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Expression of fos-related antigen-2 in rat hippocampus after middle cerebral arterial occlusion

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Abstract

AP-1 transcription factors have been shown to be induced in the brain after ischemic injury. However, their roles in neuronal survival or death have yet to be defined. Here, we report the discovery of elevated nuclear levels of fos-related antigen-2 (FRA-2) in the nuclei of hippocampal neurons seven days after middle cerebral artery occlusion (MCAO). Expression of FRA-2 and AP-1 DNA binding activity is elevated in hippocampi ipsilateral as well as contralateral to MCAO. Using Fluoro-Jade staining as a marker of neurodegeneration, FRA-2 was not found to be expressed in degenerating neurons. Thus, FRA-2 is expressed in neurons that survive ischemic insult suggesting a role for this transcription factor in neuronal adaptation to the post-injury state.

Keywords: AP-1; Stroke; Ischemia; Neuronal survival; Transcription factor; Fluoro-Jade

AP-l transcription factors, which include c-fos and fos-related antigens (FRA) as well as the Jun-related factors, are induced in neurons after ischemic insult to the brain. These gene-regulatory proteins are expressed in neurons destined to degenerate as well as ones that survive the injury, playing roles in both cellular death and survival after brain injury [2]. Differences in the composition of the AP-1 DNA binding complex may account for differences in functional outcomes associated with expression of these transcription factors.

The fos-related antigen-2 (FRA-2) protein was originally identified as a 46 kDa protein induced in growth-stimulated chicken embryo fibroblasts and, unlike Fos, exhibits delayed and prolonged kinetics [7]. Previously, we have reported that FRA-2 is induced in neurons in several models of chemically-induced brain injury [13] (Pennypacker and O'Callaghan, submitted). The expression of FRA-2 is increased from days to months depending on the severity of the injury. In this current study, we report that FRA-2 expression is increased in the hippocampus seven days after middle cerebral artery occlusion (MCAO), a model of stroke, and is localized to neurons surviving the stroke-induced injury.

The MCAO method used in this study is a variant of the procedure described earlier [3,8]. Male Sprague–Dawley rats weighing 200–250 g at time of the surgery were anesthetized with isoflurane delivered at a 5% concentration mixed with O_2 and delivered at a flow rate of 2 l/min. Using blunt dissection techniques, the right common carotid, external carotid and internal carotid were isolated. The external carotid was ligated with silk suture and an embolus inserted in retrograde fashion through the external into the internal carotid, past the base of the skull into the Circle of Willis and the origin of the middle cerebral artery (25 mm). Once in place, the embolus was anchored by a temporary suture around the external carotid. The embolus was left in place for 60 min, removed, the hole in the external carotid closed, and the incision sutured.

Nuclear protein extracts were prepared from hippocampi for immunoblot and DNA binding analyses. AP-1 DNA binding activity was assayed using the AP-1 consensus oligo-

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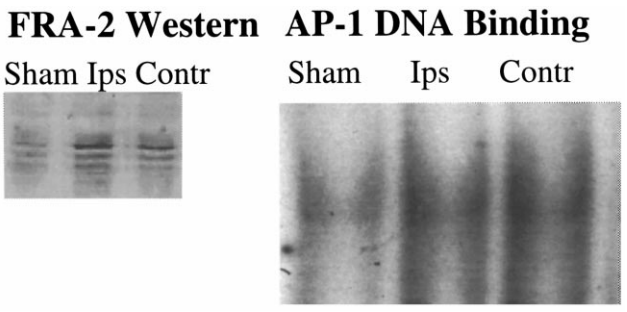

Fig. 1. Induction of FRA-2 and AP-1 DNA binding in the ipsilateral and contralateral hippocampus after MCAO. Nuclear protein extracts were prepared using hippocampi from sham-operated rats and hippocampi ipsilateral and contralateral to the MCAO seven days after surgery. Western blot analysis revealed an increase the 46 kDa FRA-2 in the ipsilateral (Ips) and contralateral (Control) hippocampi relative to the levels found in hippocampus from sham-operated rats (Sham). AP-1 DNA binding activity was increased in hippocampal nuclear extracts from MCAO-operated rats relative to sham-operated ones.

mer (22-mer; 5′-CTAGTGA*TGAGTCA*GCCGCATC-3′), containing the consensus sequence (5′-TGAGTCA-3′) [10]. For characterization of AP-1 DNA binding activity, the protein extracts were preincubated for 10 min prior to the addition of labeled probe with a 100-fold excess of unlabelled AP-1 oligomer. FRA-2 expression was determined by immunoblot and immunohistochemical analyses with antibodies against FRA-2 peptide (Santa Cruz Biotechnology, Santa Cruz, CA) using hippocampal nuclear extracts and tissue sections from MCAO and sham operated rats [13]. The above antibodies were preincubated with the antigen (10-fold excess of peptide to antibody by weight) after which immunoblot or immunohistochemical assays were performed to demonstrate specificity of the immunostaining, any protein bands that were detected were considered non-specific. Fluoro-Jade was used as a marker for neurodegeneration to determine whether the expression of FRA-2 was located in degenerating or surviving neurons in hippocampal sections from rats seven days after MCAO surgery. The Fluoro-Jade histochemical method was adapted from Schmued et al. [11]. After immunohistochemistry using diaminobenzidine, the thirty micron thick rat hippocampal sections were mounted, stained for 1 h at 4°C in a solution of 0.001% Fluoro-Jade in 0.1% acetic acid, dried and coverslipped.

Immunoblot analysis using the FRA-2 antibody revealed a 46 kDa band in nuclear extracts isolated from hippocampi removed from rats seven days after sham or MCAO surgery (Fig. 1). The expression of FRA-2 is increased in nuclear protein extracts from hippocampi ipsilateral and contralateral to the MCAO relative to protein extracts from sham-operated rats. Nuclear protein extracts containing increased FRA-2 levels also exhibit elevated AP-1 DNA binding activity (Fig. 1).

Immunohistochemical analysis revealed FRA-2 expression throughout the neuronal layers of the hippocampus. Double-immunohistochemical staining has shown that FRA-2 is specifically expressed in neurons (data not shown). Neurons of the CA1 hippocampal region from rats seven days after MCAO exhibited strong nuclear staining of FRA-2 (Fig. 2, Panel B) relative to hippocampal sections from sham-operated rats (Panel A). Fluoro-Jade was used as a marker for neurodegeneration to determine whether the expression of FRA-2 was located in degenerating or surviving neurons. Seven days after MCAO surgery sections prepared from hippocampus were immunostained using the FRA-2 antibodies followed by Fluoro-Jade histochemistry. In general, few neurons stained with Fluoro-Jade at seven days after MCAO. Within the CA1 region of the hippocampus, few neurons stained with Fluoro-Jade (Fluoro-Jade-positive neurons appear white in Fig. 3)

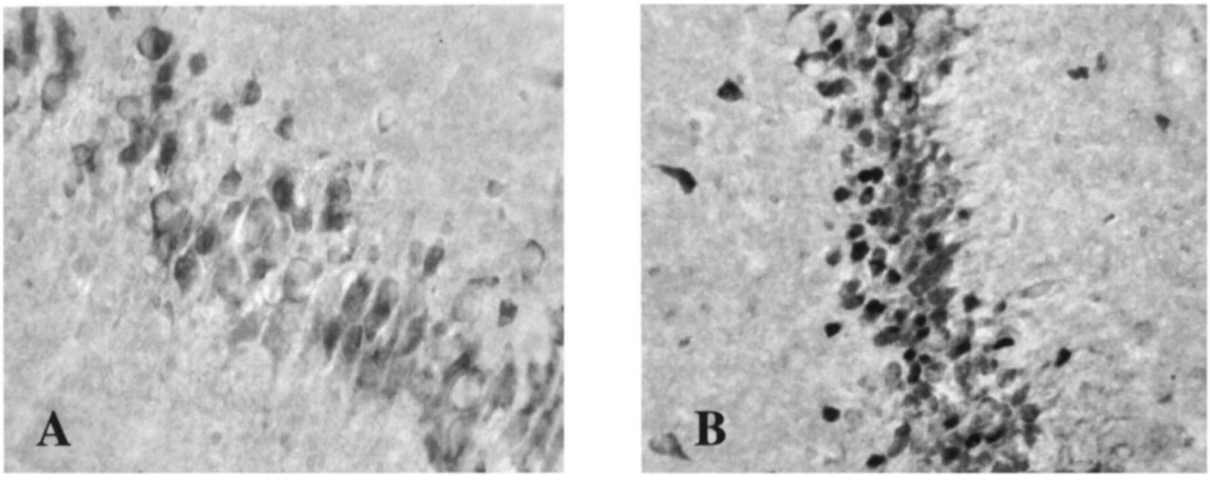

Fig. 2. Nuclear localization of FRA-2 in hippocampal neurons seven days after MCAO. Brain sections containing the hippocampus from sham and MCAO-operated rats were immunostained using antibodies recognizing FRA-2. Strong nuclear staining is detected in hippocampal neurons from rats after MCAO (Panel B; Magnification = 200×), but not after sham operation (Panel A).

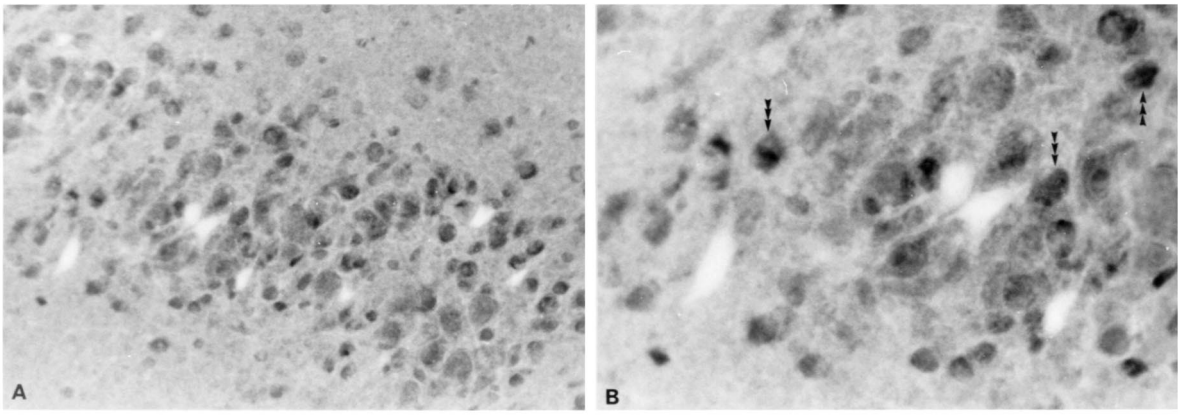

Fig. 3. FRA-2 is not expressed in degenerating neurons. After FRA-2 immunohistochemistry, hippocampal sections from rats seven days after MCAO were stained with Fluoro-Jade, a marker for degenerating neurons. A few neurons in the CA1 region of the hippocampus were Fluoro-Jade-positive, which appear white (Panel A; magnification = 200×). At higher magnification (Panel B; magnification = 400×), FRA-2-positive nuclei (arrow heads) were not localized in Fluoro-Jade-positive neurons.

while several neurons contain FRA-2-immunoreactive nuclei (Fig. 3 Panel A). At higher power, FRA-2 immunoreactivity was clearly localized to neurons that were not Fluoro-Jade-positive.

We have described the elevation of FRA-2 expression in hippocampal neurons seven days after MCAO. Previously, we have discovered that FRA-2 expression increases in neurons in several different models of chemically-induced brain injury [13] (Pennypacker and O'Callaghan, submitted). Depending on the severity of injury, the induction of FRA-2 persists from days to months after injury. Here, we have demonstrated that an increase in FRA-2 expression can be extended to an additional model of injury, ischemia. FRA-2-positive neurons survive the ischemic-induced neurodegeneration suggesting a role of FRA-2 in the adaptation of these neurons to the post-injury state. Together with our previous observations, the present findings indicate that FRA-2 expression is altered in diverse models of brain injury during periods of neuronal regeneration, suggesting a role for FRA-2 in neuroplasticity.

Of potential importance, we noted that the contralateral hippocampus also contains elevated levels of FRA-2 after MCAO, indicating that the response to brain injury is not confined to the sites of cell loss. Other molecules, such as nerve growth factor, presenilin-1 and -2, and glial fibrillary acidic protein are upregulated bilaterally [4,9]. Injury to one hemisphere of the brain could be relayed trans-synaptically to the other side suggesting the contralateral hemisphere is also involved the remodeling of the brain to compensate for the injury. Additionally, these data demonstrate that the contralateral hemisphere is not a good control tissue in models of injury.

AP-1 transcription factors play dual role in neuronal death and survival after injury to the brain [2] and this is also true for ischemic-induced neurodegeneration of the hippocampus [1]. The duality in function appears to depend on composition of the AP-1 dimer. FRA-2 is a component of the elevated AP-1 DNA binding activity after trimethyltin-induced neurodegeneration [13]. The prolonged expression of FRA-2 in neurons surviving injury suggests that the FRA-2 as a constituent of AP-1 DNA binding complex is increasing the transcriptional activity of AP-1 target genes associated with regeneration and repair in order to remodel the brain to compensate for the injury. For example, expression of neurotrophins and their receptors is induced after ischemic insult to the brain [6]. Of the many neurotrophins examined in ischemia models, nerve growth factor and basic fibroblast growth factor stand out due to their protracted expression at 7 days or more after ischemia [5,12]. Thus, ubiquitous and prolonged elevation of FRA-2 in ischemia and other brain injury models suggests that FRA-2 is an important transcription factor to activate the expression of genes, such as growth factors, to repair the brain.

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