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ORIGINAL PAPER

Preinduction of HSP70 promotes hypoxic tolerance and facilitates acclimatization to acute hypobaric hypoxia in mouse brain

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Abstract It has been shown that induction of HSP70 by administration of geranylgeranylacetone (GGA) leads to protection against ischemia/reperfusion injury. The present study was performed to determine the effect of GGA on the survival of mice and on brain damage under acute hypobaric hypoxia. The data showed that the mice injected with GGA survived significantly longer than control animals (survival time of 9.55±3.12 min, n=16 vs. controls at 4.28±4.29 min, n=15, P<0.005). Accordingly, the cellular necrosis or degeneration of the hippocampus and the cortex induced by sublethal hypoxia for 6 h could be attenuated by preinjection with GGA, especially in the CA2 and CA3 regions of the hippocampus. In addition, the activity of nitric oxide synthase (NOS) of the hippocampus and the cortex was increased after exposure to sublethal hypoxia for 6 h but could be inhibited by the preinjection of GGA. Furthermore, the expression of HSP70 was significantly increased at 1 h after GGA injection. These results suggest that administration of GGA improved survival rate and prevented acute hypoxic damage to the brain and that the underlying mechanism involved induction of HSP70 and inhibition of NOS activity.

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Keywords Acute hypobaric hypoxia · Brain damage · Geranylgeranylacetone · Heat shock protein 70 · Nitrogen monoxidum synthase · Survival

Abbreviations

GGA	Geranylgeranylacetone
HSP70	Heat shock protein 70
NOS	Nitrogen monoxidum synthase
nNOS	Neuronal NOS
eNOS	Endothelial NOS
iNOS	Inducible NOS
AMS	Acute mountain sickness
HACE	High-altitude cerebral edema
HAPE	High-altitude pulmonary edema

Introduction

Acute hypobaric hypoxia occurs frequently during ascent to high altitude (>3,500 m) by aircraft, road vehicle, or ski lift (Clarke 2006) and is one of the main causes of acute mountain sickness (AMS), high-altitude cerebral edema (HACE), and high-altitude pulmonary edema (HAPE). These syndromes can affect unacclimatized travelers shortly after ascent to high altitude (especially higher than 2,500 m). AMS is relatively common and is usually mild and self-limiting; HACE and HAPE are uncommon but life threatening. The principal symptoms of AMS are headache, nausea, vomiting, fatigue, dizziness, and sleep disturbance, although all need not be present (Murdoch 2004). HACE is considered the end stage of AMS, eventually leading to death. Studies by Hackett and Roach suggest that HACE can be caused by acute hypoxia, which induces osmotic cell

swelling, vasogenic edema, biochemical alteration of the blood-brain barrier (BBB), and brain swelling (Roach and Hackett 2001). Recovery occurs with descent, oxygen inhalation or treatment of drugs, such as ibuprofen/naproxen, nifedipine, nimodipine, dexamethasone, Acetazolamide, and Gingko biloba (Murdoch 2004; Berghold 2000; Youquan and Yang 2005). Recently, it was also found that cobalt supplementation promotes hypobaric hypoxic tolerance and facilitates acclimatization to hypobaric hypoxia in rat brain (Shrivastava et al. 2008).

Heat shock proteins (Hsp) are expressed in response to a variety of stressors. They convey protection against protein denaturation and a subsequent immediate stress (Madden et al. 2008). The 70-kDa family of HSPs, HSP70, is upregulated in response to hypoxia and involved in cell protection and survival (Das et al. 1995; Bruemmer-Smith et al. 2001; Weinstein et al. 2004). HSP70 also serves as a useful marker of the cellular response to a hypoxic insult. Recently, some studies have reported the neurobehavioral function of HSP70 during chronic hypoxia (Guanghe and Feng 2007) and the protective role of HSP70 in brain ischemia (Chen et al. 2006). However, the role of inducible HSP70 in protecting against injury of central nervous system during acute hypobaric hypoxia is unclear.

Geranylgeranylacetone (GGA), known as an antiulcer agent in Japan, was shown to induce HSP70 for the first time in 1996 (Hirakawa et al. 1996). Subsequent studies demonstrated that GGA induced HSP70 and exerted cytoprotective action against various kinds of stresses in the gastric mucosa (Hirakawa et al. 1996; Tomisato et al. 2001), liver (Yamagami et al. 2000), small intestine (Tsuruma et al. 1999), retina (Ishii et al. 2003), heart (Latchman 2001), and brain (Hiroshi and Yoshinobu. 2005). Fujiki et al. (2003) evaluated the effect of a single oral dose of GGA on the brain and showed that GGA significantly increased HSP70 immunoreactivity in the hippocampal neurons in the rats.

In addition, some studies showed that GGA could induce HSP70 to prevent the injury of organs such as hearts (Shinohara and Yoshimatsu 2007) and brains (Hiroshi and Yoshinobu 2005) from ischemia. However, most of the studies on GGA administration are focused on ischemia–reperfusion injury (Shinohara and Yoshimatsu 2007; Fan et al. 2005), middle cerebral artery, and occlusion injury (Hiroshi and Yoshinobu 2005), and there are few reports about the efficacy of GGA in facilitating acclimatization to acute hypobaric hypoxia. In the present study, we aimed to determine whether GGA preconditioning facilitates acclimatization to acute hypobaric hypoxia by inducing HSP70 expression and by inhibition of the activity of nitric oxide synthase (NOS). An attempt was also made to provide a novel way to develop drugs to prevent anoxia.

Materials and methods

Animals

Male Kunming mice (weighing 25 to 35 g) were used for the experiments. Animals were allowed unlimited access to standard laboratory chow and water and were maintained at a constant temperature (24±2°C) with a 12-h light–dark cycle. They were fasted overnight before the onset of the experiment but had free access to water. The experiments were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee of Academy of the Military Medical Science.

Treatment with GGA

GGA was provided by Eisai Co. Ltd (Tokyo, Japan). GGA as an emulsion with 0.0056% α-tocopherol and 2% gum Arabic was given intraperitoneally at a dose of 1,000 mg/kg (GGA group; Hiroshi and Yoshinobu 2005). The dose volume was 10 mL/kg. Mice in the control group were given the same dose of vehicle (2% gum Arabic in 0.0056% α-tocopherol). In the studies of lethally acute hypoxic tolerance and histological analysis, 1,000mg/kg GGA or vehicle were administered intraperitoneally 1 h before the onset of the hypoxic exposure. The HSP70 expression and NOS activity were examined 1, 7, or 1 h normoxic + 6 h sublethally acute hypoxic exposure after treatment with 1,000 mg/kg GGA or vehicle, followed by isolation of the cortex and hippocampus. We chose a dose of 1,000 mg/kg, in which GGA could show an obviously protective function against the injury of brain 1 h after administration (Hiroshi and Yoshinobu 2005).

Hypoxic exposure

Conscious mice were exposed to various hypoxic conditions in a decompression chamber (model: DYC-DWI; Guizhou Fenglei, China). Humidity in the chamber was maintained at 40–50% and temperature at 22–24°C. Mice were acclimatized in the chambers in room air for 30 min to 1 h before experiments. Two altitudes were selected based on trials and previous reports (Shelley et al. 2004) to create hypoxic conditions in the chambers: (1) the altitude of 10,000 m at a velocity of approximately 50 m/s for lethally acute hypobaric hypoxia, under which untreated mice died in a relatively uniform time period and (2) the altitude of 8,300 m at a velocity of approximately 10–20 m/s for sublethally acute hypobaric hypoxia, which, when administered via either an environmental chamber or a ventilator, was severe enough to induce typical hypoxic organ injuries yet allowed the mice to survive so that hypoxia-induced pathological changes could be analyzed.

Determination of the tolerance to lethally acute hypobaric hypoxia

Survival under lethally acute hypobaric hypoxia was examined in control mice and mice pretreated with GGA (1,000 mg/kg). Control mice and mice pretreated with GGA were intraperitoneally injected with vehicle or 1,000 mg/kg GGA, respectively, 1 h before the onset of the lethally acute hypoxic exposure. The above two groups were then exposed to simulated acute hypobaric hypoxia of 10,000 m at a velocity of 50 m/s for 15 min. The time of death was measured after achieving the altitude and was defined as the time of the last breath by an investigator who was blindfolded. The survival rate was defined as the ratio (the number of surviving mice/ the number of total mice).

Histological analysis

After the exposure to sublethally acute hypoxia for 6 h, animals were immediately anesthetized by an injection of sodium pentobarbital (50 mg/g, i.p.) and were then perfused with ice-cold phosphate-buffered saline followed by 4% paraformaldehyde via the left ventricle of the heart. The whole brain was removed and postfixed in 4% paraformaldehyde in 15% cane sugar for 24 h, followed by dehydration in 30% cane sugar for 12 h. For histological analysis with Nissl's staining, consecutive coronal sections of 20 μm in thickness were prepared using frozen sectioning technique (model: E, Thermo, USA). The cortex above the hippocampus and dorsal hippocampus were examined, and neural damage was evaluated in each hemisphere.

HSP70 expression studies

After exposure to 1, 7, or 1 h normoxia + 6 h sublethally acute hypoxia, animals were immediately anesthetized by an injection of sodium pentobarbital (50 mg/g, i.p.). The cortex and hippocampus were removed and ground to extract the total protein. The protein (100 μg) was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose membrane. The membrane was blocked with 5% dried skim milk (Applygen) in 0.1% phosphate-buffered saline-Tween, washed, and probed with mouse monoclonal antibody of HSP70 (1:1,000, Calbiochem). The membrane was washed with phosphate-buffered saline-Tween (0.1%) and incubated with antimouse IgG horseradish peroxidase conjugate (1:1,000) for 2 h at room temperature. The membrane was then incubated with chemiluminescent substrate (Santa Cruz) and the bands were developed using X-ray films (Kodak, Rochester, NY, USA).

TNOS and iNOS activity assay

NO synthases (NOS) comprise constitutive (neuronal NOS (nNOS) and endothelial NOS (eNOS)) and inducible forms (iNOS). Total NOS (TNOS) and iNOS activity of mice exposed to 1, 7, or 1 h normoxia + 6 h sublethally acute hypoxia were determined using a Jiancheng kit (Jiancheng Ltd., Nanjing, China) following the manufacturer's instructions.

Statistical analysis

Comparisons among groups were made with a one-way analysis of variance followed by unpaired Student's t tests with the Bonferroni correction. Comparisons between control and mice pretreated with 1,000 mg/kg GGA or normoxic and hypoxic mice were made with unpaired Student's t test. A P value of <0.05 was considered significant.

Results

Effect of GGA pretreatment on tolerance to lethally acute hypoxia

Mice exposed to simulated acute hypobaric hypoxia of 10,000 m at a velocity of about 50 m/s in a decompression chamber were monitored for survival for 15 min. Two thirds of the control mice died within 3 min (Fig. 1; average survival time of 4.28±4.29 min, $n=15$), and the survival rate decreased to 6.67% 15 min after the mice were exposed to simulated acute hypobaric hypoxia of 10,000 m, indicating that this level of acute hypobaric hypoxia was lethal to most of the Kunming mice. Surprisingly, the mice pretreated with GGA (1,000 mg/kg) 1 h before exposure to acute hypobaric hypoxia survived more than 5 min (Fig. 1; average survival time of 9.55±3.12, $n=16$, $P<0.005$ vs. controls) and the survival rate significantly improved by about three times (Fig. 1; the survival rate increased to 18.75%) as compared to the control mice. Thus, these data suggest that a potent, intrinsic protective mechanism can be acutely induced by GGA and renders the entire organism more resistant to acute hypobaric hypoxia.

Histological analysis in hippocampus and cortex

Understanding that the hippocampus and cortex are highly sensitive to acute hypoxia, we examined whether 1,000 mg/kg GGA acted to reduce the acute hypobaric hypoxia-induced neuronal cell death in hippocampus and cortex with Nissl's staining. The mice were exposed to simulated acute hypobaric hypoxia of 8,300 m (sublethal hypoxia) at a velocity of about 10–20 m/s for 6 h. The neurons in the

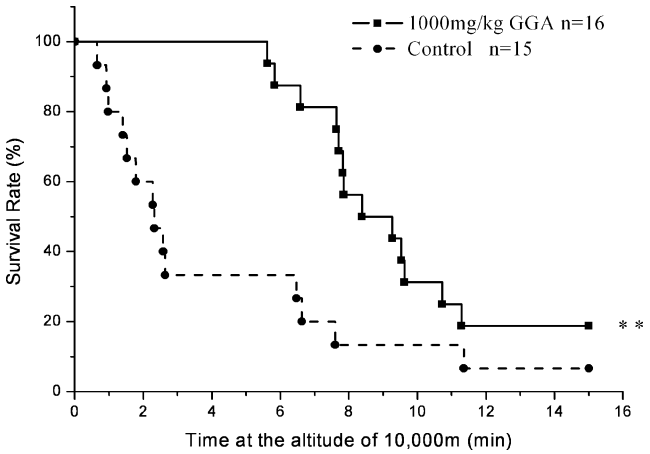

Fig. 1 Kaplan–Meier survival plots of conscious mice exposed to lethally acute hypobaric hypoxia after 1,000 mg/kg GGA administration. The animals were exposed to simulated acute hypobaric hypoxia at 10,000 m (lethal hypoxia) at a velocity of about 50 m/s at 21°C in a decompression chamber 1 h after 1,000 mg/kg GGA administration. Mice were monitored for survival for 15 min immediately after exposure to acute hypobaric hypoxia. Control mice received vehicle alone 1 h before being exposed to acute hypobaric hypoxia; $^{**}P < 0.005$ versus control. Control group: $n=15$; 1,000 mg/kg GGA group: $n=16$

cortex of mice exposed to hypobaric hypoxia were shrunken and slightly scattered (Fig. 2E,F). However, 1,000 mg/kg GGA pretreatment 1 h before sublethally acute hypobaric hypoxic exposure obviously reduced the shrinking of neurons in the cortex (Fig. 2G,H). In hippocampal CA_2 and CA_3 subfields of mice exposed to sublethally acute hypobaric hypoxia, the neurons were significantly shrunken, irregularly arranged, and weakly stained, which indicated that neurons were diffusely deteriorated or dead and that a great many Nissl bodies had been lost in these neurons (Fig. 2M,N,U,V).This result was consistent with the previous studies about the hypobaric hypoxic injury in brain (Maiti et al. 2007, 2008), indicating that the animals had been in the later phase of edema after sublethal hypoxic exposure for 6 h in this model (Castejón and Arismendi 2006). Pretreatment of 1,000 mg/kg GGA significantly attenuated the deterioration of the neurons, which were less shrunken, arranged more regularly, and stained more intensively (Fig. 2O,P,W,X). In addition, almost all of the neurons appeared normal in hippocampal CA2 and CA3 subfields of mice pretreated with 1,000 mg/kg GGA in normoxia (Fig. 2K,L,S,T).

Effect of 1,000 mg/kg GGA pretreatment on HSP70 expression

To investigate the mechanisms underlying the protective effect of this novel treatment using GGA administration, we examined the level of HSP70 expression, which has been shown previously to be induced by GGA (Hirakawa et al. 1996). We found that the expression of HSP70 was significantly elevated in both cortexes (Fig. 3a,c) and in the hippocampus (Fig. 3b,d) with 1 h normoxic exposure after 1,000 mg/kg GGA administration. The above data indicated that GGA induced the expression of HSP70, which might be involved in the protective role of GGA against acute hypoxic injury.

Effect of 1,000 mg/kg GGA pretreatment on NOS activity

It has been reported that nitric oxide synthase induces edema of brain (Roach and Hackett 2001) and causes neuronal damage following hypoxia–ischemia (HI; Peeters-Scholte et al. 1997; Samdani et al. 1997) and that HSP70 reduces the activity of iNOS (Hauser et al. 1996; Scarim et al. 1998). Thus, we further examined whether HSP70 induced by 1,000 mg/kg GGA pretreatment could reduce the activity of TNOS or iNOS. As expected, the appreciably increased activity of iNOS induced by sublethally acute hypobaric hypoxia at 8,300 m was significantly reduced to that of control values by 1,000 mg/kg GGA pretreatment in the cortex (Fig. 4c). Similarly, 1,000 mg/kg GGA pretreatment also reduced the slightly increased activity of iNOS or TNOS following sublethally acute hypoxia, although there were no significant changes between the control group and the 1,000 mg/kg GGA administration group in the activity of iNOS in the hippocampus (Fig. 4d) and TNOS in the cortex (Fig. 4a) or the hippocampus (Fig. 4b). In addition, there were no significant changes in TNOS and iNOS activity in the cortex or the hippocampus after 1 or 7 h normoxia (Fig. 4), which indicated that 1,000 mg/kg GGA administration alone doest not obviously increase the activity of NOS, the marker of tissue injury.

Discussion

The present study shows that GGA pretreatment significantly improved tolerance to acute hypoxia as revealed by increased survival rate and survival time and attenuated the tissue damage in cortex and hippocampus caused by acute hypobaric hypoxia, as revealed by the histological analysis with Nissl's staining. GGA administration also resulted in a significant increase in HSP70 expression and a decrease in the activity of iNOS and TNOS. All these underlying molecular changes were responsible for the observed tolerance to acute hypoxia and for the attenuation of the acute hypoxic damage in cortex and hippocampus. To the best of our knowledge, we have demonstrated for the first time the efficacy of GGA preconditioning in inducing tolerance to acute hypobaric hypoxia and in promoting acclimatization of the brain to acute hypoxia.

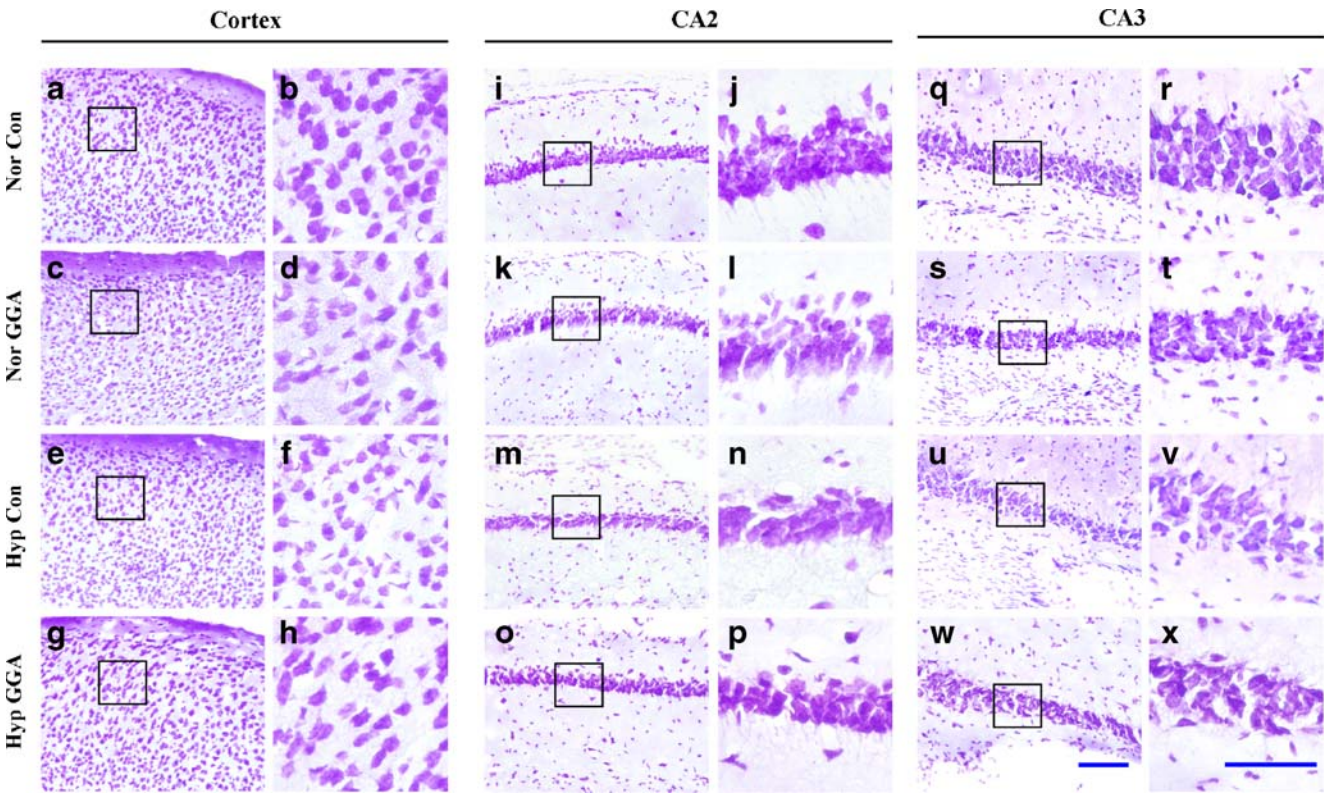

Fig. 2 Prevention of acute hypobaric hypoxia-induced neuronal damage or death in cortex and hippocampus by 1,000 mg/kg GGA pretreatment 1 h before exposure to sublethally acute hypobaric hypoxia. The animals were exposed to normoxia or simulated sublethally acute hypobaric hypoxia of 8,300 m at a velocity of about 10–20 m/s in a decompression chamber 1 h after 1,000 mg/kg GGA or vehicle administration for 6 h. Representative Nissl's stains in the cortex (*A–H*), the CA_2 region (*I–P*), and the CA_3 region (*Q–X*) of hippocampus in the condition of vehicle administration in normoxia (*A*, *B*, *I*, *J*, *Q*, *R*), 1,000 mg/kg GGA administration in normoxia (*C*, *D*, *K*, *L*, *S*, *T*), vehicle administration 1 h before sublethally acute hypoxia (*E*, *F*, *M*, *N*, *U*, *V*), 1,000 mg/kg GGA administration 1 h before sublethally acute hypoxia (*G*, *H*, *O*, *P*, *W*, *X*) are shown. *Scale bars* are 100 μm in photos of *A*, *C*, *E*, *G*; *J*, *K*, *M*, *O*; and *Q*, *S*, *U*, *W*. *Scale bars* are 50 μm in photos of *B*, *D*, *F*, *H*; *J*, *L*, *N*, *P*; and *R*, *T*, *V*, *X*. *Nor* normoxia, *Hyp* hypoxia, *Con* control, *GGA* 1,000 mg/kg GGA. $n=3$ in each group

In the present study, we sought to determine whether GGA pretreatment would promote the tolerance to acute hypoxia. We started administration of 1,000 mg/kg GGA 1 h before exposing the animals to acute hypobaric hypoxia so that the protective mechanism was ready when animals were exposed to high altitude hypoxia. We exposed the animals to a very high altitude of 10,000 m, because smaller animals have higher capillary density in tissues, which makes them more resistant to hypoxia than man (Shrivastava et al. 2008). This level of altitude was previously demonstrated to be lethal to mice or rats (Shelley et al. 2004; Shrivastava et al. 2008), so we considered survival rate and survival time as parameters to measure the acute hypoxic tolerance of mice. Interestingly, we found that 1,000 mg/kg GGA pretreatment obviously enhanced hypoxic tolerance as revealed by an increase in survival rate and survival time by about three and two times, respectively, compared to the control mice.

Subsequently, we further determined whether GGA could attenuate the acute hypobaric hypoxic damage at the cellular and tissue level. It has been demonstrated that severe and chronic (5,500 m, for 3–4 days) hypoxia/ischemia caused neuronal death in the deep and peripheral brain structures such as CA3, CA4, and dentate gyrus of the hippocampus and thalamus, cerebral cortex, and striatum (Freyaldenhoven et al. 1997; Gibson et al. 1981; Naghdi et al. 2003; Smith et al. 1993). Indeed, neurons in the hippocampus and cortex were also highly susceptible to hypoxic injury (Beal 1995; Cervos-Navarro and Diemer 1991; Choi 1996; Pulsinelli 1985). There were also some previous reports showed that the hypobaric hypoxia could induce neuronal pyknosis, cell shrinkage, and tangle in cortex, hippocampus, and striatum (Maiti et al. 2007, 2008). Hence, we examined the morphological changes of neurons in the cortex and hippocampus of mice exposed to an altitude of 8,300 m for 6 h to determine the efficacy of the protective role of GGA in preventing neuronal damage caused by acute hypobaric hypoxia. Exposure to the altitude of 8,300 m for 6 h was previously demonstrated to be severe enough to induce brain damage in mice

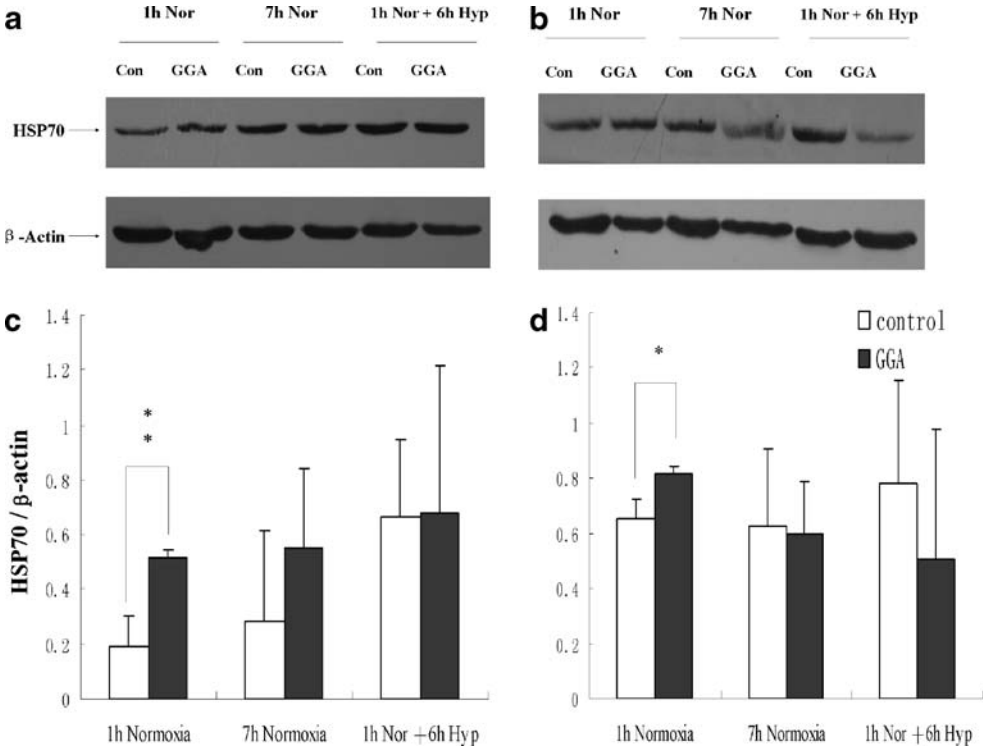

Fig. 3 Effect of 1,000 mg/kg GGA pretreatment on the expression of HSP70, in cortex or hippocampus of mice exposed to sublethally acute hypobaric hypoxia. Cortex and hippocampus of mice were isolated immediately after 1, 7, or 1 h normoxic + 6 h sublethally acute hypoxic (simulated acute hypobaric hypoxia of 8,300 m at a velocity of about 10–20 m/s) exposure 1 h after 1,000 mg/kg GGA administration. A representative Western blot of HSP70 and β-actin expression in cortex (**a**) or hippocampus (**b**) is shown. HSP70 levels were quantified densitometrically and expressed as relative density (HSP70/β-actin), respectively, in cortex (**c**) and hippocampus (**d**). Data were obtained from three separated experiments and are expressed as means ± SD. *Nor* normoxia, *Hyp* hypoxia, *Con* control, *GGA* 1,000 mg/kg GGA. **P*<0.05, ***P*<0.01. *n*=3 in each group

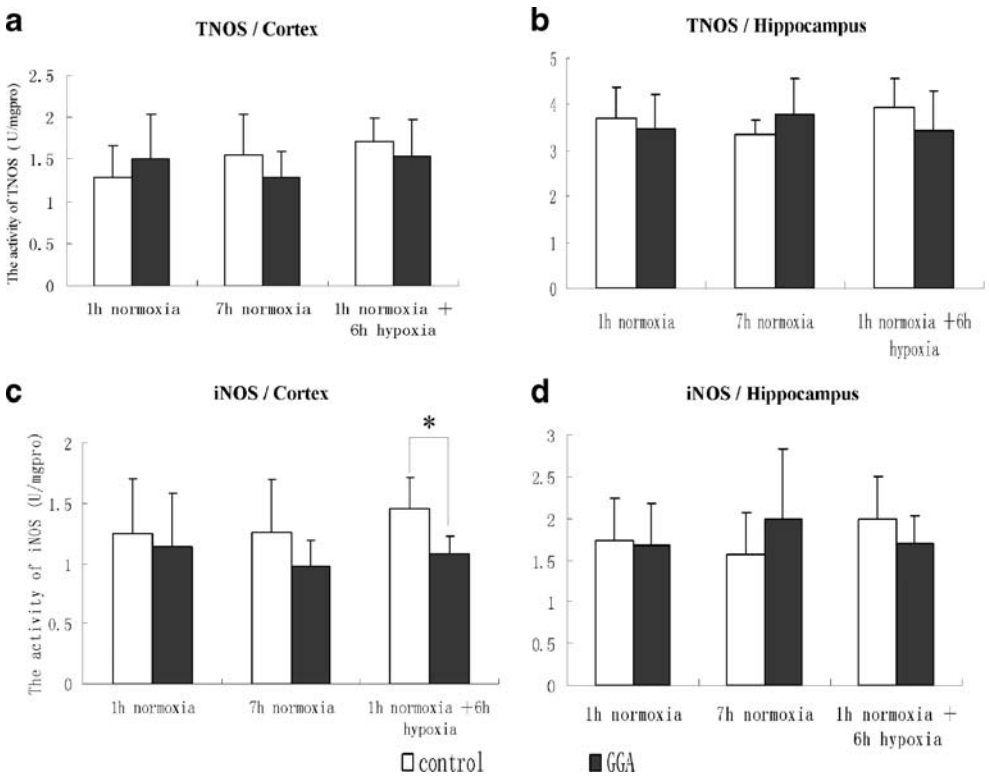

Fig. 4 Effect of 1,000 mg/kg GGA pretreatment on the activity of nitric oxide synthase, in cortex or hippocampus of mice exposed to sublethally acute hypobaric hypoxia. Cortex and hippocampus of mice were isolated immediately after 1, 7, or 1 h normoxic + 6 h sublethally acute hypoxic (simulated acute hypobaric hypoxia of 8,300 m at a velocity of 10–20 m/s) exposure 1 h after 1,000 mg/kg GGA administration. The total nitric oxide synthase (*TNOS*; **a**, **b**) and inducible nitric oxide synthase (*iNOS*; **c**, **d**) activities in cortex (**a**, **c**) and hippocampus (**b**, **d**) were measured as a marker, inducing brain edema and neuronal damage. All the results are expressed as mean ± SD after six individual experiments. *Nor* normoxia, *Hyp* hypoxia, *Con* control, *GGA* 1,000 mg/kg GGA. **P*<0.05. *n*=3 in each group

(Shelley et al. 2004). Data showed that GGA appeared to have an obvious protective function at the cellular and tissular level, especially in hippocampus, as revealed by the significantly improved neuronal morphology in hippocampus and cortex.

Maintaining homeostasis is critical for cell function and survival. In some cases, a reduction in oxygen supply triggers cellular adaptive responses that minimize the deleterious effects of hypoxia (Semenza 1999; Lopez-Barneo et al. 2001). Under hypoxia stress, cells transactivate a variety of genes in order to adapt to altered metabolic status or, alternatively, to induce irreversible cell toxicity. It was previously reported that HSP70 serves as a key modulator of cellular responses to hypoxia (Mohan et al. 2001; Rafiee et al. 2003; Guanghe and Feng 2007), and hypoxia-induced HSP70 expression suggests a positive correlation between stress protein expression and protection against myocardial damage (Dillmann and Mestril 1995).

Therefore, we further investigated whether GGA preinduced HSP70 led to the improved tolerance of mice to acute hypobaric hypoxia and attenuated acute hypoxic damage in hippocampus and cortex. We found that the expression of HSP70 significantly increased in the cortex and hippocampus 1 h after GGA pretreatment. This result suggested that HSP70 preinduced by GGA might be one of major reasons for acute hypobaric hypoxic tolerance. Previous studies reported that HSP70 upregulation required 8 or 24 h after GGA administration in normal condition (Nakada et al. 2005; Minoru et al. 2003). On the other hand, there are some studies demonstrated that the upregulation of HSP70 can be observed within 2 or 4 h after GGA administration (Kazuhiko et al. 2000; Hiroshi and Yoshinobu 2005; Ikeyama et al. 2001). Therefore, the time course of HSP70 induction following GGA is widely various, depending upon the dose, the way of administration, and the tested organs. In the present study, we found that the HSP70 can be induced as early as 1 h after 1,000 mg/kg GGA administration. This suggested that the early induction of HSP70 expression may be caused by the high dose of GGA administration. In addition, one previous study has also demonstrated that 1,000 mg/kg GGA significantly reduced infarct volume when administered 1 h prior to the onset of permanent focal cerebral ischemia (Hiroshi and Yoshinobu 2005).

NOS comprise a constitutive (nNOS and eNOS) and an inducible form (iNOS). In fact, there is already evidence that acute hypoxia is implicated in upregulation of iNOS (Melillo et al. 1995), nNOS, and eNOS (Gess et al. 1997). Nitric oxide (NO), the product of NOS, has been implicated in the pathophysiology of headache and BBB permeability (Roach and Hackett 2001) and is one of the most important initiators of the neuronal damage following HI (Peeters-Scholte et al. 1997; Samdani et al. 1997). It has also been found that inhibition of nNOS and iNOS following hypoxia–ischemia has long-term neuroprotective effects (van den Tweel et al. 2002). Moreover, it has been reported that HSP70 inhibited iNOS expression to prevent NO-mediated damage, such as hypotension (Hauser et al. 1996) and cytokine-induced islet damage (Scarim et al. 1998).

Hence, we investigated whether HSP70 preinduced by GGA could inhibit NOS to prevent the brain damage mediated by NOS which was elevated by acute hypobaric hypoxia. We found that preinduced HSP70 obviously inhibited the iNOS in the cortex, which was elevated in the control mice after hypobaric hypoxic exposure. In addition, the inhibited iNOS 1 h normoxia + 6 h hypoxia after GGA administration may be the effect of HSP70 induced 1 h after GGA administration, because a previous study has reported that the effect of inhibiting iNOS by HSP70 might be followed by the induction of HSP70 and could last for a long time (Wong et al. 1995). This result indicated that the decreased iNOS may be one of the underlying mechanisms why preinduced HSP70 improved tolerance to hypobaric hypoxia and attenuated acute hypobaric hypoxic damages to the brain.

Conclusion

Although the mechanisms are not fully elucidated, GGA pretreatment markedly increased acute hypobaric hypoxic tolerance and attenuated acute hypoxic damage to the brain compared to the control mice. We show that the preinduced HSP70 and inhibited iNOS may be the primary underlying mechanism of improved acute hypobaric hypoxic tolerance. Increased expression of HSP70 maintains homeostasis of organisms and triggers cellular adaptive responses to acute hypobaric hypoxia. Inhibition of iNOS could modulate the vascular tone and BBB permeability to prevent tissue damages caused by acute hypobaric hypoxia. The findings of this study will help in the design and development of novel therapeutic strategies to use GGA as drug for promoting acclimatization to high altitude and preventing HACE.

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