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BRAIN RESEARCH

Research Report

Very brief focal ischemia simulating transient ischemic attacks (TIAs) can injure brain and induce Hsp70 protein☆

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ABSTRACT

This study examined very brief focal ischemia that simulates transient ischemic attacks (TIAs) that occur in humans. Adult rats were subjected to sham operations or 5 min, 10 min, or 2 h of middle cerebral artery (MCA) ischemia using the suture (thread) model. Hsp70 protein was induced 24 h, 48 h and 72 h later in neurons throughout the entire MCA territory in many but not all animals. Following 5- and 10-minute MCA occlusions, 9 of 32 animals (28%) had microinfarcts mostly in dorsal lateral striatum. Uncommon Hsp70 stained intracellular cytoplasmic inclusions, some of which co-localized with activated caspase-3, were detected in microglia, macrophages, astrocytes and oligodendrocytes. Hsp70 stained neurons were TUNEL negative at 24 h and 48 h whereas some Hsp70 stained neurons were TUNEL positive at 72 h after reperfusion. Hsp70 positive, activated "bushy" microglia and Hsp70 negative, activated "polarized" or rod-shaped microglia were located outside of the microinfarcts. Thus, experimental focal ischemia simulating TIAs can: induce Hsp70 protein throughout the ischemic vessel territory; produce Hsp70 protein positive glial inclusions; activate Hsp70 positive and negative microglia; and cause microinfarcts in some animals.

1. Introduction

The classic definition of a transient ischemic attack (TIA) is a temporary focal neurological deficit caused by brief interruption of local cerebral blood flow (Johnston, 2002). The symptoms of a TIA in the carotid territory typically last 5 to 10 min in humans and by definition last less than 24 h (Pessin et al., 1977). As many as a third of TIA patients eventually go on to have ischemic strokes (Rothwell et al., 2006). Recently, magnetic resonance diffusion-weighted imaging (DWI) has been used to assess cerebral ischemia. DWI measures the Brownian motion of water protons in tissue. When sodium-potassium pumps fail, water moves from the extracellular space to the more restricted intracellular space. This restricted proton motion causes an ischemic lesion to appear bright on DWI (Davis et al., 2006). Though TIAs have not been confirmed pathologically to be associated with cell death in brain, as many as 25–40% of TIA patients have DWI abnormalities on MRI brain scans (Nagura et al., 2003; Rothwell et al., 2006). Animal models show that these DWI changes occur within minutes of the onset of ischemia (Moseley et al. 1990; Li et al., 2000; Tong and Albers, 2000). These results suggest that some classical TIA patients may have brain infarction. Though TIA is a common cerebrovascular disorder (Johnston et al., 2003; Rothwell et al., 2006), there are few experimental studies directed only at 5–10-minute durations of focal ischemia.

Ischemic brain damage can be prevented or at least significantly reduced when there is a preceding brief ischemic

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period that does not exceed the "threshold for tissue damage"–a phenomenon termed "ischemic preconditioning" (Sommer, 2008). There are some reports of using 3–20 min of focal ischemia, but almost all of these short durations of ischemia are used for the induction of "preconditioning" or "ischemia-induced tolerance" (Chen et al., 1996; Li et al., 2006; Currie et al., 2000; Puisieux et al., 2004; Shimizu et al., 2001; Dhodda et al., 2004; Naylor et al., 2005). Focal ischemia as short as 3 min produced preconditioning (Puisieux et al., 2004), with three 10-minute episodes of preconditioning appearing to produce the best protection against focal infarction (Chen et al., 1996; Li et al., 2006).

One study has shown that 10 min of focal ischemia induced Hsp70 mRNA 24 h later in brain (Soriano et al., 1995). Since ischemia produces a translation block that may or may not affect synthesis of heat shock proteins (Vass et al., 1988; Gonzalez et al., 1989; Kinouchi et al., 1993a), this study specifically examined Hsp70 protein expression following very brief focal ischemia.

Given the clinical importance of TIAs and the fact that few studies have studied TIAs experimentally, we initiated these studies. The suture model was used, since it is well suited for producing 5 or 10 min of focal ischemia with reperfusion (Longa et al., 1989). We examined Hsp70 protein expression following the brief ischemia, and searched for evidence of infarction using Nissl, NeuN and GFAP staining and searched for evidence of isolated cell death using TUNEL and cleaved, activated Caspase-3 staining.

2. Results

2.1. *Induction of Hsp70 protein and microinfarction following brief focal ischemia*

Hsp70 protein was expressed in the brain of animals subjected to 5 min, 10 min and 2 h of focal, middle cerebral artery (MCA) ischemia (Figs. 1A–O) and not in animals subjected to sham operations (Figs. 1P–R). Five minutes of focal MCA ischemia induced Hsp70 protein in both striatum and cerebral cortex in much of the MCA territory (Figs. 1A–C). Following 10 min of focal MCA ischemia, Hsp70 was similarly expressed in the cortex and striatum in much of the MCA territory (Fig. 1D–L). The maximal induction of Hsp70 following 10 min of ischemia was at 24 h (Figs. 1D–F) with less Hsp70 at 48 h (Figs. 1G–I) and 72 h (Figs. 1J–L). Two hours of MCA ischemia produced infarction of striatum and cortex, with most of the Hsp70 protein expressed at the margins of the infarct (Figs. 1M–O). At 24 h following 5 min of focal ischemia Hsp70 protein was expressed primarily in neurons in the cortex (Fig. 1B) and striatum (Fig. 1C). Similarly, following 10 min of MCA ischemia Hsp70 protein was expressed primarily in neurons in cortex and striatum at 24 h (Figs. 1E, F), 48 h (Figs. 1H, I) and 72 h (Figs. 1K, L). The Hsp70 positive neurons in cortex were distributed throughout all cortical layers (I–VI) following both 5 and 10 min of MCA ischemia at 24 to 72 h of reperfusion (Figs. 1A, D, G, J). Following 2 h of ischemia with a 24 h reperfusion, little Hsp70 protein was expressed in the core of large middle cerebral artery infarctions (Fig. 1M). Hsp70 protein was expressed at the margins of the infarction (Fig. 1M) in microglial cells and in neurons (Figs. 1N, O).

Of the 8 animals subjected to 5 min of ischemia, 5 (63%) showed Hsp70 staining 24 h later. Of the 8 animals subjected to 2 h of ischemia, 6 (75%) showed Hsp70 staining 24 h later. Following 10 min of ischemia 87.5%, 75.0%, and 50.0% of animals ($n=8$ at each time) expressed Hsp70 protein at 24 h, 48 h, and 72 h later, respectively.

Western blot analysis (Fig. 2A) confirmed that the maximal induction of Hsp70 protein at 24 h of reperfusion occurred following 10 min and 120 min (2 h) MCA occlusions (Figs. 2A, B). Following 10 min MCA ischemia, the expression of Hsp70 protein was maximal at 24 h and decreased at 48 h and 72 h reperfusion (Figs. 2A, C).

Microinfarcts, not visible with the naked eye, were noted in 9 of the 32 rats (28%) subjected to 5 or 10 min of ischemia (Figs. 3B, D, F). Following 5 min of focal ischemia 2/8 of the animals had microinfarcts at 24 h. The areas of microinfarction were limited to the dorsal lateral striatum. Following 10 min of focal ischemia 3/8 of the animals had microinfarcts at 24 h, 2/8 at 48 h, and 2/8 at 72 h. These microinfarcts following 10 min of focal ischemia mainly involved the dorsal lateral striatum but occasionally involved adjacent cortex. Microinfarcts in ischemic striatum showed no NeuN staining of neuronal nuclei (Fig. 3B) compared to normal NeuN staining of neuronal nuclei in the contralateral non-ischemic striatum (Fig. 3A). Microinfarcts in ischemic striatum showed no GFAP stained astrocytes (Fig. 3D) compared to normal GFAP stained astrocytes in the non-ischemic, contralateral striatum (Fig. 3C). These same regions in dorsal lateral striatum showed disintegration of cells in the microinfarcts with Nissl staining (Fig. 3F) compared to normal Nissl staining in the contralateral non-ischemic striatum (Fig. 3E). Similar findings were observed in the scattered cortical microinfarcts following 10 min of focal ischemia (not shown).

The animals with 2 h occlusions had large MCA infarcts involving the striatum and cortex as well as microinfarcts (Fig. 3G) involving the cortex. Microinfarcts were often observed around the large infarcts following 2 h MCA occlusion. Macrophages expressed Hsp70 protein in the core of the microinfarct (Fig. 3G, red arrows). Hsp70 stained microglia surrounded the infarct and had a "bushy" appearance (Figs. 3G, H, yellow arrows). Some Hsp70 stained neurons

Fig. 1 – Hsp70 immunostaining following very brief Middle Cerebral Artery Occlusions (MCAO). (A–C) Five minute MCAO followed by 24 h reperfusion. Note Hsp70 immunostaining in cortex and striatum. (D–F) Ten minute MCAO followed by 24 h reperfusion. (G–I) Ten minute MCAO followed by 48 h reperfusion. (J–L) Ten minute MCAO followed by 72 h reperfusion. (M–O) Two hour MCAO followed by 24 h reperfusion. (P–R) Sham-operated control. Sham operated control cortex (Q) and control striatum (R) showed no staining. Following MCAO neuronal and glial cells expressed Hsp70 protein in the cortex (B, E, H, K, and N) and striatum (C, F, I, L, and O) following various durations of ischemia (5 min, 10 min and 2 h) and reperfusion (24 h, 48 h and 72 h). Red bar=3 mm, black bar=25 μm.