Role of phospholipase D and osteopontin in reactive glial cells after transient forebrain ischemia

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Abstract

Transient forebrain ischemia results in delayed neuronal death in the CA1 region of the hippocampus after injury, which is, at least in part, a consequence of excessive generation of reactive oxygen species. Previous *in vitro* studies using cell cultures or brain slices have demonstrated that phospholipase D (PLD) in the nervous system is involved in the signaling mechanism in response to a variety of agonists. Several recent studies have shown that reactive oxygen species stimulate phospholipase D (PLD) activity in several kinds of cells. Therefore, this raises the possibility that PLD activity is enhanced in the ischemic brain.

Meanwhile, osteopontin (OPN) was initially identified as a sialoglycoprotein in bone, but has since been found in various tissues. Although not much is known about its function, OPN seems to play an important role in inflammation and tissue repair. Recently, it was reported that OPN was upregulated in the activated microglia after focal brain ischemia, suggesting that OPN might play a role in wound healing after a focal stroke.

In the present study, it was investigated whether the expression of PLD1 in the hippocampus is altered after transient brain ischemia using immunohistochemistry, Western blot analysis and an assay for PLD activity. In addition, this study was performed to investigate the spatial and temporal expression of OPN mRNA after global forebrain ischemia and to determine whether this phenomenon was associated

spatiotemporally with microglial reaction. Experiments were carried out using a four-vessel occlusion model for forebrain ischemia.

In the control hippocampus, PLD1 immunoreactivity was very low. After ischemia, in parallel with the results of Western blot analysis and the PLD activity assay, immunohistochemical analysis of PLD1 demonstrated that the immunoreactive proteins peaked at 7 to 14 days and were most prominent in the CA1 and the dentate hilar region. The temporal and spatial patterns of immunoreactivity of both PLD1 and glial fibrillary acidic protein (GFAP) were very similar, indicating that reactive astrocytes express PLD1, which was confirmed by double staining for PLD1 and GFAP. These results demonstrate that reactive astrocytes upregulate PLD *in vivo* after injury in the adult rat hippocampus.

The transient induction of OPN mRNA after global ischemia occurred earlier in the striatum than in the hippocampus. It was pronounced in the dorsomedial striatum close to the lateral ventricle and in the CA1 subfield and the subiculum of the hippocampus before microglial cells became more reactive. It also could be detected in the dentate hilus and to a marginal extent in the CA3. These results suggest that the hippocampus and the striatum following global forebrain ischemia upregulate OPN mRNA in different spatiotemporal profiles.