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Research Report

Hypoxic preconditioning up-regulates glucose transport activity and glucose transporter (GLUT1 and GLUT3) gene expression after acute anoxic exposure in the cultured rat hippocampal neurons and astrocytes

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ABSTRACT

Hypoxic preconditioning has been shown to increase the hypoxic tolerance of brain neurons. However, the mechanism underlying the increased hypoxic tolerance has not been well elucidated. Since anaerobic glycolysis is the only pathway for a vertebrate cell to produce energy under anoxic conditions, which needs a large amount of glucose, we hypothesize that glucose transport, the rate-limiting step for glucose metabolism, plays a critical role in the hypoxic tolerance induced by hypoxic preconditioning. In this study, the effects of hypoxic preconditioning on glucose transport activity and the gene expression of two major forms of glucose transporters (GLUT1 and GLUT3) in the brain were investigated in cultured rat hippocampal neurons and astrocytes. The neuronal and astroglial cultures were preconditioned for 6 days by intermittently exposing the cells to sublethal hypoxic gas mixture (1% O_2/10% CO_2/89% N_2) for 20 min each day. 24 h after the last hypoxic exposure, the cells were exposed to a lethal anoxic gas mixture (10% CO_2/90% N_2) for 6 h and the uptake rate of [^{3}H] 2-deoxyglucose (2-DG) and the levels of GLUT1 and GLUT3 glucose transporter mRNAs in the cells were examined immediately after anoxic exposure. The neurons and astrocytes preconditioned with hypoxia showed higher 2-DG uptake rates than the non-preconditioned cells. Compatible with the change in 2-DG uptake, hypoxic preconditioning also increased GLUT1 mRNA levels in the astrocytes and GLUT1 and GLUT3 mRNA levels in the neurons. The neurons preconditioned by hypoxia displayed increased anoxic tolerance. However, when glucose uptake in the neurons was blocked by cytochalasin B, the anoxic tolerance was almost abolished. These results suggest that glucose transport is critical to neuronal survival during anoxic exposure and the increased glucose transport activity is probably one of the important mechanisms for the enhanced hypoxic tolerance induced by hypoxic preconditioning.

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1. Introduction

The availability of glucose as a universal substrate for aerobic and anaerobic metabolism is of supreme importance in maintenance of cellular energy homeostasis. The initial step in the metabolism of glucose is its transport across the plasma membrane, a step that is rate-limiting in a vast majority of cells and tissues (Elbrink and Bihler, 1975; Ismail-Beigi, 1993). Hence, control of glucose transport is a key step in the action of a variety of factors that influence the cellular metabolism of glucose. Of particular importance is the role of stimulation of glucose transport in the adaptive response of cells and tissues to hypoxia.

Owing to the polar characteristics, glucose transport across the plasma membrane is mediated by a facilitated diffusion-type transport system designated glucose transporters (GLUT). 13 facilitative glucose transporters have been identified (Wood and Trayhurn, 2003), in which glucose transporter 1 (GLUT1) and glucose transporter 3 (GLUT3) predominate in the brain (Vannucci et al., 1997). GLUT1 is localized in cerebrovascular endothelial cells, astrocytes, and oligodendrocytes, while glucose transporter 3 (GLUT3) is specifically expressed in neurons (Vannucci et al., 1997; Yu and Ding, 1998). In special conditions, the stressed neurons also express GLUT1 (Vannucci et al., 1997).

Hypoxic preconditioning has been shown to produce tolerance against hypoxia–ischemia brain injury (Bergeron et al., 2000; Cantagrel et al., 2003; Jones and Bergeron, 2001; Kulinskii et al., 2002; Lin et al., 2000; Ohtsuki et al., 1996; Prass et al., 2003; Schurr, 2002). The induced tolerance has also been observed in rat hippocampal slices preconditioned by anoxia (Omata et al., 2002) and in primary neuronal cultures pre-exposed to oxygen-glucose deprivation (Bruer et al., 1997; Grabb et al., 2002; Khaspekov et al., 1998; Liu et al., 2000; Meloni et al., 2002; Wu et al., 2004). However, the mechanisms for this neuroprotection have not been thoroughly clarified. Because it generally takes several hours to days after the preconditioning episode before a state of tolerance is attained, the preconditioning stimulus is likely to involve adaptive changes in gene expressions. Since anaerobic glycolysis is the only pathway for a vertebrate cell to produce energy under anoxic conditions, which needs a large amount of glucose being transported into the cell, and glucose transport plays a critical role in the resistance of brain cells to hypoxia (Zhang et al., 1999), we hypothesize that glucose transport, the rate-limiting step for glucose metabolism, plays a key role in the hypoxic tolerance induced by hypoxic preconditioning. In neonatal rat brain, hypoxic preconditioning has been reported to increase the expression of GLUT1 mRNA and protein (Jones and Bergeron, 2001) and to afford about 95% of brain protection (Bergeron et al., 2000). Whether the expression of GLUT3, another major form of glucose transporter in the brain, is also affected by hypoxic preconditioning, and to what extent glucose transport activity may contribute to the hypoxic tolerance induced by hypoxic preconditioning have not been investigated. Moreover, direct evidence that hypoxic preconditioning may influence the glucose transport activity of neurons and astrocytes has not been obtained. In the present study, we investigated the effects of hypoxic preconditioning on the glucose transport activity and the gene expression of glucose transporters (GLUT1 and GLUT3) in the cultured rat hippocampal neurons and astrocytes. We found that intermittent hypoxic preconditioning increased the levels of GLUT1 and GLUT3 mRNAs in the neurons and the levels of GLUT1 mRNA in the astrocytes. In addition, intermittent hypoxic preconditioning also increased the glucose transport activity in the neurons and astrocytes, which appeared to play a crucial role in the hypoxic preconditioning-induced neuronal resistance to hypoxia.

2. Materials and methods

2.1. Cell culture

Newborn Wistar rats were used within 24 h after birth. The brains were removed from the skull and the bilateral hippocampi were isolated. The hippocampi were then chopped into approximately 2-mm-thick pieces, which were digested at 37 °C for 30 min with 0.25% trypsin in Hank's buffer. The tissue pieces were washed with Dulbecco's modified Eagle's medium (DMEM, Sigma) containing 10% heat-inactivated horse serum (HyClone), 10% fetal calf serum (HyClone), 100 U/ml of penicillin, and 100 μg/ml of streptomycin. The DMEM was modified by adding the following extra ingredients: 0.192 mg/ml of glutamine, 3.7 mg/ml of $NaHCO_3$, and 5 mg/ml of glucose (the final concentration being 6 mg/ml). The hippocampal cells were dissociated by gently pipetting the tissue pieces with a fire-polished Pasteur pipette. To prepare neuronal cultures, the cells were plated at a density of 1×10^5 cells/cm^2 in a poly-L-lysine-coated 35-mm-diameter culture dish (Nunc, Denmark) and cultured at 36 °C in a 10% CO_2 incubator. The culture medium was replaced within 24 h with fresh DMEM containing 10% horse serum. On day three, the cultures were treated with cytosine arabinoside (3 μg/ml, Sigma) to suppress proliferation of non-neuronal cells. The cells were allowed to grow for 10–12 days with periodic changes of the medium. Astroglial cultures were prepared in a similar fashion, except that 10% fetal bovine serum was substituted for horse serum and cytosine arabinoside was omitted from the culture medium. A secondary culture, usually on day 7, was performed once the cells were confluent, with a density of 1×10^4 cells/cm^2 in a poly-L-lysine-coated 35-mm-diameter culture dish. Cells were allowed to grow additional 8 days with periodic changes of the medium.

2.2. Hypoxic preconditioning and acute anoxia

Hypoxic preconditioning and acute anoxia were performed according to the methods described previously with slight modifications (Wu et al., 2004). Experiments for the primarily cultured neurons were started the fourth day after plating, and for the secondarily cultured astrocytes, the second day after plating. The neurons and astrocytes were divided into 4 groups: (1) Control group, cells were continually cultured in normoxic gas mixture of 90% air and 10% CO_2 for 7 days plus 6 h without any treatment; (2) Hypoxic preconditioning (HP) group, cells were placed in a 2000-cm^3 airtight chamber, partially submerged in a 42 °C water bath, to maintain a

constant thermal environment at 36 °C ambient temperature. A gas mixture of 1% O_2/10% CO_2/89% N_2 was delivered into the chamber at a flow rate of 200 ml/min. The neurons and the astrocytes were exposed to the gas mixture for 20 min each day and then returned to normoxic conditions immediately. This procedure was repeated for 6 days. 28 h after the last exposure to the hypoxic gas mixture, the cells were assayed; (3) Acute anoxia (AA) group, cells were cultured in normoxic gas mixture for 7 days and then were exposed to a lethal anoxic gas of 0% O_2/10% CO_2/90% N_2 for 6 h; (4) Hypoxic preconditioning plus acute anoxia (HP + AA) group, cells were intermittently hypoxia-preconditioned for 6 days. 24 h after the last hypoxic exposure, the cells were exposed to the lethal anoxic gas for 6 h. Immediately after acute anoxic exposure, all the cells were assayed for either glucose transport activity, GLUT1 and GLUT3 mRNA expression, and cell viability.

2.3. *Measurement of glucose transport activity*

2-Deoxyglucose uptake was used to evaluate the glucose transport activity in neurons and astrocytes according to the method described previously (Hara et al., 1989). The medium was aspirated and the cells were rinsed gently in PBS. Cells were incubated for 5 min at 37 °C in PBS containing 0.5 mmol/l 2-DG and [^{3}H]-2-DG (1 μCi/ml, Amersham Pharmacia Biotech). The cells were then washed repeatedly with ice-cold PBS and solubilized by incubation in 0.2 N NaOH for 1 h at room temperature. Data for specific activity were used to calculate glucose uptake in nanomoles per milligram of protein.

2.4. *Northern blot analysis of mRNA expression*

In order to measure mRNA expression, the total RNA of the cell cultures was extracted with RNAgents Total RNA Isolation System (Promega). 10 μg of total RNA was separated by electrophoresis on a 1% agarose-formaldehyde gel, transferred onto a nylon membrane, and hybridized with rat GLUT1 cDNA, rat GLUT3 cDNA, and β-actin cDNA, which were labeled with [^{32}P]-dCTP using Prime-a-Gene Labeling System (Promega). The cDNAs, which encode amino acids 208–393 of rat GLUT1 and amino acids 204–382 of rat GLUT3, were generated by RT-PCR and used as probes.

The membrane was washed at room temperature (2 × SSC + 0.1% SDS, once, 0.5 × SSC + 0.1% SDS, once, and 0.1 × SSC + 0.1% SDS, twice, 15 min each wash) and autoradiographed. The bands were scanned and quantified by an image analyzer (Glyko BandScan). The Northern bands of GLUT1 and GLUT3 were normalized with the values of β-actin bands.

2.5. *Assessment of cell viability*

Cell viability after treatments was assessed using trypan blue exclusion test according to the method described previously (Maiese et al., 1993). Briefly, the cells were incubated with 0.4% trypan blue for 3 min. The cells that excluded trypan blue were taken as viable cells. For each dish, eight fields were randomly selected and counted for both viable cells and dead cells. For each experimental group, 5 dishes were counted and calculated.

2.6. *Statistics*

All data are expressed as means ± SD. Statistical significance was determined by Student's *t* test and $P < 0.05$ was accepted as significant.

3. Results

3.1. *Hypoxic preconditioning up-regulates glucose transport activity*

The neurons and astrocytes were intermittently preconditioned with hypoxic gas mixture for 6 days. 24 h later, they were exposed to anoxic gas mixture for 6 h. Immediately after completion of anoxic gas exposure, 2-DG uptake rates of the neurons and astrocytes were measured. As shown in Fig. 1, hypoxic preconditioning did not apparently change the 2-DG uptake rates of the neurons and astrocytes when compared with the control values. The 2-DG uptake rates of the control neurons and the hypoxia-preconditioned neurons were 5.50 ± 1.17 nM/min/mg and 5.61 ± 1.26 nM/min/mg, respectively. The 2-DG uptake rates of the control astrocytes and hypoxia-preconditioned astrocytes were 8.12 ± 1.83 nM/min/mg and 8.24 ± 1.89 nM/min/mg, respectively. However, 6 h anoxic exposure significantly enhanced the 2-DG uptake rates in both the preconditioned and non-preconditioned neurons and astrocytes. Very interestingly, the preconditioned neurons and astrocytes showed much higher 2-DG uptake rates when compared with the non-preconditioned cells. The 2-DG uptake rates in the non-preconditioned and the preconditioned neurons were increased by 1.4-fold (7.31 ± 1.58 nM/min/mg) and 1.9-fold (10.11 ± 3.38 nM/min/mg), respectively. The 2-

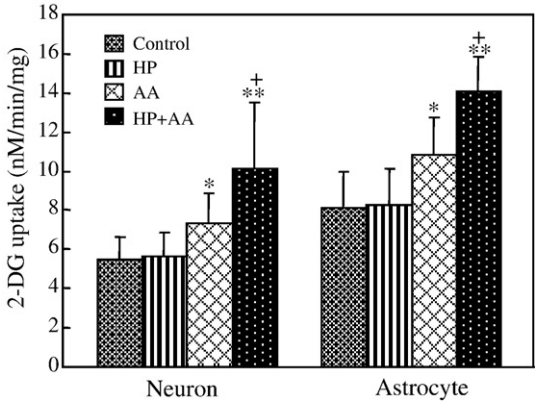

Fig. 1 – Effect of hypoxic preconditioning on 2-DG uptake rates in the cultured neurons and astrocytes. Hypoxic preconditioning itself did not apparently affect the 2-DG uptake rates in the neurons and astrocytes. However, 6 h anoxic exposure significantly increased the 2-DG uptake rates in both the neurons and the astrocytes. The increase in the 2-DG uptake rate was much higher in the hypoxia-preconditioned cells than the non-preconditioned ones. $^*P < 0.05$, $^{**}P < 0.01$, compared with control group; $^+P < 0.01$, compared with only acute anoxia group; $n = 7$; AA: acute anoxia; HP: hypoxic preconditioning.

DG uptake rates in the non-preconditioned and the preconditioned astrocytes were increased by 1.3-fold (10.80 ± 1.95 nM/min/mg) and 1.7-fold (14.08 ± 1.77 nM/min/mg), respectively. These results indicated that the glucose transport activity of the hypoxia-preconditioned hippocampal neurons and astrocytes was up-regulated during anoxic exposure.

3.2. *Hypoxic preconditioning increases the levels of GLUT1 and GLUT3 mRNAs*

GLUT1 and GLUT3 are two major forms of glucose transporters in the brain (Vannucci et al., 1997). Whether intermittent hypoxic preconditioning may affect the gene expression of GLUT1 and GLUT3 in the cultured neurons and astrocytes was investigated. As shown in Fig. 2, hypoxic preconditioning did not apparently change the levels of GLUT1 and GLUT3 mRNAs in the neurons as well as the levels of GLUT1 mRNA in the astrocytes. However, the levels of GLUT1 and GLUT3 mRNAs in the neurons significantly increased after anoxic exposure. Compared with the non-hypoxia-preconditioned neurons, the hypoxia-preconditioned neurons expressed much higher GLUT1 and GLUT3 mRNAs. A similar change in the expression of GLUT1 mRNA in the astrocytes was also observed. The preconditioned astrocytes expressed much higher GLUT1 mRNA than the non-preconditioned ones. No GLUT3 mRNA was detected in the astrocytes (Fig. 3).

3.3. *Cytochalasin B abolishes the hypoxic tolerance induced by hypoxic preconditioning*

Since the hypoxia-preconditioned neurons displayed increased glucose transport activity during anoxia, we tested its role in hypoxic tolerance by using cytochalasin B, a specific glucose transport inhibitor, to inhibit glucose transport. 30 min before anoxic exposure, 10 μM of cytochalasin B (Sigma) was added to the medium of either the preconditioned or non-preconditioned neurons. This concentration of cytochalasin B (CB) has been demonstrated to inhibit 88% of glucose transport of cultured neurons (Hara et al., 1989). As have shown in many other studies (Bruer et al., 1997; Grabb et al., 2002; Khaspekov et al., 1998; Liu et al., 2000; Meloni et al., 2002; Wu et al., 2004), hypoxic preconditioning increased the hypoxic tolerance of the hippocampal neurons. In CB free conditions, 6 h anoxic exposure decreased the cell survival rate from 96.4 ± 1.4% to 83.4 ± 2.9% for the non-hypoxia-preconditioned neurons while only to 92.0 ± 2.2% for the hypoxia-preconditioned neurons. In the presence of CB, although as high as 95.4 ± 2.1% of the neurons were still alive after 6 h normoxic exposure, only 62.1 ± 5.9% of the preconditioned neurons and 59.0 ± 3.8% of the non-preconditioned neurons survived after anoxic exposure (Figs. 4 and 5). These results indicate that inhibition of glucose uptake almost completely abolished the hypoxic tolerance induced by hypoxic preconditioning and made the cells even more vulnerable to anoxia.

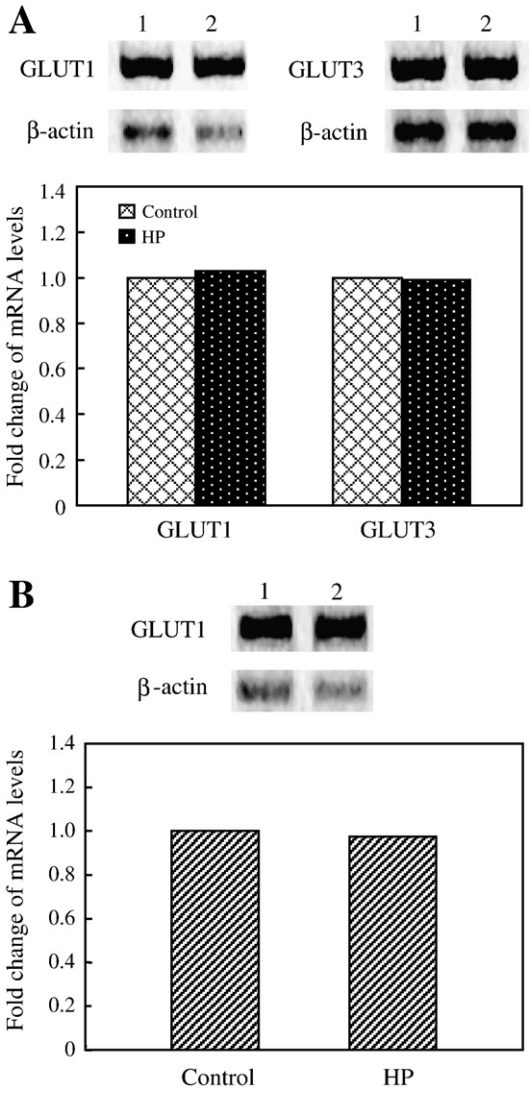

Fig. 2 – Effect of hypoxic preconditioning on the expression of GLUT1 and GLUT3 mRNAs in normoxic conditions. (A) The upper panel shows the bands for Northern hybridization in control neurons (1) and hypoxia-preconditioned neurons (2). The lower panel shows the relative optical densities of the bands. There was no apparent difference of GLUT1 and GLUT3 mRNA expression between the hypoxia-preconditioned neurons and non-hypoxia-preconditioned neurons. (B) The upper panel shows the bands for Northern hybridization in control astrocytes (1) and hypoxia-preconditioned astrocytes (2). The lower panel shows the relative optical densities of the bands. There was no apparent difference of GLUT1 mRNA expression between the hypoxia-preconditioned astrocytes and non-hypoxia-preconditioned astrocytes.

4. Discussion

In this study, we provide direct evidence that hypoxic preconditioning increases the glucose transport activity during anoxic exposure in the cultured hippocampal neurons and astrocytes. In addition, the levels of GLUT1 and GLUT3 mRNA in the preconditioned neurons as well as the levels of GLUT1 mRNA in the preconditioned astrocytes were also increased during anoxic exposure. The detailed mechanisms for the increased glucose transport activity in the preconditioned

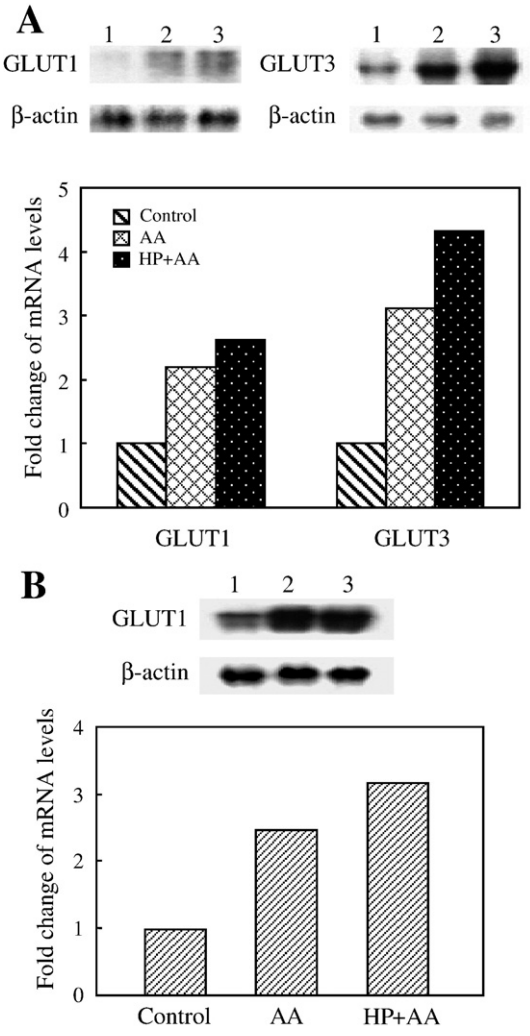

Fig. 3 – Effect of hypoxic preconditioning on the expression of GLUT1 and GLUT3 mRNAs during anoxic exposure. (A) The upper panel shows the bands for Northern hybridization in control (1), non-preconditioned (2), and hypoxia-preconditioned (3) neurons after 6 h anoxic exposure. The lower panel shows the relative optical densities of the bands. The levels of GLUT1 and GLUT3 mRNAs in both the preconditioned and non-preconditioned neurons were elevated after anoxic exposure. However, the preconditioned neurons expressed much higher GLUT1 and GLUT3 mRNAs than the non-preconditioned ones. (B) The upper panel shows the bands for Northern hybridization in control (1), non-preconditioned (2), and hypoxia-preconditioned (3) astrocytes after 6 h anoxic exposure. The lower panel shows the relative optical densities of the bands. The levels of GLUT1 mRNA in both the preconditioned and non-preconditioned astrocytes were elevated after anoxic exposure. However, the preconditioned astrocytes expressed more GLUT1 mRNA than the non-preconditioned ones. AA: acute anoxia; HP: hypoxic preconditioning.

neurons and astrocytes remain unknown. One possibility is the stimulation of GLUT3 and/or GLUT1 synthesis since the change of GLUT3 and/or GLUT1 mRNAs in the preconditioned neurons and astrocytes is quite similar to that of glucose transport activity. To demonstrate this possibility, changes in the levels of GLUT3 and/or GLUT1 proteins should be further investigated. Another possibility for the increased glucose transport activity is the stimulation of intrinsic activity of GLUT3 and/or GLUT1 proteins. In that case, glucose transporters preexisting in the plasma membrane is activated, which will cause increased glucose transport activity without change in the number of glucose transporters in plasma membrane (Zhang et al., 1999). It has been demonstrated that the intrinsic activity of GLUT1 and GLUT3 was increased within 1 h with a maximum increase after 6 h upon stimulation of hemopoietic growth factor or interleukin-3 (Ismail-Beigi, 1993; McCoy et al., 1997). Further study is needed to show whether or not the intrinsic activity of GLUT1 and GLUT3 is modulated by hypoxic preconditioning.

It is unknown what signal transduction pathway mediates the up-regulation of GLUT3 and/or GLUT1 mRNAs by hypoxic preconditioning. One of the possible regulators is hypoxia inducible factor 1 (HIF-1), which plays a central role in the oxygen transduction pathway (Semenza, 2000). HIF-1 is an upstream regulator for a series of hypoxia-responsive genes including GLUT1, GLUT3, EPO, VEGF, and some glycolytic enzymes such as lactate hydroxylase A and phosphofructokinase L (Semenza, 2000). In the neonatal rat brain, HIF-1 and its target genes including GLUT1 were increased by hypoxic

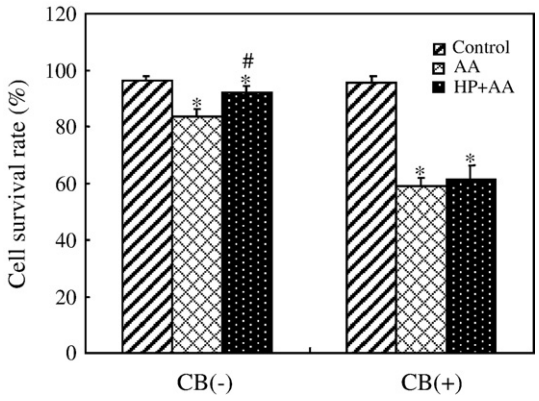

Fig. 4 – Effect of cytochalasin B on hypoxic preconditioning-induced neuronal hypoxic tolerance. 10 μM of cytochalasin B was added to the culture medium 30 min before anoxic exposure and was allowed to be present in the medium during anoxic exposure. In the cytochalasin B free conditions, 6 h acute anoxic exposure led to dramatic reduction of the cell survival rate in the non-preconditioned neurons. However, hypoxic preconditioning significantly protected the neurons from the injury, as shown by the higher survival rate in the preconditioned neurons. In the presence of cytochalasin B, both the hypoxia-preconditioned and non-preconditioned neurons represented a dramatic fall in the hypoxic tolerance, as shown by the significant reduction of the survival rate after acute anoxic exposure. There was no significant difference in the cell survival rate between the hypoxia-preconditioned and non-preconditioned neurons. *$P < 0.01$, compared with control group; #$P < 0.01$, compared with acute anoxia group; $n = 5$; AA: acute anoxia; HP: hypoxic preconditioning.

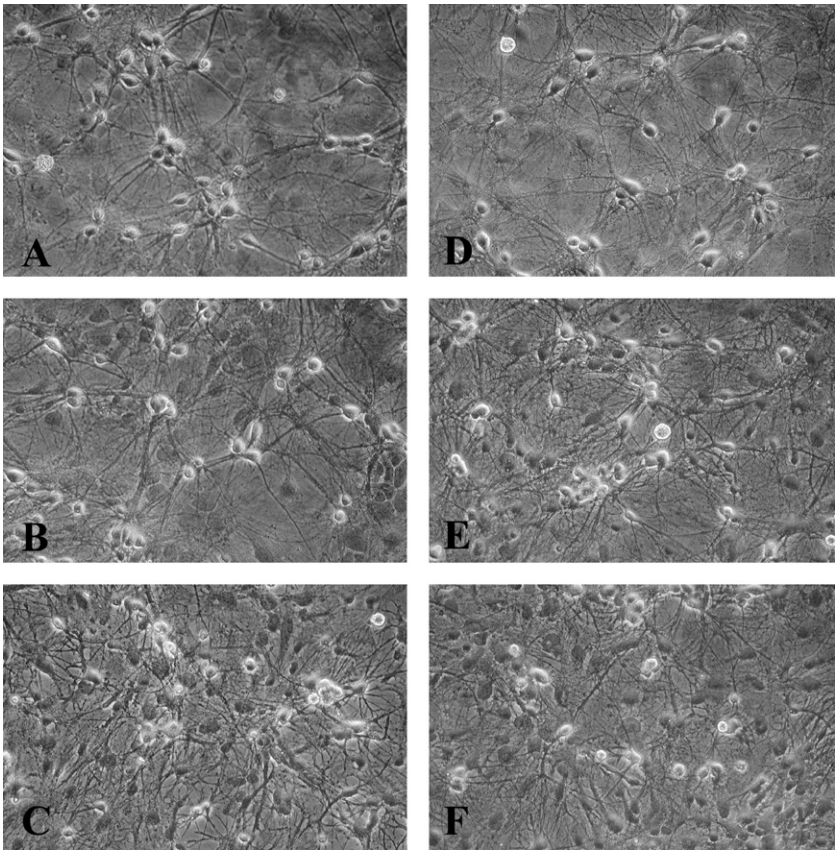

Fig. 5 – Photomicrographs showing the effect of cytochalasin B on hypoxic preconditioning-induced neuronal hypoxic tolerance. (A and D) Non-preconditioned (A) and preconditioned (D) neurons treated by 10 μM cytochalasin B (CB). Most of the neurons are alive. The living neurons under the phase contrast microscope have bosomy cell bodies, smooth cell surfaces, intact processes, and bright halos (arrows). There was no apparent morphological difference between CB-treated and CB-untreated neurons. (B and E) Non-preconditioned (B) and preconditioned (E) neurons after 6 h anoxic exposure. Some of the neurons were damaged after anoxic exposure. The dead neurons have flat cell bodies, ruptured processes, and rough cell surfaces without halos (arrowheads). More preconditioned neurons survived anoxic exposure. (C and F) Cytochalasin-B-treated non-preconditioned (C) and preconditioned (F) neurons after anoxic exposure. In the presence of CB, most of the neurons were damaged in either non-preconditioned or preconditioned groups after 6 h anoxic exposure. Scale bar = 50 μm.

preconditioning (Bergeron et al., 2000; Jones and Bergeron, 2001), raising a high possibility that HIF-1 may mediate the up-regulation of GLUT1 and GLUT3 mRNAs in the preconditioned cells. However, the possibility for the involvement of other hypoxia-responsive transduction pathways such as PKC and MAPK (Carini et al., 2001) pathways in the up-regulation of GLUT1 and GLUT3 mRNAs in the preconditioned cells cannot be excluded.

Glucose transport is the rate-limiting step for cellular metabolism of glucose. Because of the low ATP production rate, a cell may need more glucose to maintain its ATP levels by glycolysis in anoxic conditions. Therefore, increased glucose transport activity may play a pivotal role in the adaptive response to anoxic conditions. This has been demonstrated by previous studies showing the role of activation of glucose transport and glycolytic activity in the hypoxic tolerance of cells (Lin et al., 2000; Malhotra and Brosius, 1999). Indeed, we observed that the hypoxia-preconditioned neurons with higher glucose transport activity were more tolerant to anoxia than the non-preconditioned neurons with lower glucose transport activity. This result was further convinced by the observations that cytochalasin B, a specific inhibitor of glucose transporters, almost completely abolished the induced anoxic tolerance of the preconditioned neurons. Although several other mechanisms have been suggested in the hypoxic tolerance induced by hypoxic preconditioning (Bossenmeyer and Daval, 1998; Duan et al., 1999; Newby et al., 1990; Ohtsuki et al., 1996; Xie et al., 1999; Ying et al., 1997; Zhang and Behrooz, 1999), our results indicate that the increased glucose transport activity is crucial in the induced hypoxic tolerance.

The role of the increased glucose uptake of astrocytes in the hypoxic tolerance of neurons needs to be further investigated. Increased glucose uptake of astrocytes in anoxic conditions indicates an up-regulation of glycolysis in the cells, which may lead to accumulation of lactate and acidosis, and has been thought to be detrimental to neurons (Siesjo, 1981; Siesjo, 1988). However, recent studies have demonstrated that brain lactate produced in hypoxic conditions is an obligatory aerobic energy substrate for the functional recovery of neurons after hypoxia (Schurr, 2002). Therefore, the increased glucose transport activity in astrocytes may be not only protective to

astrocytes themselves during anoxia, but also beneficial to the functional recovery of the neurons after anoxia in in vivo brain.

In summary, we provide direct evidence that hypoxic preconditioning up-regulates glucose transport activity in the cultured hippocampal neurons and astrocytes. Hypoxic preconditioning also promotes the expression of GLUT1 mRNA in both the astrocytes and neurons and the expression of GLUT3 mRNA in only the neurons. The increased glucose transport activity may play a pivotal role in the hypoxic tolerance induced by hypoxic preconditioning. The mechanisms for the increased glucose transport activity and the elevated expression of GLUT1 and GLUT3 mRNAs induced by hypoxic preconditioning need to be further investigated.

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