai, China).

Experimental rats

36 female albino rats weighing 220–240 g were received from the animal facility of the Neurology Department of the Affiliated Hospital of Qingdao University. The rats were grouped into six homogeneous groups with each group containing six rats. The rats had ad libitum access to water and food and were kept in an experimental animal facility with a standard 12-h light/dark cycle. All the animals kept for adaptive feeding for 7 days before the experiment. All experiments involving rats were monitored and approved by the Ethics Committee of the Affiliated Hospital of Qingdao University.

Experimental groups and treatment

The experimental groups were as follows: group I, sham; group II, sham + PTZ; group III, sham + HCA + PTZ; group IV, OVX; group V, OVX + PTZ; and group VI, OVX + HCA + PTZ. Normal saline was administered to the rats in groups I, II, IV, and V for 45 consecutive days, whereas HCA [homocarnosine (1 mM)–carnosine–(1 mM)–anserine (1 mM)] was administered orally to the rats in groups III and VI for 45 consecutive days. At the end of 45 days, PTZ (90 mg/kg body weight) was administered to the rats in groups II, III, V, and VI through intraperitoneal injection. Generalized tonic-clonic seizure (GTCS) and minimal clonic seizure (MCS) activity were determined (Hosseini et al. 2009). Rats were sacrificed by decapitation

following anesthetization [intraperitoneal administration of ketamine (100 mg/kg)/xylazine (10 mg/kg)]. Blood was collected, and the hippocampal and cortical regions were surgically removed and excised. Tissues were washed with normal saline, homogenized in buffer (0.1 M Tris–HCl, pH 7.4), and centrifuged at 1200 rpm for 10 min. The supernatant was taken and centrifuged again at 100,000g for 60 min, and the resulting cytosolic fractions were obtained for the determination of biochemical markers (Jindao et al. 2017).

Determination of biochemical markers

Catalase and superoxide dismutase (SOD) levels were measured as previously described (Meng et al. 2002; Iwase et al. 2013). Glutathione peroxidase (Gpx) activity, lipid peroxidation, and reduced glutathione (GSH) levels were determined according to the method previously described by Power and Blumbergs (2009). The total thiol content in hippocampal and cortical tissue was also determined (Khodabandehloo et al. 2013).

Behavioral assessment

Following PTZ administration, rats were kept in a Plexiglas chamber (30×30×30 cm each) and observed for 1 h. GTCS and latency to the first MCS, the incidence of MCS and GTCS, and mortality rate were calculated as criteria of the behavioral response to PTZ administration (Ebrahimzadeh Bideskan et al. 2011; Hosseini et al. 2009).

Statistical analysis

All experimental outcomes are presented as the mean ± the standard deviation (SD). Analysis of variance (ANOVA) was performed for multiple comparisons. A *P* value < 0.05 was considered to indicate statistical significance.

Results

We investigated the protective effect of HCA on brain oxidative damage in a PTZ-induced model of epilepsy using OVX rats. Biochemical markers were significantly altered in the brain tissue of OVX rats. Indeed, lipid peroxidation, GSH, SOD, Gpx, catalase, and thiol levels were altered in OVX rats compared to sham rats (Tables 1, 2, *P* < 0.05). Administration of PTZ significantly increased lipid peroxidation, whereas GSH, Gpx, SOD, catalase, and thiol levels were significantly reduced in the sham + PTZ group (Table 1, *P* < 0.05). Furthermore, PTZ administration aggravated the effects of ovariectomy on brain hippocampal tissue by increasing lipid peroxidation and reducing GSH, Gpx, catalase, and thiol levels (Table 1; Fig. 1, *P* < 0.05).

Table 1 Effects of L-homocarnosine, L-carnosine and anserine (HCA) on MDA, GSH, SOD, Gpx and catalase in the brain hippocampal tissue of PTZ-treated OVX rats

Biochemical markers	Sham	Sham + PTZ	Sham + HCA + PTZ	OVX	OVX + PTZ	OVX + HCA + PTZ
MDA (nmol/g)	8.43 ± 0.57	17.25 ± 1.2 ^a	11.51 ± 0.8 ^{a,b}	13.3 ± 0.9 ^{a,b}	16.84 ± 1.1 ^{a,c,d}	9.2 ± 0.7 ^{b,d,e}
GSH (nmol/g)	0.41 ± 0.005	0.18 ± 0.001 ^a	0.28 ± 0.001 ^{a,b}	0.24 ± 0.002 ^{a,b}	0.16 ± 0.002 ^{a,c,d}	0.38 ± 0.003 ^{b,c,d,e}
SOD (U/g)	301 ± 17.6	104.4 ± 8.5 ^a	197.8 ± 14.5 ^{a,b}	141.6 ± 10.2 ^{a,b,c}	97.8 ± 8.2 ^{a,c,d}	275.3 ± 17.2 ^{b,c,d,e}
Gpx (U/g)	0.39 ± 0.005	0.15 ± 0.005 ^a	0.22 ± 0.004 ^a	0.19 ± 0.005 ^a	0.16 ± 0.003 ^a	0.37 ± 0.005 ^{b,c,d,e}
Catalase (U/g)	9.1 ± 0.6	3.2 ± 0.13 ^a	4.7 ± 0.15 ^{a,b}	3.9 ± 0.14 ^a	3.1 ± 0.11 ^{a,c,d}	8.8 ± 0.23 ^{b,c,d,e}

^a*P* < 0.05 vs. sham^b*P* < 0.05 vs. sham + PTZ^c*P* < 0.05 vs. sham + HCA + PTZ^d*P* < 0.05 vs. OVX^e*P* < 0.05 vs. OVX + PTZ**Table 2** Effects of L-homocarnosine, L-carnosine, anserine (HCA) on MDA, GSH, SOD, Gpx and catalase in the brain cortical tissue of PTZ-treated OVX rats

Biochemical markers	Sham	Sham + PTZ	Sham + HCA + PTZ	OVX	OVX + PTZ	OVX + HCA + PTZ
MDA (nmol/g)	8.47 ± 0.557	17.44 ± 1.12 ^a	11.82 ± 0.7 ^{a,b}	13.2 ± 0.8 ^{a,b}	16.93 ± 1.3 ^{a,c}	9.1 ± 0.8 ^{b,c,d,e}
GSH (nmol/g)	0.44 ± 0.004	0.19 ± 0.005 ^a	0.25 ± 0.005 ^{a,b}	0.22 ± 0.005 ^a	0.17 ± 0.005 ^{a,c,d}	0.40 ± 0.004 ^{b,c,d,e}
SOD (U/g)	305.4 ± 21.2	107.6 ± 8.9 ^a	199.2 ± 15.6 ^{a,b}	145.68 ± 11.5 ^{a,b,c}	94.3 ± 8.8 ^{a,b,d}	283.5 ± 17.5 ^{b,c,d,e}
Gpx (U/g)	0.42 ± 0.007	0.17 ± 0.004 ^a	0.20 ± 0.009 ^a	0.18 ± 0.008 ^a	0.15 ± 0.005 ^{a,c}	0.38 ± 0.004 ^{b,c,d,e}
Catalase (U/g)	9.3 ± 0.7	3.1 ± 0.18 ^a	4.5 ± 0.15 ^{a,b}	3.8 ± 0.16 ^{a,b,c}	3.2 ± 0.1 ^{a,c}	8.9 ± 0.2 ^{b,c,d,e}

^a*P* < 0.05 vs. sham^b*P* < 0.05 vs. sham + PTZ^c*P* < 0.05 vs. sham + HCA + PTZ^d*P* < 0.05 vs. OVX^e*P* < 0.05 vs. OVX + PTZ

Administration of HCA substantially reduced hippocampal MDA to 11.51 and 9.2 nmol/g in the sham + HCA + PTZ group and OVX + HCA + PTZ group, respectively. Moreover, HCA administration significantly increased hippocampal GSH, SOD, Gpx, and catalase levels in the sham + HCA + PTZ and OVX + HCA + PTZ groups (Table 1, *P* < 0.05). After HCA treatment, the hippocampal thiol levels of the sham + HCA + PTZ and OVX + HCA + PTZ groups increased to 24.58 and 23.83 mM, respectively (Fig. 1, *P* < 0.05). Additionally, lipid peroxidation, GSH, SOD, Gpx, catalase, and thiol levels were significantly altered in the cortical tissue of OVX rats compared to that of sham rats (Table 2, *P* < 0.05). PTZ significantly increased lipid peroxidation, whereas GSH, Gpx, SOD, catalase, and thiol levels were significantly reduced in the cortical tissue of the sham–PTZ group (Table 2, *P* < 0.05). PTZ administration aggravated the effects of ovariectomy on brain cortical tissue by increasing lipid peroxidation and reducing GSH, Gpx, catalase, and thiol levels (Table 2; Fig. 1, *P* < 0.05). HCA treatment resulted in a significant reduction in cortical MDA content to 11.82 and 9.1 nmol/g in the sham + HCA + PTZ group and

the OVX + HCA + PTZ group, respectively. HCA administration significantly reduced lipid peroxidation and increased hippocampal GSH, SOD, Gpx, and catalase levels in cortical tissue of the sham + HCA + PTZ and OVX + HCA + PTZ groups (Table 2, *P* < 0.05). The cortical thiol levels of the HCA + PTZ and OVX + HCA + PTZ groups increased to 19.7 and 22.6 mM, respectively, after administration of HCA (Fig. 1, *P* < 0.05).

Seizure incidence in the form of GTCS and MCS was directly associated with PTZ administration in OVX rats. OVX exerted a significant protective effect against PTZ-induced seizure activity via elevation of GTCS latency (Figs. 2, 3, *P* < 0.05). HCA supplementation substantially increased MCS and GTCS latency in the OVX–PTZ and sham–PTZ groups (Figs. 2, 3, *P* < 0.05).

Fig. 1 Effect of L-homocarnosine, L-carnosine, and anserine (HCA) on thiol levels in the hippocampal and cortical tissue of PTZ-treated OVX rats. Thiol level is expressed in mM, and the experimental values are presented as the mean $\pm$ the standard deviation (SD). ^a $P < 0.05$ vs. sham, ^b $P < 0.05$ vs. sham + PTZ, ^c $P < 0.05$ vs. sham + HCA + PTZ, ^d $P < 0.05$ vs. OVX and ^e $P < 0.05$ vs. OVX + PTZ

Fig. 2 Effect of L-homocarnosine, L-carnosine, and anserine (HCA) on MCS latency in PTZ-treated OVX rats. Experimental values are presented as the mean $\pm$ the standard deviation (SD). ^a $P < 0.05$ vs. sham, ^b $P < 0.05$ vs. sham + PTZ, ^c $P < 0.05$ vs. sham + HCA + PTZ, ^d $P < 0.05$ vs. OVX and ^e $P < 0.05$ vs. OVX + PTZ

Fig. 3 Effect of L-homocarnosine, L-carnosine, and anserine (HCA) on GTCS latency in PTZ-treated OVX rats. Experimental values are presented as the mean $\pm$ the standard deviation (SD). ^a $P < 0.05$ vs. sham, ^b $P < 0.05$ vs. sham + PTZ, ^c $P < 0.05$ vs. sham + HCA + PTZ, ^d $P < 0.05$ vs. OVX and ^e $P < 0.05$ vs. OVX + PTZ

Discussion

We investigated the protective effects of HCA on brain oxidative damage in a PTZ-induced model of epilepsy in OVX rats. Oxidative metabolism is directly linked with neurodegenerative and neurological disorders (Uttara et al. 2009). Several researchers have reported elevated free radicals in the context of seizures, implicating the pathological role of oxidative stress in epileptic seizures (Patel 2004; Gupta et al. 2003). In our study, we demonstrated a reduction in GSH, catalase, Gpx, and thiol levels and an elevation in lipid peroxidation in the hippocampal and cortical tissues of PTZ-treated sham and OVX rats. Several researchers have reported elevated levels of hydroxyl radicals, reactive oxygen species (ROS), hydrogen peroxide, and superoxide anions in the brain during seizures (Sudha et al. 2001; Rodrigues et al. 2012).

Reilly et al. (2011) previously demonstrated that increased oxidative damage and free radical production in the brain results in cognitive and psychiatric issues such as memory loss, depression, and anxiety. Brain oxidative damage during epileptic seizures is partially or wholly associated with reduced lifespan (Maldonado et al. 2010), and Liang et al. (2007) proposed that oxidative stress acts as a connecting point between seizures and aging. Our results are in line with previous reports, suggesting that oxidative stress in the brain is associated with seizures in PTZ-treated rats. PTZ-induced seizures are widely used to investigate the effects of natural compounds and drugs on seizures (Hosseini et al. 2009). Czapiński et al.

(2005) reported that PTZ exposure increased glutamate neurotransmission and reduced gamma-aminobutyric acid (GABA) levels and that PTZ induced neurotoxic effects in the central nervous system by elevating levels of oxidative stress and ROS (Liu et al. 2012).

Promyslov and Mirzoian (1976) investigated the active role of L-homocarnosine on brain tissue. Reduced levels of L-homocarnosine were observed in human glial tumors compared to normal brain tissue, whereas elevated L-homocarnosine levels were found in the animal brain tissue of an experimental traumatic brain model (Promyslov and Mirzoian 1976). Moreover, Kohen et al. (1988) showed that HCA exerted antioxidant effects in the brain, and Tabakman et al. (2002) demonstrated the neuroprotective potential of HCA in ischemia-induced PC12 cells. L-Homocarnosine and L-carnosine were also shown to exert protective effects against DNA damage (Kang 2010), and carnosine supplementation was reported to protect rat brain endothelial cells by scavenging 4-hydroxynonenol and MDA. Moreover, L-carnosine exerted neuroprotective effects in Parkinson's disease (Zhao et al. 2017), and both anserine and L-carnosine were shown to exert similar effects in an animal model of focal ischemia.

Conclusion

In summary, our experimental data indicate that combined supplementation of HCA substantially increases anticonvulsant activity. Moreover, HCA treatment reduces oxidative

damage by reducing lipid peroxidation and increasing antioxidant levels in the brain of a PTZ-induced seizure rodent model.

Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

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