

Molecular Mechanisms Leading to Neuroprotection/Ischemic Tolerance: Effect of Preconditioning on the Stress Reaction of Endoplasmic Reticulum

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Abstract Ischemic tolerance can be developed by prior ischemic non-injurious stimulus preconditioning. The molecular mechanisms underlying ischemic tolerance are not yet fully understood. The purpose of this study is to evaluate the effect of preconditioning/preischemia on ischemic brain injury. We examined the endoplasmic reticulum stress response (unfolded protein response (UPR)) by measuring the mRNA and protein levels of specific genes such as ATF6, GRP78, and XBP1 after 15 min 4-VO ischemia and different times of reperfusion (1, 3, and 24 h). The data from the group of naïve ischemic rats were compared with data from the group of preconditioned animals. The results of the experiments showed significant changes in the gene expression at the mRNA level in the all ischemic/reperfusion phases. The influence of preischemia on protein level of XBP was significant in later ischemic times and at 3 h, the reperfusion reached 230% of the controls. The protein levels of GRP78 in preischemic animals showed a significant increase in ischemic and reperfusion times. They exceeded to 50% levels of corresponding naïve ischemic/reperfusion groups. Preconditioning also induced remarkable changes in the levels of ATF6 protein in the ischemic phase (about 170%).

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The levels of ATF6 remained elevated in earlier reperfusion times (37 and 62%, respectively) and persisted significantly elevated after 24 h of reperfusion. This data suggest that preconditioning paradigm (preischemia) underlies its neuroprotective effect by the attenuation of ER stress response after acute ischemic/reperfusion insult.

Keywords Unfolded protein response ·
Global brain ischemia · Preischemia · Ischemic tolerance

Introduction

Ischemic brain damage is a very severe event with multiple, parallel and sequential pathogenesis (Endres et al. 2008). Transient global brain ischemia, arising in humans, can be a consequence of cardiac arrest or can be induced experimentally in animals. After days of reperfusion in humans and rodents, it leads to selective cell death of hippocampal CA1 pyramidal neurons. Interruption of blood flow initiates high-energy metabolic failure, ATP depletion, ion imbalance, and other biochemical changes. Those changes include an increase of free radicals, mitochondrial dysfunction, lactic acidosis, and inhibition of proteosynthesis as a consequence of endoplasmic reticular (ER) stress (DeGracia et al. 2002). Other neurons such as CA3 or parietal cortical pyramidal neurons are much less vulnerable. Since hippocampal CA1 neuronal death usually occurs 2–3 days after an initial ischemic insult, this phenomenon is commonly referred to as delayed neuronal death.

To avoid ischemia-induced injury, several neuroprotective agents and protocols were tested. The brain resistance to ischemic injury or ischemic tolerance can be transiently augmented by prior exposure to a non-injurious stimulus.

The concept of ischemic preconditioning was introduced in the late 1980s by Murry et al. (1986) on the heart and later on the brain by Schurr et al. (1986) and Kitagawa et al. (1991). The molecular mechanisms underlying ischemic tolerance are not yet fully understood. However, two windows have been identified. One window represents a very rapid and short-lasting post-translational changes. The second window develops slowly (over days) as a robust and longlasting transcriptional change after an initial insult (Gidday 2006).

The endoplasmic reticulum reacts to the interruption of blood flow by the unfolded protein response (UPR). It can be highly variable depending on the dosage, duration of ischemic treatment (Imaizumi et al. 2001), and intensity of UPR signals (Yoshida et al. 2003). However, when ER stress is too severe and prolonged, apoptosis is induced. Various enzymes and transcription factors include the double stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK; Harding et al. 1999), transcription factors ATF4, ATF6 (activating transcription factor 6) and the inositol-requiring enzyme IRE1 (Shen et al. 2001) which are involved in the UPR. In the physiological state, PERK, ATF6, and IRE1 activity is suppressed by binding of the ER chaperone: glucose regulated protein 78 (GRP78). Morimoto et al. (2007) reported that the induction of GRP78 prevents neuronal damage which is induced by ER stress. The increase in GRP78 (BiP) expression may correlate with the degree of neuroprotection. Under ER dysfunction, GRP78 dissociates from PERK, ATF6, and IRE1. It binds to the unfolded proteins to facilitate refolding. Dissociation of GRP78 from PERK and IRE1 triggers activation of PERK and IRE1. It activates ATF6 subsequently and induces expression of ER stress genes. ATF6 is a key transcription factor in the resolution of the mammalian UPR. Unlike IRE1 and PERK, there is no evidence that ATF6 is involved in proapoptotic pathways (Yoshida et al. 2001). Haze et al. (1999) showed that ATF6 appears to be turned over fairly quickly. Its half-life within the cell is 2 h. After being activated, IRE1 is turned into an endonuclease that specifically cuts out a sequence of 26 bases from the coding region of X-box protein 1 (*XBPI*) mRNA (Calfon et al. 2002). Processed *XBPI* mRNA is translated into a new protein—54 kDa. It functions as a transcription factor specific for ER stress genes including GRP78 and GRP94. Paschen (2003a) have reported that *XBPI* mRNA was induced at 6 h after cerebral ischemia and reperfusion.

As shown by previous studies, the changes of the UPR gene expression induced by transient ischemia occur mostly during the first 24 h (Paschen 2003b) or the first few days after the insult (Qi et al. 2004). In line with this, we have decided to measure changes in mRNA and protein levels of GRP78, ATF6, and XBPI (UPR reaction) after

15 min of global ischemia and 1, 3, and 24 h reperfusion. In addition, we have focused our attention on the effect of preconditioning on the stress reaction of endoplasmic reticulum induced by ischemic/reperfusion insult.

Materials and Methods

Animal Model of Ischemia and Experimental Design

Adult male Wistar rats (mean body weight 320 g) used for the experiments were housed in a menagerie under standard conditions with a temperature of $22 \pm 2^\circ\text{C}$, and periodical variation in daylight at 12-h intervals. Food and water were provided ad libitum.

Global forebrain ischemia was induced by the standard four-vessel occlusion model as was recently described (Lehotský et al. 2004; Sivoňová et al. 2008; Uríková et al. 2006; Urban et al. 2008). Briefly, on day 1, both vertebral arteries were irreversibly occluded for 10 min. After anaesthesia with 2.5% halothane in a mixture of oxygen/nitrous oxide (30%/70%), thermocoagulation was used through the alar foramina. There was no visible influence on the animals. On day 2, both common carotid arteries were occluded for 15 min by small atraumatic clips under anaesthesia with 2.5% halothane in a mixture of oxygen/nitrous oxide (30%/70%). Two minutes before carotid occlusion, the halothane was removed from the mixture. Normothermic conditions (37°C) were monitored by a microthermistor placed in the ear. Temperature was maintained using a homeothermic blanket. Sham control naïve animals (C, $n = 6$) were prepared in the same way without carotid occlusion. The naïve rats then underwent 15 min of ischemia (I, $n = 6$). It was followed by 1, 3, and 24 h of reperfusion (from I1R to I24R, respectively, each group $n = 6$). Criteria for forebrain ischemia were loss of the righting reflex, mydriasis, and paw extension. The rats that became unresponsive and lost the righting reflex during bilateral carotid artery occlusion and showed no seizures, during and after ischemia, were used for the experiments. Only such animals are considered to have met the criteria for adequate ischemia (Pulsinelli et al. 1982). All rats used reached the criteria for global forebrain ischemia. They were divided into groups for the experiments mentioned above in the same way as non-treated animals (C, I, I1R-I24R, in each group $n = 6$).

Ischemic preconditioning was induced by 5 min of sublethal ischemia followed by 2 days of reperfusion in a preischemic group of rats. Rats then underwent lethal ischemia in a duration of 15 min as mentioned above (I, $n = 6$), followed by 1, 3, or 24 h of reperfusion (from I1R to I24R, respectively, each group $n = 6$). After ischemia and specific timing of reperfusion, animals were

sacrificed by decapitation. The hippocampus was dissected and processed immediately. Control animals (C) for both the naive ischemic group and preischemic group underwent the same procedure except of carotid occlusion.

After decapitation, the whole brain was extracted in a RNA-ase free condition, then we isolated the hippocampi. The hippocampus was used for Western blot analysis and for mRNA analysis. All sections were weighed and stored at -80°C .

Real-Time PCR

For maximal proof of changes in mRNA levels, we decided to use real-time PCR with SYBr green. We performed four analyses for each gene per animal (in each group $n = 6$). Total RNA was harvested by phenol-chloroform isolation using TRI reagent (Biotech). First-strand cDNA synthesis was performed using RevertAid H minus First strand cDNA Synthesis Kit (Fermentas Life sciences). Real-time PCR was made using primers:

Gene	Forward primer sequence	Reverse primer sequence
Xbp1	TGTCACCTCCCCAGAACATCT	CAGGGTCCAACCTGTCCAGAA
Grp78	TGATAATCAGCCCACCGTAACA	GGAGGGATTCCAGTCAGATCAA
Atf6	GGAAGTTACCAAGGCTTCTTTGAC	TGGGTGGTAGCTGGTAATAGCA
18S	AACGAACGAGACTCTGGCATG	CGGACATCTAAGGGCATCACA

All fragments had 100 bp and the S18 was used as house-keeping gene. Melting points for specific gene were analyzed as follows: XBP1 74.3°C , ATF6 75.2°C , GRP78 74.6°C , and GADD153 76.7°C . The PCR was conducted for 40 cycles (50°C for 2 min, 95°C for 10 min, and 95°C for 15 s and 60°C for 1 min) by 7500 real-time PCR System (Applied Biosystems). Each sample was loaded at least four times.

Western Blot Analysis

Hippocampi from sham control, ischemic and preconditioned animals were homogenized and resolved by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). After Western blotting (Trans blot SD semi-dry transfer cell, Bio-Rad), blots on nitrocellulose membrane were probed with goat polyclonal antibodies against GRP78 (sc-1051), XBP1 (sc-32138), and ATF6 (sc-30597, all from Santa Cruz Biotechnology) at room temperature for 2 h in dilution 1:500. GRP 78 antibodies are specific for detection of GRP 78 of mouse, rat, and human origin, XBP1 are specific for detection of XBP1 of mouse, human, and rat origin, ATF6 antibodies are specific for detection of ATF6 of

mouse, rat, and human origin. After being washed with 0.05% phosphate buffered saline (PBS)-Tween, the membranes were incubated with a donkey anti-goat secondary antibody conjugated with horseradish peroxidase (sc-2033, 1:3000, Santa Cruz Biotechnology) for 1 h and washed again with PBS-Tween. Finally, the membranes were developed with a SuperSignal West Pico Chemiluminescence Substrate (ECL system from Pierce, #34080) and detected by Molecular Imager Gel Doc XR System (Bio-Rad). Spot analysis was made using Gene Tools (SynGene). We analyzed six animals per experimental group. To reduce differences among animals, sample loading on SDS-PAGE and variability due to ECL detection, Western blots were performed by loading the same amount of proteins for each reperfusion time point per animal, at least four times.

Data Analysis

Data from naïve sham control, ischemic/reperfusion and preconditioned animals were examined by one-way

ANOVA with Tukey's post hoc test to analyze the significance of differences between groups (for either mRNA or protein levels). One-way ANOVA with the paired Student's *t*-test was used to compare results between different times of reperfusion within the same experimental group (for either mRNA or protein levels). Data were presented as mean percent \pm SD.

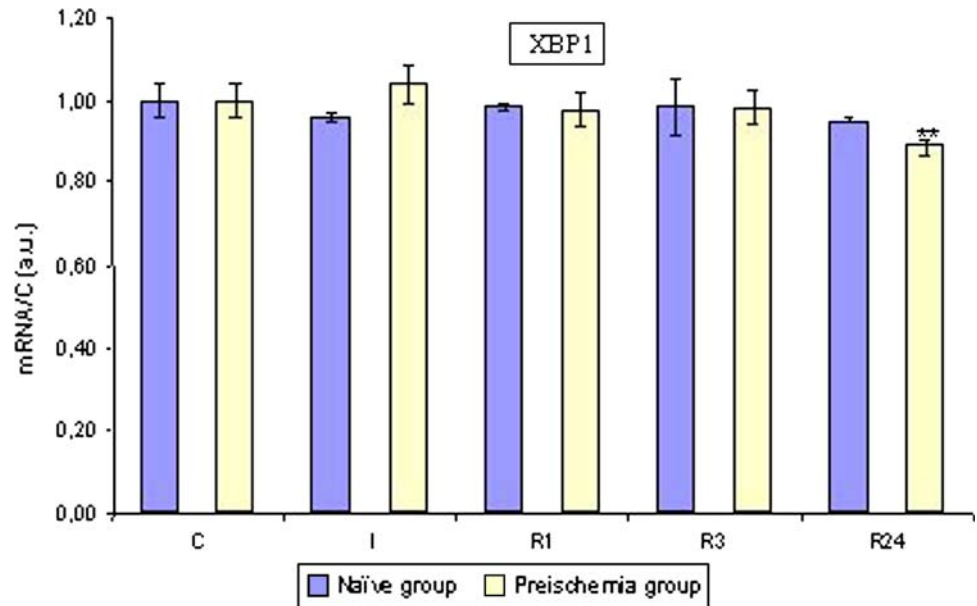
Results

In this study we have analyzed both the mRNA and the protein levels of ER stress genes after ischemic/reperfusion damage (I/R) in naïve and preconditioned groups of rats.

XBP1

In the naïve ischemic group of animals, the mRNA level (Fig. 1) did not change significantly and persisted unchanged in all analyzed periods. In the preischemic animal group, the mRNA level showed in ischemic time only slight, not significant differences compared to

Fig. 1 mRNA levels of *XBPI* in rat hippocampus after 15 min ischemia and 1, 3, and 24 h reperfusion, comparison between naïve ischemic groups and with 5 min preischemia (preischemia group) following 15 min ischemia (I) and 1, 3, and 24 h reperfusion (R1, R3, and R24). Results are presented as mean \pm SEM for $n = 6$, normalized to control levels. $^{***}P < 0.01$ significantly different as compared to naïve and preischemic controls (C), respectively



controls, followed by a significant decrease at 24 h of reperfusion (24 h) (by about $12.8 \pm 1.4\%$ compared to controls).

The level of XBPI protein in naïve ischemic group of animals (Fig. 2) showed a significant decrease in ischemic phase (I) (by about $39.2 \pm 1.6\%$ compared to

controls) and reached significant elevation at later reperfusion periods (R3 and R24) (by $82 \pm 2.4\%$ and $24.1 \pm 1.6\%$, respectively, compared to controls). The influence of preischemia on protein level was significant mainly in later ischemic times. The protein level reached maximum at 3 h of reperfusion (R3) (about 230% of

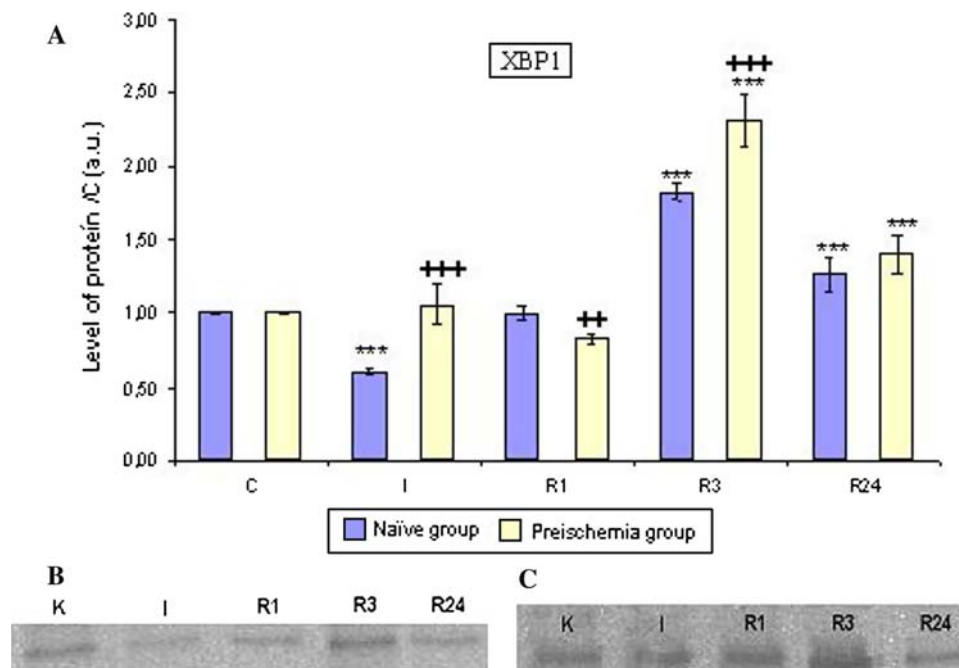


Fig. 2 Protein levels of XBPI in rat hippocampus after 15 min ischemia and 1, 3, and 24 h reperfusion. **a** Comparison between naïve ischemic groups and with 5 min preischemia (preischemia group) following 15 min ischemia (I) and 1, 3, and 24 h reperfusion (R1, R3, and R24), respectively. Results are presented as mean \pm SEM for $n = 6$, normalized to control levels. $^{***}P < 0.01$ significantly different as compared to preischemia control animals.

$^{+++}P < 0.001$ mean significantly different between naïve ischemic and preischemia animals in the same time points. **b** Detected bands for protein XBPI in naïve ischemic animals (K control, I 15 min ischemia, IR, 3R, and 24R reperfusion from 1, 3, and 24 h). **c** Detected bands for protein XBPI in preischemia group of animals (K control, I 15 min ischemia, IR, 3R, and 24R reperfusion from 1, 3, and 24 h)

controls) and persistently elevated in later reperfusion (R24) (by $40.3 \pm 4.9\%$ in compared to controls).

GRP78

In naïve ischemic group of animals, the mRNA level for *GRP78* (Fig. 3) showed maximal differences in later reperfusion phases. At 3 and 24 h (R3 and R24) of reperfusion mRNA level reached non-significant elevation (by about 10.8 and 11.3% compared to controls). Effect of preischemia (preischemic group) was documented in all analyzed periods and was expressed by the elevated mRNA levels in reperfusion period by about 11.7 ± 3.6 at first hour (R1) and by about $8.7 \pm 1.8\%$ at 24 h of reperfusion (R24) in comparison to mRNA levels in corresponding ischemic/reperfusion times.

The level of GRP78 protein (Fig. 4) in naïve ischemic animals showed a rapid increase in ischemic time (I) (by about 217% of controls) and remained elevated at 3 and 24 h of reperfusion (R3 and R24) (about 213 and 43%, respectively, compared to controls).

Increased mRNA values in preconditioned animals (preischemic group) also corresponded with the significant increase of the levels of GRP78 protein (Fig. 4). The changes are documented in the ischemic phase (I) and also in all reperfusion times (R1, R3, and R24) (by about 250% of controls and about 50% of corresponding ischemic/reperfusion times).

ATF6

In naïve ischemic animals, the level of mRNA for *ATF6* (Fig. 5) showed a non-significant decrease in ischemic

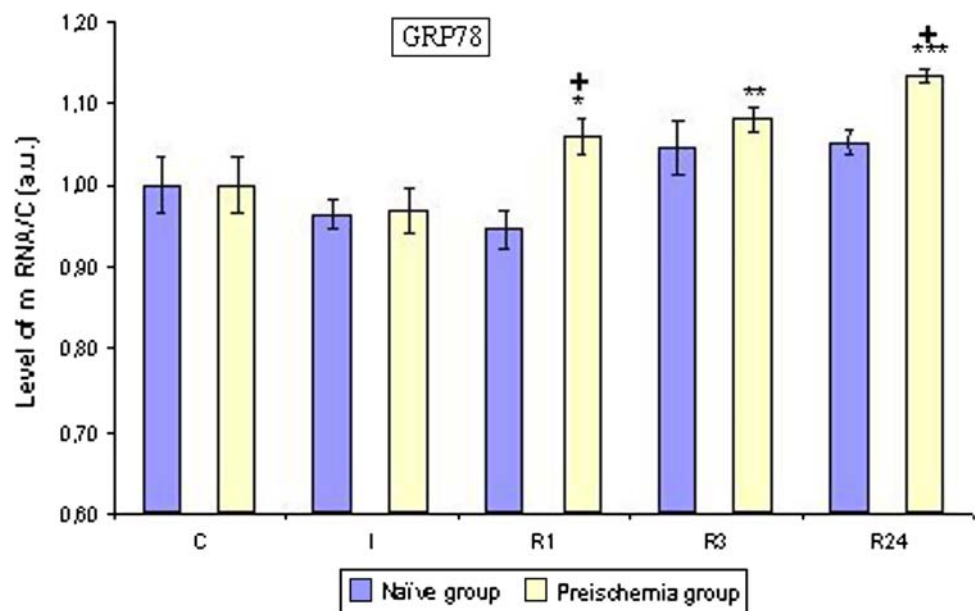
phase (I), followed by gradual elevation which reached to a significant increase at 24 h of reperfusion (R24) ($9.2 \pm 4\%$ higher than control). Preconditioning did not significantly change mRNA levels in all analyzed periods. Similarly to naïve ischemic animals, in the preischemic group the mRNA levels for ATF6 in ischemic phase (I) and early reperfusion times showed depression and at 24 h of reperfusion (R24) it reached a significant increase (by $9.2 \pm 2.9\%$ of controls).

Protein level in naïve ischemic animals followed the mRNA levels, ischemia (I) induced changes in all analyzed periods (R1 and R3) except of 24 h of reperfusion (R24), when the level of p90ATF6 non-significantly increased. On the other hand, preconditioning induced remarkable changes in the protein levels. In preischemic animals (Fig. 6), the levels of p90ATF6 at ischemic phase (I) significantly increased (about 170%) in comparison to controls, which remained elevated in earlier reperfusion times (R1 and R3) (about 37 and 62% higher than in controls). After 24 h of reperfusion (R24), it significantly elevated and reached 15% over the control level.

Discussion

Ischemic tolerance induced by short-term sublethal ischemia saves most of the pyramidal neurons in the hippocampus from the neuronal death induced by short (6–10 min) global and focal ischemia (Coimbra and Wieloch 1994; Ohtsuki et al. 1996; Danielisová et al. 2005). In this study, we were interested whether preconditioning induced by preischemic treatment prior to the global ischemia/reperfusion would affect the expression of genes coding for

Fig. 3 mRNA levels of *GRP78* in rat hippocampus after 15 min ischemia and 1, 3, and 24 h reperfusion, comparison between naïve ischemic groups and with 5 min preischemia following 15 min ischemia (I) and 1, 3, and 24 h reperfusion (R1, R3, and R24), respectively. Results are presented as mean \pm SEM for $n = 6$, normalized to control levels. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ significantly different as compared to naïve and preischemic controls (C), respectively. + $P < 0.05$ mean significantly different between naïve ischemic and preischemic animals in the same time points



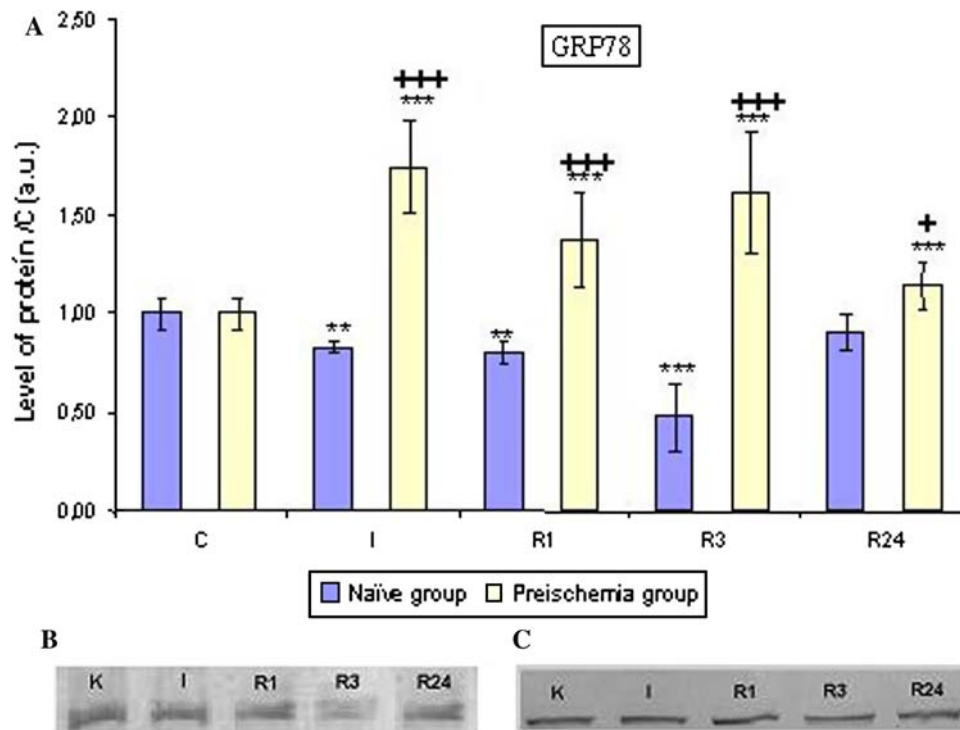


Fig. 4 Protein levels of GRP78 in rat hippocampus after 15 min ischemia and 1, 3, and 24 h reperfusion. **a** Comparison between naïve ischemic groups and with 5 min preischemia following 15 min ischemia and 1, 3, and 24 h reperfusion, respectively. Results are presented as mean \pm SEM for $n = 6$, normalized to control levels. *** $P < 0.001$ significantly different as compared to naïve and preischemic controls, respectively. +++ $P < 0.001$ mean significantly

different between naïve ischemic and preischemic animals in the same time points. **b** Detected bands for protein GRP78 in naïve ischemic animals (*K* control, *I* 15 min ischemia, *1R*, *3R*, and *24R* reperfusion from 1, 3, and 24 h). **c** Detected bands for protein GRP78 in preischemic group of animals (*K* control, *I* 15 min ischemia, *1R*, *3R*, and *24R* reperfusion from 1, 3, and 24 h)

the main proteins involved in the unfolded protein response of ER in hippocampal neurons.

In general, I/R injury initiates suppression of global proteosynthesis, which is practically recovered in the reperfusion period with the exception in the most vulnerable neurons, such as pyramidal cells of CA1 hippocampal region (de la Vega et al. 2001). On the other hand, ischemia is one of the strongest stimuli of gene induction in the brain. Different gene systems related to reperfusion processes of brain injury, repair, and recovery are modulated (Gidday 2006).

The results of our experiments showed that the level of XBP1 protein was elevated in both animal groups. In the preischemic group, XBP1 reached 230% of control in later reperfusion phases. Cerebral ischemia induces the strong but transient inhibition of translation which prevents the expression of key effector UPR proteins such as XBP1, GRP78, or ATF4. They hindered recovery from ischemia-induced ER dysfunction (Kumar et al. 2003; Paschen 2003a) which possibly leads to a pro-apoptotic phenotype (DeGracia and Montie 2004). The genetic response is based on XBP1 mRNA processing, resulting in synthesis of a new XBP1 protein. However, probably due to the

transient pattern of translation suppression, the rise of XBP1 protein was documented in preischemic experiments only in later reperfusion phase. Similar to our data, Thue-rauf et al. (2006) found that myocardial ischemia/hypoxia activates UPR with the concomitant increased expression of XBP1 protein and XBP1-inducible protein. They contribute to protection of the myocardium during hypoxia. Also, the results of Paschen (2003a) by semi-quantitative RT-PCR showed a marked increase of *XBP1* mRNA levels after focal ischemia in the cerebral cortex.

The results of real-time PCR measurement showed an increased mRNA level of *GRP78*, at later phases of reperfusion in non-treated animals. Preischemia induced further elevation of mRNA levels in reperfusion periods, which corresponded with the significant increase of the level of GRP78 protein. GRP78 is a member of the 70-kDa heat shock protein family that acts as a molecular chaperone in the folding and assembly of newly synthesized proteins within the ER. As shown by Yu et al. (1999) the suppression of GRP78 expression enhances apoptosis and disruption of cellular calcium homeostasis in hippocampal neurons that are exposed to excitotoxic and oxidative insults. This indicates that a raised level of GRP78 makes

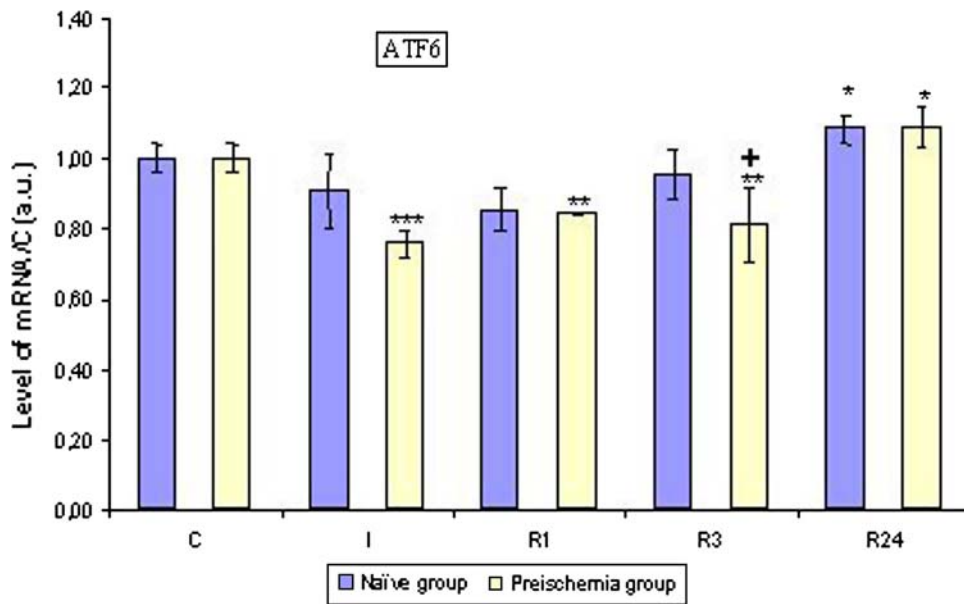


Fig. 5 mRNA levels of *ATF6* in rat hippocampus after 15 min ischemia (I) and 1, 3, and 24 h reperfusion (R1, R3, and R24), comparison between mRNA levels of *XBPI* in rat hippocampus after 15 min ischemia and 1, 3, and 24 h reperfusion, comparison between naïve ischemic groups and with 5 min preconditioning following 15 min ischemia and 1, 3, and 24 h reperfusion, respectively. Results

are presented as mean ± SEM for $n = 6$, normalized to control levels (C). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ significantly different as compared to naïve and preischemic controls, respectively. + $P < 0.05$ mean significantly different between naïve ischemic and preischemic animals in the same time points

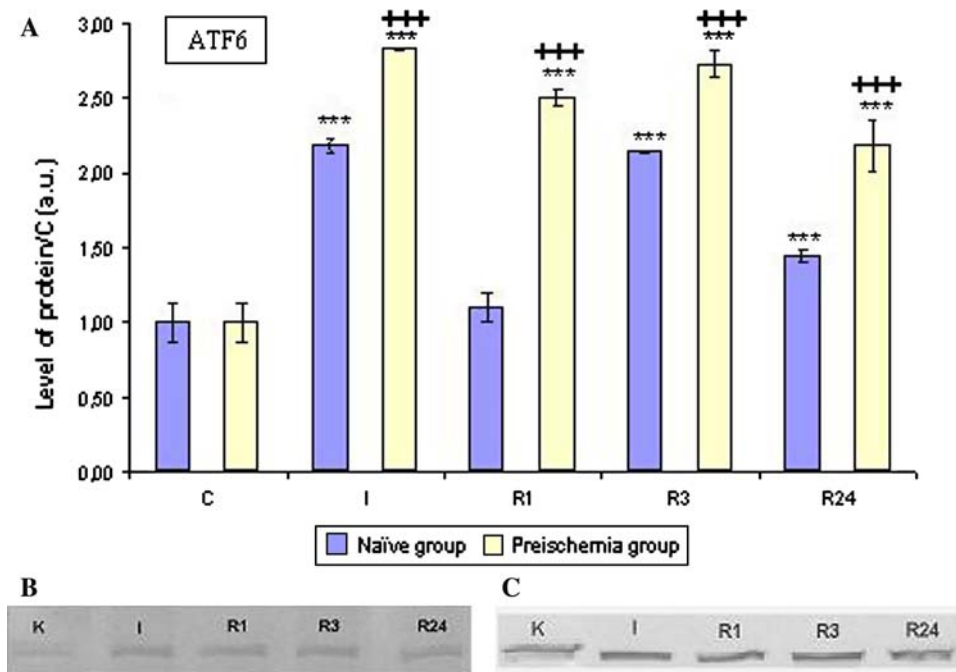


Fig. 6 Protein levels of *ATF6* in rat hippocampus after 15 min ischemia and 1, 3, and 24 h reperfusion. **a** Comparison between naïve ischemic groups and with 5 min preconditioning (preischemia group) following 15 min ischemia and 1, 3, and 24 h reperfusion, respectively. Results are presented as mean ± SEM for $n = 6$, normalized to control levels. ** $P < 0.01$, *** $P < 0.001$ significantly different as compared to naïve and preischemia controls, respectively. + $P < 0.05$,

+++ $P < 0.001$ mean significantly different between naïve ischemic and preischemia animals in the same time points. **b** Detected bands for protein *ATF6* in naïve ischemic animals (K control, I 15 min ischemia, IR, 3R, and 24R reperfusion from 1, 3, and 24 h). **c** Detected bands for protein *ATF6* in preischemia group of animals (K control, I 15 min ischemia, IR, 3R, and 24R reperfusion from 1, 3, and 24 h)

cells more resistant to the stressful conditions (Aoki et al. 2001). Morimoto et al. (2007) used the focal ischemia model to detect the maximum level of *GRP78* mRNA after 6 h of reperfusion, which also supports our results. In our experiments, we found elevated protein levels of GRP78 in the non-treated ischemic animals. However, preischemia induced a higher elevation of protein level. Our results are similar to the findings of Hayashi et al. (2003) and García et al. (2004), who used a model of ischemic preconditioning in rats. They documented an increase in GRP78 expression after 2 days of preconditioning. Authors proposed that the development of tolerance includes changes in PERK/GRP78 association, which were responsible for the decrease in eIF2a phosphorylation induced by preconditioning. On the other hand, Burda et al. (2003) failed to find any differences in the level of GRP78 protein in rats that were with or without acquired ischemic tolerance. This was probably due to exposure of very short reperfusion times.

In a screen for compounds that induced the ER-mediated chaperone GRP78 (78 kDa glucose-regulated protein, BiP), BiP inducer X (BIX) was recently identified. BIX preferentially induced GRP78 with slight inductions of GRP94, calreticulin, and C/EBP homologous protein. Remarkably, the induction of BiP mRNA by BIX was mediated by the activation of ER stress response elements upstream to the BiP gene, through the ATF6 pathway (Kudo et al. 2008).

ATF6 is an ER-membrane-bound transcription factor activated by ER stress. It is specialized in the regulation of ER quality control proteins (Adachi et al. 2008). The results of our experiments showed elevated mRNA expressions of ATF6, only in later reperfusion times. Consequently, the levels of mRNA for GRP78 were increased only slightly compared to controls. However, preconditioning induced a remarkable elevation of p90ATF6 protein at ischemic phase and significantly elevated in reperfusion. The IRE1 pathway mediates transcriptional induction of not only ER quality control proteins (molecular chaperones, folding enzymes, and components of ER-associated degradation) but also proteins working at various stages of secretion. The PERK pathway is responsible for translational control and also participates in transcriptional control in mammals.

The minimum level of mRNA was probably due to pro-survival mechanism through the inhibition of pro-apoptotic protein GADD153. GADD153 usually acts as a transcription factor of UPR genes (Kumar et al. 2003). Interestingly, Haze et al. (1999) found that the overexpression of full-length ATF6 activates transcription of the GRP78 gene. We also showed significant higher levels of ATF6 in preischemic animals in comparison to the non-treated group both in ischemic and reperfusion periods. Explanation of

generally higher levels of protein p90ATF6 in preischemic group is probably connected with an increased promoter activity of GADD153 to UPR genes (Oyadomari and Mori 2004).

Our results indicate that global ischemia/reperfusion initiates time-dependent differences in endoplasmic reticular gene expression at both the mRNA and protein levels and that endoplasmic gene expression is affected by preischemic treatment. The difference between naïve ischemic and preischemic animals was detected in both the mRNA and the protein levels of all products of ER stress response. This suggests that the preischemic treatment may exert a role in the attenuation of ER stress response in the neuroprotective phenomenon of acquired ischemic tolerance. Changes in gene expression of the key proteins provides an insight into ER stress pathways. It also might suggest possible targets of future therapeutic interventions to enhance recovery after stroke.

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