

Expression of the Endoplasmic Reticulum Chaperone GRP94 Gene in Ischemic Gerbil Brain

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GRP94 (glucose regulated protein 94) gene expression in the ischemic-hippocampus of gerbils, which was induced by a temporary occlusion of the bilateral common carotid arteries (CCAs), was tested by Northern blot analysis. The maximum GRP94 gene expression level was detected at the occipital lobe 10 min after the induction of ischemia. In the hippocampus, GRP94 gene expression reached a maximum 15 min after inducing ischemia. Following reperfusion, the maximum expression level was shown at 12 h and continuing thereafter.

Key words: Glucose-Regulated Protein 94 (GRP94), Common Carotid Artery (CCA), Ischemia

Introduction

Brain ischemia initiates a complex cascade of metabolic events, several of which involve the enhanced production of a specific protein family, so-called the heat shock proteins, including the endoplasmic reticulum (ER) chaperones (Chen *et al.*, 1996). Although, under normal conditions, the ER chaperones act as intracellular housekeepers and are synthesized in response to various physiochemical or environmental stimuli (Fewell *et al.*, 2001). The elevation of the ER chaperone expression levels has been shown to be a major symptom of brain damage after transient focal ischemia that maintains the intracellular homeostasis of neurons in experimental animals (Kuznetsov *et al.*, 1996). The ER harbors an array of chaperones including the glucose-regulated protein 94 (GRP94), GRP78 (BiP), protein disulfide isomerase (PDI), calreticulin, calnexin, ERp29 and others (Kim and Arvan, 1998; Kwon *et al.*, 2000). GRP94 is one of the major ER chaperones, and is induced by the accumulation of unfolded proteins in the ER. The highly conserved ER retention motif of KDEL (Lys-Asp-Glu-Leu) is present at the C-terminus of GRP94 (Mazzarella and Green, 1987). This sug-

gests that it might associate transiently with a variety of newly synthesized secretory and membrane proteins or permanently with mutant or defective proteins. Recently, there have been a number of studies on GRP94 structure/function relationships, including the biochemical and biophysical analyses, cell biological analyses, immunological function in the regulation of the cellular immune responses (Csermely *et al.*, 1998). However, in the context of brain ischemia, the precise function of ER chaperones is unclear, especially GRP94 is associated with induction of ischemia. Therefore, this study examined one of the functions of GRP94 gene expression in an ischemic gerbil brain, the hippocampus.

Materials and Methods

Male Mongolian gerbils (*Meriones unguiculatus*; 60–80 g) were anesthetized with halothane (1.5 % in 70 % N₂O and 30 % O₂) during the following procedures to induce transient global cerebral ischemia. Both common carotid arteries (CCAs) were exposed by a ventral midline incision and separated carefully from the adjacent veins and nerves. Surgical clips transiently clamped the ves-

sels and the halothane level was reduced to 0.75 %. After removing the clips, the restored blood flow was visually confirmed. The gerbils were kept under a heating lamp at 30 °C until they regain consciousness, and rectal temperature was monitored continuously during ischemia. The brains were removed as quickly as possible under standard conditions (Hermann *et al.*, 1998).

For the Northern blot analysis, the total RNA samples were taken from each group (20 μ g each), electrophoresed on 1 % formaldehyde-agarose gels, and transferred onto a nylon membrane (Boehringer GmbH, Germany). The total RNA from each sample was isolated using a RNeasyTM Kit (Tel-Test, INC, Texas, USA) according to the manufacture's instructions. After baking at 68 °C for 2 h, the resulting membrane was hybridized with [α -³²P]-dCTP-labeled mouse GRP94 cDNA (Mazzarella and Green, 1987) in a SDS buffer [7 % SDS, 50 % formamide, 5 \times SSC (44 g NaCl, 22 g sodium citrate/1 l water), 2 % blocking reagent, and 50 mM sodium phosphate] at 50 °C overnight. The membrane was rinsed twice with 2 \times SSC and 0.1 % SDS, and then exposed to X-ray film at - 80 °C overnight and analyzed using an image analyzing system.

Results and Discussion

In order to determine the expression level of the GRP94 gene in the ischemic gerbil brain induced by the CCAs was tested. After inducing ischemia for 10 min, the brain was dissected into the different lobes and the total RNA was isolated as described in the Method section. 20 μ g of the total RNA was used in the Northern blot analysis, and the results are shown in Fig. 1. GRP94 gene expression was detected in all the lobes tested. While the strongest GRP94 gene expression in the ischemia-induced gerbil brain was shown in the occipital lobe, the minimum expression was found in the parietal lobe, and its expression in the occipital was approximately 3 times higher than its expression level in the other lobes tested. Here, it is unclear why GRP94 gene expression was the most sensitive to ischemia in the occipital lobe. However, it was also reported that an ischemia-responsive protein (irp94), one of the HSP110 families, was also strongly expressed at the occipital lobe (Koh *et al.*, 2000). This suggests that although

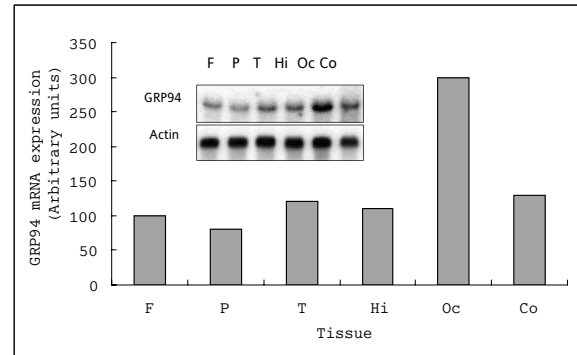


Fig. 1. The location of GRP94 gene expression in the gerbil brain after ischemia-induction. Each lane contained 20 μ g of the total RNA, upper panel, and at the lower panel, mouse actin was used to indicate the equivalence of the load on the gel. The GRP94 band intensities were measured using the ImageQuant software package. All experiments were performed in duplicate. F; frontal lobe. P; parietal lobe. T; temporal lobe. Hi; hippocampus. Oc; occipital lobe. Co; cerebellum.

GRP94 is an omnipresent protein under non-stimulated conditions, its expression is largely enhanced in the gerbil brain particularly in the occipital lobe after an ischemic injury.

GRP94 gene expression was examined in the hippocampus of gerbils as a function of the different clamping times of both CCAs. As shown in Fig. 2, after 5 min of ischemia, GRP94 gene expression began to increase gradually until 10 min and its maximum expression level occurred at 15 min, and continuing thereafter. The relative

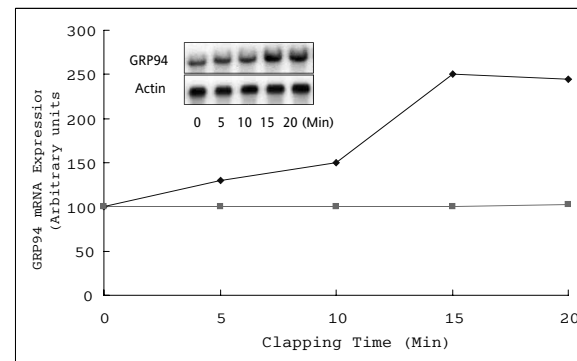


Fig. 2. GRP94 gene expression by reperfusion. In the hippocampus, GRP94 gene expression was tested during various ischemia-induction. Each lane was induced after 0, 5, 10, 15, and 20 min of ischemia induction. Northern blotting conditions are same with Fig. 1.

value of the maximum GRP94 gene expression level was approximately 2.5 times higher than the expression of the control. It is already well documented that a GRP94 belongs to the ER molecular chaperone family and plays important role in cellular protection against rapid external stimuli (*e.g.* heat, heavy metal, metabolically toxic matters, or malformed protein accumulation) (Kwon *et al.*, 1999; Sorger and Pelham, 1987). The overexpression of the cytoplasmic heat shock proteins (hsp) protects the host cells or/and by maintaining homeostasis (Massa *et al.*, 1995). However, until the effects of ischemia on the expression of the ER chaperones are uncertain. This study did not find any direct evidences showing that increased GRP94 gene expression was directly associated with ischemic damage and/or tolerance by GRP94 as an ER chaperone. However, the result suggests that the GRP94 gene expression might play a pivotal role in minimizing the cell damage caused by factors induced by ischemia.

Reperfusion experiments were conducted in order to determine if GRP94 gene expression is associated with rehabilitating the ischemic-damaged cells in the hippocampus. After both CCAs were clamped for 10 min, the brains were reperused for set times during the following 48 h. The results are shown in the Fig. 3. GRP94 gene expression in the hippocampus increased after reperfusion during the first 12 h, and decreasing thereafter. The relative maximum value of GRP94 gene expression at 12 h was approximately 1.2 times higher than its expression level at 1 h and continuing thereafter. Although it has already been reported that the cytoplasmic hsp has a recovery function of a damaged cell (Hanninen *et al.*, 1999), this study it the first to show that GRP94 as an ER chaperone,

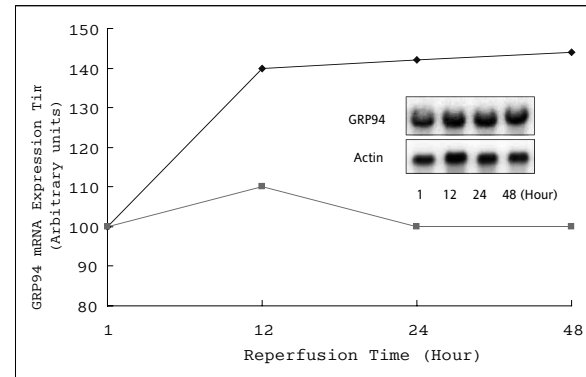


Fig. 3. GRP94 gene expression by different clamping times. GRP94 gene expression in the hippocampus following various reperfusion times after 15 min of ischemia. Lanes 1, 12, 24, 48 h indicate each reperfusion time. Northern blotting conditions are same with Fig. 1.

which might assist in rehabilitating damaged cells including ischemia. In this respect, the GRP94 gene expression associated with ischemia is of great interest because it can be applied to a therapeutic strategy in ischemic injuries. In future, to understand more the relationships both GRP94 and ischemia, we have to get several important information that how was GRP94 mRNA increased? During ischemia-reperfusion, what becomes ER-stress? Why do increase of GRP94 gene expression levels connect to the function of GRP94?

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