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Short Communication

Prolonged bihemispheric alterations in unfolded protein response related gene expression after experimental stroke

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ABSTRACT

After ischemia, endoplasmic reticulum (ER) stress pathways are activated that include unfolded protein response (UPR) and protein synthesis inhibition (PSI). Both of these mechanisms aim to restore ER functioning mainly by inhibition of translation and increased processing of excess proteins in ER. We were interested in the role of these pathways during spontaneous recovery after transient middle cerebral artery occlusion (MCAO) in rats. The spontaneous recovery of rats was assessed with a limb-placing test. The expression of ER-stress-related genes (IRE1, ATF6, GRP78, eif2α, ATF4, PERK) was studied by using in situ hybridization in different brain areas on post-operative days 2, 7, 14 and 28. Elevated signals were detected in striatum contralateral to the lesion on days 2 (PERK and IRE1) and 14 post-ischemia (IRE1). Gene expression was elevated on day 7 in the striatum ipsilateral to the lesion (ATF6 and GRP78) and on day 14 (GRP78) post-ischemia. Furthermore, elevated levels of GRP78 were detected on day 14 after ischemia in the ipsilateral sensorimotor cortex. These results suggest that altered gene expression related to unfolded protein response may be more long lasting than expected following focal cerebral ischemia. In addition, these results show that the response to ER stress differs ipsi- and contralaterally after MCAO in rats. Since these differences are detected in both hemispheres only in areas adjacent to the lesion, UPR may contribute to spontaneous recovery after MCAO in rats.

Endoplasmic reticulum (ER) is able to activate signal transduction pathways in ER stress conditions (Schröder and Kaufman, 2005). In ER stress, the protein folding capacity is exceeded and ER cannot function in normal physiological state. ER stress can be caused by several insults, such as ischemia or ER-related diseases that increase unfolded polypeptides or folding-incompetent proteins in ER (Schröder and Kaufman, 2005). Such events activate unfolded protein response (UPR) signal transduction pathways. UPR is characterized by activation of three ER-resident kinases, PKR-like ER kinase (PERK), IRE1 and ATF6 (Schröder and Kaufman, 2005). PERK induces phosphorylation of eIF2α, resulting in shutdown of translation at the initiation step. This stress response is needed to stop new synthesis of proteins that cannot be correctly folded in the ER and thus to protect cells from the effect of unfolded proteins. IRE1 is turned on after activation into an endonuclease that cuts

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out a sequence of 26 bases from the coding region of xbp1 mRNA. Processed xbp1 mRNA is translated into the respective protein, an active transcription factor specific for ER stress genes such as GRP78. ATF6 is a key transcription factor in the resolution of the mammalian UPR, and unlike IRE1 and PERK, there is no evidence that ATF6 is involved in proapoptotic pathways (Paschen, 2003a; Schröder and Kaufman, 2005). Shutdown of translation is a common response of cells to a severe form of stress. Protein synthesis inhibition is found to occur in brain immediately on reperfusion after ischemia and involves alterations in several genes related to initiation of translation, e.g. eukaryotic initiation factor 2 (eIF2) (DeGracia et al., 2002). Phosphorylation of eIF2α leads to translation of ATF4, which is an activator of CHOP-mediated apoptotic pathways (DeGracia et al., 2002). Repression of protein synthesis protects cells against protein misfolding during endoplasmic reticulum (ER) stress, nutrient deprivation and oxidative stress, all of which are consequences of ischemia. Ischemia studies conducted in vivo report changes in UPR gene expression only during the first 24 h (Paschen, 2003a) or the first few days after the insult (Qi et al., 2004). After transient cerebral ischemia, translational recovery is found only in cells that survive the interruption of blood supply, suggesting that restoration of translation critically determines the final result (Paschen, 2003b).

Functional recovery after stroke most likely occurs as the result of brain plasticity. Brain plasticity involves mechanisms such as increases in dendritic length, increase of spine density and changes in neuronal circuitry in cerebral cortical areas during recovery (Kolb et al., 2001). In addition, increased dendritic branching, increased numbers of synapses and perforated synapses per neuron and increased number of synapses with multiple synaptic boutons are found in motor cortex during the recovery process (Nudo et al., 2001). These changes correlate with functional recovery after focal ischemic injury, especially when rats are housed in an enriched environment in which they have additional social, sensory and motor experiences (Biernaskie and Corbett, 2001; Biernaskie et al., 2004). While changes in gene expression have been extensively studied after different restorative interventions (e.g. stem cells) (Loring et al., 2001), the molecular mechanisms underlying *spontaneous* recovery have not received much attention.

We were interested in the unfolded protein response at different time points during spontaneous recovery after focal ischemia in rats. We hypothesized that there are changes in gene expression coding for the main proteins involved in UPR during spontaneous recovery after ischemia. Changes in gene expression of key proteins are expected to provide insight into these unknown pathways and suggest possible targets of future therapeutic interventions to enhance recovery after stroke.

In limb-placing test there was a significant difference revealed between sham-operated and MCAO groups on day 2 (7.8 ± 0.4, $P < 0.0001$) and on day 7 (12.2 ± 1.1, $P = 0.0026$). Rats spontaneously recovered in limb functioning almost to the same level as sham-operated animals during 4 weeks (Fig. 1). Cresyl violet stain showed an extensive cortical and striatal damage in rats (Fig. 2) in all groups.

There was no significant difference in gene expression of ATF4 and eIF2α between ischemic and sham-operated rats on days 2, 7, 14 or 28 post-surgery in any of the brain regions (hippocampus, CA1, CA3, dentate gyrus, motor and sensorimotor cortex and striatum, see ROIs in Fig. 3) studied from either hemisphere nor between the hemispheres.

Gene expression of IRE1 (Fig. 4, 5) was significantly different between ipsi- and contralateral hemispheres ($F = 13.46$, $P = 0.002$), being significantly higher in the contralateral striatum (opposite hemisphere to the lesion) on days 2 (174.5% ± 14.5, $P = 0.036$) and 14 (180.7% ± 48.2, $P = 0.042$). There was no significant alteration detected in any other brain regions.

Gene expression of PERK (Fig. 4, 5) was significantly ($F = 5.59$, $P = 0.032$) elevated in the contralateral striatum. Effect of group approached significance ($P = 0.059$).

Gene expression of ATF6 (Fig. 4, 5) was significantly elevated ($F = 9.68$, $P = 0.0001$) in the ipsilateral striatum, while a group effect revealed significant elevation on day 7 after ischemia (159.8% ± 33.3, $P = 0.0032$).

GRP78 gene expression was elevated in the ipsilateral striatum ($F = 24.54$, $P = 0.00017$). A significant group effect ($F = 4.29$, $P = 0.016$) revealed elevation on days 7 (179.9% ± 34.8, $P = 0.036$) and 14 post-ischemia (235.8% ± 74.3, $P = 0.003$) (Fig. 4). The effect was also significant in a group × side comparison ($F = 4.84$, $P = 0.01$), which indicates that gene expression was significantly greater in ipsilateral striatum on day 14 (235.8% ± 74.3 $P = 0.001$). There was also a significant elevation in gene expression of GRP78 detected in the ipsilateral sensorimotor cortex ($F = 6.48$, $P = 0.022$). An effect of group ($F = 3.19$, $P = 0.044$) revealed elevation of GRP78 (Fig. 4, 5) on day 14 post-ischemia (155.2% ± 27.0, $P = 0.011$).

Our results show spontaneous recovery of function in limb-placing task by day 14 after ischemia. The improvement in limb function occurred simultaneously with

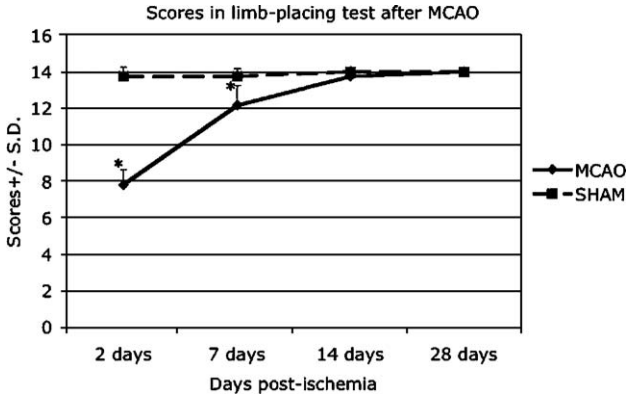

Fig. 1 – Performance of rats in the limb-placing test after transient MCAO. Values are means of test scores ± SD. The ischemic rats impaired significantly on days 2 and 7 post-ischemia when compared to different time points and performance of sham-operated rats ($P = 0.0001$).