

Heat-Shock Proteins Induced by Transient Brain Ischemia

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INTRODUCTION

Heat-shock proteins, as well as other proteins common to the heat shock response, are induced in rodent brain following transient ischemia (Dienel et al., 1986; Jacewicz et al., 1986; Kiessling et al., 1986; Nowak, 1985). The function of the heat-shock proteins induced is a key to be cleared.

INDUCTION OF HSP AFTER ISCHEMIA

The 70 kDa heat shock protein, HSP 70, was induced and localized in gerbil brain at intervals after 10 min of transient ischemia (Vass et al., 1988). Cerebral ischemia was produced by occlusion of bilateral common carotid artery. The localization of HSP 70 was evaluated using a monoclonal antibody specific for stress-inducible forms of HSP 70-related proteins. Induced immunoreactivity was found only in neurons, primarily in hippocampus, striatum, entorhinal cortex and some neocortical regions. HSP 70 accumulation was minimal in hippocampal CA1 neurons, but was most pronounced in dentate granule cells and CA3 neurons. The time course of HSP 70 immunoreactivity through dorsal hippocampus was that: by 12-16 hr dentate granule cells were positive. At 24 hr occasional positive cells were evident in CA3. Immunoreactivity in CA3 was most intense at 48 hr. A few scattered neurons in CA1 showed staining at this time. At 96 hr hippocampal immunoreactivity was greatly reduced, but was still evident in CA3.

Induction of mRNA encoding HSP 70 was most pronounced in hippocampal CA1 neurons in brain received 5 min ischemia evaluated by *in situ* hybridization using ³⁵S-labeled oligonucleotide probe selective for stress-inducible members of this gene family (Nowak, 1991). Postischemic HSP 70 mRNA induction in hippocampus is documented. Strong hybridization was evident in dentate granule cells as well as in all hippocampal pyramidal cell fields within 3 h recirculation. Hybridization was absent from dentate granule cells and

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greatly diminished in CA3 neurons by 24 h. The most persistent induction occurred in CA1, which showed significant hybridization through at least 48 hr. Hybridization was no longer detected in the CA1 sector at 4 days.

HSP 70-mRNA sequences are strongly induced in CA1 neurons that rarely show significant protein immunoreactivity (Vass et al., 1988; Nowak, 1989). In other cell populations that express the protein, there is a considerable lag between the interval of maximal HSP 70-mRNA induction at 3-12h (Nowak, 1991, Nowak, 1985; Nowak et al., 1990a) and the delayed appearance of HSP-70 protein detected, e. g., 48 h in CA3 (Vass et al., 1988; Nowak, 1989).

INDUCED TOLERANCE TO ISCHEMIA

Ischemia for up to 2 min was a non-lethal, reversible ischemic stress. Minor 2-min ischemia transiently perturbs cellular metabolism (Ljunggren et al., 1974; Nowak et al., 1985), but neuronal necrosis never occurs (Hatakeyama et al., 1988). Animals received 5-min ischemia showed necrotic lesions in the CA1 area of hippocampus 7 days following recirculation (Izumiya and Kogure, 1988; Kirino, 1982; Paschen et al., 1984; Kitagawa et al., 1990b).

A prior milder ischemic treatment made tolerative property of neurons to a subsequent lethal ischemic stress in gerbils (Kitagawa et al, 1990a). Animals, which received single 2-min ischemia 1 day before or 2 days before 5-min ischemia, showed no or mild necrotic lesions and exhibited an insufficient but clear tolerance for ischemic neuronal death. Animals, which received 2-min ischemia twice at 1-day interval 2 days before 5-min ischemia, showed no or mild, necrotic lesions, and this ischemic treatment induced tolerance for delayed neuronal death. More than 1-day interval between non-lethal and lethal ischemia were needed for induction of tolerance.

Tolerance induced by prior mild ischemia for succeeding lethal ischemia was analyzed by measuring changes in the number of neurons (Kirino et al., 1991). The number of intact neurons in the hippocampal CA1 subfield was counted, and the density of neurons per mm of the pyramidal cell layer was calculated. The neuronal density of sham-operated animals (without ischemia) was 255 per 1-mm length of CA1 pyramidal cell layer. Most of the CA1 pyramidal cells were destroyed by single ischemia for 5 min. The overall CA1 neuronal density in the single-ischemia group was 11/mm. Two minutes of ischemia did not produce serious tissue damage in the brain. One day, 2 days, or 4 days following the first brief ischemia, the animals (double-ischemia group) were subjected to the second ischemia for 5 min. Gerbils pretreated with brief ischemia for 2 min and then subjected to the second ischemia for 5 min with a 1 day interval did not develop severe neuronal damage in the CA1 subfield, the CA1 neuronal density was 127/mm (69% of the normal). As the interval was elongated up to 4 days, there was a substantial preservation of CA1 pyramidal cells. The CA1 neuronal density was 176/mm (69% of the normal).

Immunostaining using monoclonal antibody raised against HSP 70 revealed an increase in HSP 70 in the CA1 area following 2 min of ischemia. Very brief ischemia induces heat-

shock proteins and, presumably, thereby renders neurons more tolerant to subsequent metabolic stress.

The effects of mild and non-lethal ischemic insult on neuronal death following subsequent lethal ischemic stress in various gerbil brain regions (Kitagawa et al. 1991a). Single 10-min ischemia consistently caused neuronal damage in the hippocampal CA1, CA2, CA3 and CA4, layer III/IV of the cerebral cortex, dorsolateral part of the caudoputamen and ventrolateral part of the thalamus. On the other hand, in double ischemia groups, 2-min ischemic insult 2 days before 10-min ischemia exhibited significant protection in the CA1 and CA3 of the hippocampus, the cerebral cortex, the caudoputamen and the thalamus. Five-min ischemic insult 2 days before 10-min ischemia also showed protective effect in the same areas as those of 2-min ischemia except for the CA1 region of the hippocampus, while 1-min ischemia induced no tolerance in any regions. In the immunoblot analysis, both 2- and 5-min ischemia caused increased synthesis of HSP 72 in the hippocampus, but 1-min ischemia did not. The ischemic tolerance was widely found in the brain and ischemic treatment severe enough to cause HSP72 synthesis might be needed for induction of ischemic tolerance. Neurons in the brain other than CA1 neurons of the hippocampus could induce the ischemic tolerance by preceding non-lethal ischemic stress.

FUNCTION OF HEAT-SHOCK PROTEINS

Living organisms, when exposed to sublethal environmental stress, respond by initiating the synthesis of several proteins. Heat-shock proteins are induced by various treatments of cells and organs of mammals other than heat; amino acid analogues, transition metals, oxidizing agents, poisons, anoxia, and tissue damage can all induce these proteins (Ananthan et al., 1986; Ashburner and Bonner, 1979; Subjeck and Thung-Tai, 1986). Heat-Shock proteins appear to exhibit a high degree of conservation from bacteria to mammalian cells (Bardwell and Craig, 1984; Hunt and Morimoto, 1985). Heat-shock proteins are considered to work by association with other proteins, thereby controlling protein conformation, stabilization, or transport to specific loci in the cell. Metabolic stress causes a decrease in free HSP 70 proteins, and this reduction seems to induce HSP 70 synthesis (Beckmann et al., 1990). This phenomenon is one of the typical metabolic changes in stress response. Stress response seems to be vital for cell recovery and survival after they are confronted with noxious insults (Barbe et al., 1988; Landry et al., 1982). The heat-shock response seems to be a way by which cells protect themselves against different stresses.

An example of induced tolerance to metabolic stress is the acquisition of heat tolerance by pretreating cells with sublethal hyperthermia (Gerner and Schneider, 1975). The phenomenon of induced tolerance was also observed in *in vivo* mammalian cells. Rats received hyperthermic treatment showed that the retina was less sensitive to light damage compared to untreated controls (Barbe et al., 1988). This change was correlated to protein synthesis in the retina after hyperthermia. They indicated a concomitant increase in major heat-shock proteins, especially HSP 70. Production of HSPs in response to elevated temperature and

other metabolic stress plays a role in enhancing survival during and after stress (Barbe et al., 1988, Riablow et al., 1988).

Cerebral ischemia induces selective gene expression and protein synthesis (Jorgensen et al., 1989; Kiessling et al., 1986; Nowak, 1985), and it is conceivable that neuronal cells like other types of cells respond and resist the suffering detrimental stress (Gerner and Schneider, 1974; Li and Hahn, 1978; Li and Werb, 1982, Li et al., 1983; Riablow et al., 1988).

When neurons are prepared with the production of HSPs, neurons could be more tolerant to subsequent ischemia. These experimental results suggest that failure of stress response could be a cause of ischemic neuronal damage in the hippocampal CA1 subfield.

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