Changes in the Level of Glial Fibrillary Acidic Protein (GFAP) after Mild and Severe Focal Cerebral Ischemia

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Abstract

In the present study, we examined the temporal and spatial expression profiles of GFAP mRNA and protein in a focal cerebral ischemia model with ischemic injury confined to the cerebral cortex in the right middle cerebral artery (MCA) territory. Northern blot analysis showed a respective 5.5-fold and 7.2-fold increase in the GFAP mRNA in the ischemic right MCA cortex in rats subjected to 30-min (mild) or 60-min (severe) ischemia followed by 72-hr reperfusion. The GFAP mRNA signal remained elevated up to 2-week reperfusion. Interestingly, increased GFAP mRNA signal was clearly demonstrated for the first time in the left MCA cortex. A significant 1.5-fold and 5-fold increase was observed after 72-hr reperfusion following mild and severe ischemia, respectively. However, unlike the ischemic right MCA cortex, this induction was transient in the non-ischemic left MCA counterpart. In situ hybridization studies further revealed characteristic spatial induction profile following mild vs. severe ischemia. In mild ischemia, following 24-hr reperfusion, increase in GFAP mRNA was observed mainly within the ischemic right MCA cortex. Following 72-hr reperfusion, GFAP mRNA signal was observed in virtually the entire ischemic cortex, particularly the amygdala region, then gradually reduced and restricted to right MCA territory and subcortical thalamic nucleus following 2-week reperfusion. On the other hand, in severe ischemia, following 24-hr reperfusion increased GFAP mRNA signal was observed in area surrounding right MCA territory (infarct region) and outer cortical layers within the right MCA territory. Following 72-hr reperfusion, no signal was detected within right MCA cortex; however, increased GFAP signal was detected throughout the remaining ipsilateral cortex and subcortical region, as well as the contralateral cerebral cortex. GFAP mRNA signals then gradually reduced its intensity and was restricted to area surrounding necrosis and ipsilateral thalamic nucleus following 2-week reperfusion. GFAP-like immunoreactivity was also detected in area expressing GFAP mRNA. It is very likely that de novo synthesis was responsible for this increase. In summary, increased GFAP signal was noted in both ipsilateral and contralateral cerebral following mild and severe ischemia. Although the temporal induction profile for mild vs. severe ischemia was similar, the spatial induction profile was different. The mechanism leading to this differential induction and their physiological and functional significance are not clear at present. It is very likely that some local factors may involve, nevertheless, the detailed mechanisms remain to be fully explored.

Key Words: astrocyte, gliosis, stroke, gene expression, rats

Introduction

In response to a variety of brain injuries, astrocytes are known to divide, leading to an increase in their number (hyperplasia) and the size of nucleus, cell body and processes (hypertrophy), as well as an

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increase in glial fibrillary acidic protein (GFAP) immunoreactivity (5, 30, 32, 45). These morphological features are characteristics of reactive gliosis. Reactive astrogliosis after brain injury may bear important consequences in effective neural repair by providing growth factors for neuronal regeneration or by blocking axonal regrowth through activating the physiological stop pathway (4, 9, 16, 38, 41). However, the molecular mechanisms underlying these responses as well as their functional roles are poorly understood (34, 39, 46).

GFAP is the product of a type III intermediate filament (IF) gene that is specifically expressed in mature astrocyte and has been used extensively as a cell-specific marker for astrocyte in studies of development as well as injury. During CNS development in the rodents, the emergence of GFAP is probably related to the reorganization of the cytoskeleton accompanying the major changes in cell shape (29). Furthermore, GFAP-null mice appear normal in development, reproduction, without shortening of life span or gross morphological abnormalities in CNS (12,35). In contrast, little is known about the significance of dramatic increase in both the number of intermediate filaments and the quantity of GFAP during reactive gliosis.

Petito and Babiak (36) reported the metabolic activity of astrocytes was increased in post-ischemic, non-infarcted brain. Kindy et al (15) reported an increase in GFAP mRNA and protein in the ischemic lesion area in a transient gerbil ischemia model. GFAP immunoreactivity was found in the boundary zone to the infarct in a transient rat MCAO model (6,20). Areas where GFAP expression is lost are destined to become necrotic. Valentim et al (43) reported that significant increases in GFAP phosphorylation were noted in the vulnerable CA1 region after global ischemia. Furthermore, Yamashita et al (47) reported an early and wide spread increase of GFAP mRNA in the non-ischemic areas including the contralateral hemisphere, and a delayed circumscribed expression in the peri-infarct border zone after 1 week, in a permanent rat MCAO model. However, this induction of GFAP mRNA was only slightly attenuated by glutamate receptor antagonists MK-801 and NBQX, which did not qualitatively affect the topical expression pattern. Furthermore, post-traumatic reactive gliosis was evident in GFAP knock out mice suggesting that GFAP up regulation, a hallmark of reactive gliosis, may not be required for astrocytic response to injury (35). In order to provide more information concerning the mechanism of reactive astrogliosis and the role of GFAP after brain injury, we examine the expression of GFAP at both mRNA and protein levels, after mild (30-min) and severe (60-min) focal cerebral ischemia by Northern blot, in situ hybridization and immunohistochemical staining.

Material and Methods

Stroke Model

The focal cerebral ischemia/reperfusion model in the rat has been described previously (7,23). Briefly, male Long-Evans rats weighing 250 to 300 g were anaesthetized with chloral hydrate (360 mg/kg; i.p.). The trunk of the right middle cerebral artery (MCA) was identified and ligated with a 10-0 suture. Both common carotid arteries (CCAs) were then occluded with nontraumatic aneurysm clips. After a predetermined duration of ischemia (30-min or 60-min), the aneurysm clips and suture were removed. Under these conditions, ischemia for 60-min results in a consistently large area of infarction which is confined to the right MCA cortex, whereas small or no infarction is observed following mild ischemia induced for 30-min (28). After ischemic insult, rats were maintained in an air-ventilated incubator at 24. 0 ± 0.5°C for various reperfusion periods (0-, 0.5-, 1-, 1.5-, 4-, 12-, 24-, 72-, 168- and 336-hr) and were provided with water and lab chow ad libitum. At the end of experiments, rats were killed either by transcardial perfusion with normal saline followed by cold 4% paraformaldehyde, and brains were removed and cryoprotected in 30% sucrose at 4°C overnight, or by decapitation where the cerebral cortices were rapidly dissected out and frozen in liquid nitrogen.

RNA Isolation and Northern Blot Analysis

RNA isolation and Northern blot analysis have been described previously (22). Brief, total RNA samples (10 μg/lane) were applied on 1.2% agarose gel in the presence of 2.2 M formaldehyde. After electrophoresis, the gel was transblotted onto Nytran membranes. Membranes were prehybridized at 60°C in a solution containing 1%SDS, 1M NaCl, 10% dextran sulfate and 100 μg/ml of ssDNA. ^{32}P-labelled GFAP probe, 1×10^6cpm/ml, was added directly to the prehybridization solution. Radioactive probes were prepared by a random-primer labeling method (Amersham). After hybridization for 24 hr at 60°C, membranes were washed twice in 2X SSC at room temperature for 5 min each, followed by two 30-min washes at 60°C in 2X SSC/1% SDS and two 30-min washes at 60°C in 0.1X SSC. Membranes were then exposed to X-Omat/XB-1 (Kodak). The radioactive bands were quantified by densimeter.

In Situ Hybridization

In situ hybridization to detect the regional

distribution of GFAP mRNA signal has been described previously (24). Briefly, brain sections were subjected to 0.001% proteinase K digestion at 37°C for 30 min, then immersed in 0.1M triethanolamine with 0.25% acetic acid anhydride at room temperature for 10 min, and subsequently dehydrated in 50, 70, 95 and 100% ethanol (3 min each). Hybridization was carried out in a solution containing 12.5 M formamide, 10 % dextran sulfate, 0.3 M NaCl, 1X Denhardt's solution, 10 mM Tris-Cl 8.0, 500 μg/ml ssDNA, 100 μg/ml tRNA, 20 mM DTT and 10^7 cpm/ml of probes at 55°C overnight. cDNA probes were labeled with ^{35}S-dCTP using the random-primer labeling method (Amersham). Slides were then washed sequentially in 2X, 1X, 0.2X and 0.1X SSC containing 1 mM DTT at 55°C for 30 min each, followed by dehydration in 50, 70, 95 and 100% ethanol (3 min each). Brain sections were exposed to BioMax-MR (Kodak) and autoradiographs were analyzed with Adobe photoshop 4.0 (Adobe).

Immunohistochemical Staining

Protocol to detect GFAP-like immunoreactivity has been described previously (27). Briefly, brain slices of 25 μm thickness were frozen-sectioned by cryostat and incubated in a free-floating manner. The endogenous peroxidase was blocked with 0.3% hydrogen peroxide for 30 min at room temperature. Preincubation with 3% normal goat serum (NGS) containing 0.2% Triton X-100 was carried out at room temperature for 60 min to block nonspecific binding of immunoglobin G (IgG). Mouse monoclonal anti-GFAP antibody (Boehringer Mannhein), which reacts with rat astrocytes, was used in the present study. Sections were incubated with anti-GFAP antibody diluted in 1% NGS (1:10 dilution) at 4°C overnight, then rinsed with PBS for 30 min, and incubated in biotinylated goat anti-mouse IgG for 1 h. After several rinses with PBS, sections were incubated in avidin horseradish peroxidase complex for 1 h, then incubated in 3,3'-diaminobenzidine (DAB; 0.5mg/ml) in the presence of 0.009% H_2O_2 and subsequently mounted on gelatinized slides. The specificity of each antiserum was demonstrated by the absence of immunoreactivity when the diluted primary antiserum was pre-absorbed by the respective antigen or was replaced by normal serum. For another negative control, sections were incubated with primary antibody denatured by heat. The results of these immunohistochemical controls were consistently negative.

Chemicals

All chemicals were of reagent quality and purchased either from E. Merck or Sigma Chemical Co unless otherwise indicated.

Statistics

One-way analysis of variance (ANOVA) was used to compare the temporal expression of GFAP mRNA levels. Significance for differences between two groups was further analyzed by post-hoc Tukey's protected t tests using the statistical software (GB-STAT 5.0.4, Dynamic Microsystem Inc). A value of $P < 0.05$ was considered significant.

Results

Analysis of GFAP mRNA Levels in Ischemic Cerebral Cortex by Northern Blot

The extensive conservation of mouse GFAP cDNA sequence among several mammalian species enable us to analyze the level of rat GFAP mRNA using a probe derived from mice (18,19). In the present study, a 1.1 kb Hind III fragment from the 2.7 kb mouse GFAP cDNA hybridized to a single 2.7 kb RNA species in total RNA extracted from rat ischemic cortex (Fig. 1). Fig. 1 shows that this 2.7 kb GFAP mRNA transcript was constitutively expressed in the cortex. Focal cerebral ischemia lasting 30 min (mild) resulted in a significant induction of GFAP mRNA in the ischemic cerebral cortex (Fig. 1A) by one-way ANOVA ($p<0.0001$). Significant increase in GFAP mRNA was observed starting 12-hr reperfusion and remained elevated up to 2-week reperfusion. Quantitative analysis indicated a 5.5-fold increase at the peak level as compared to sham-operated control (Fig. 1E). Interestingly, GFAP mRNA was also increased in the contralateral cerebral cortex (Fig. 1C); however, significant increase in the GFAP mRNA was only observed following 72-hr reperfusion (Fig. 1E) and to a lesser extent (~ 1.5-fold increase). On the other hand, ischemia lasting 60-min (severe) resulted in a significant induction of GFAP mRNA in the ischemic cerebral cortex (Fig. 2A) by one-way ANOVA ($p<0.0001$). Significant increase in GFAP mRNA was observed starting after 12-hr reperfusion and maintained for up to 2-week. Quantitative analysis indicates a 7.2-fold increase at the peak level as compared to the sham-operated control (Fig. 2E). Interestingly, similar induction profile was also observed in the contralateral cerebral cortex (Fig. 2C). Although, significant increase was only observed at 24- and 72-hr reperfusion (Fig. 2E) the degree of increase was quite high following 72-hr reperfusion (~ 5-fold increase). Although the induction profile was similar, the level of GFAP mRNA was higher in rats subjected to 60-min ischemia

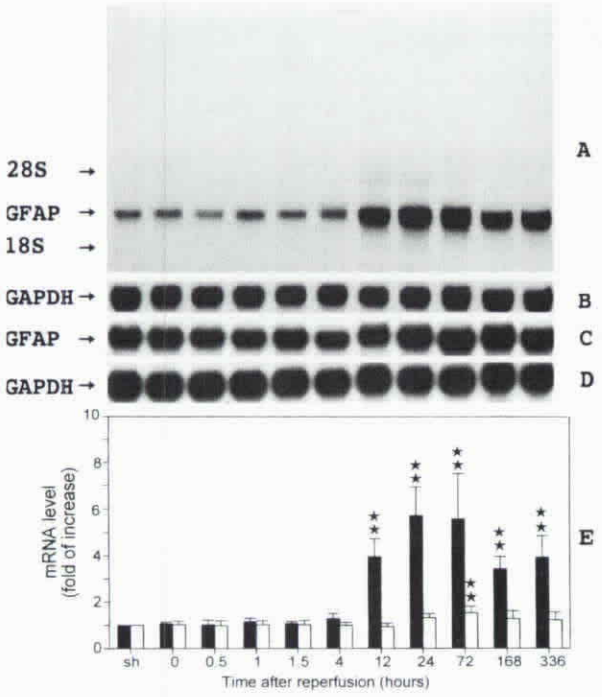

Fig. 1. Time-course of GFAP mRNA expression in the cerebral cortex following 30-min ischemia. Total RNA (10 μg) extracted from cerebral cortex of rats subjected to 30-min ischemia and various reperfusion periods were hybridized with ^{32}P-labeled GFAP probe as described in the text. Ischemia causes a delayed induction of GFAP mRNA in both ipsilateral (A) and contralateral (C) cerebral cortex. The same membrane was subsequently stripped and rehybridized with GAPDH (B&D, respectively) to serve as internal control. The radioactive bands of GFAP mRNA were quantified and normalized with those derived from GAPDH mRNA (E). Filled (■) and empty (❑ bar represent value from ipsilateral and contralateral cerebral cortex, respectively. Values obtained from sham-operated control were arbitrarily defined as 1. Data are mean ± SD from 4 animals. ★★ & ★ indicate $p<0.01$ & 0.05, respectively, as compared to the sham-operated control. Sh denotes sham-operated control; 0 denotes 30-min ischemia with no reperfusion; 0.5, 1, 1.5, 4, 12, 24, 72, 168 and 336 denote 30-min ischemia followed by 0.5, 1, 1.5, 4, 12, 24, 72, 168 and 336 hr of reperfusion, respectively.

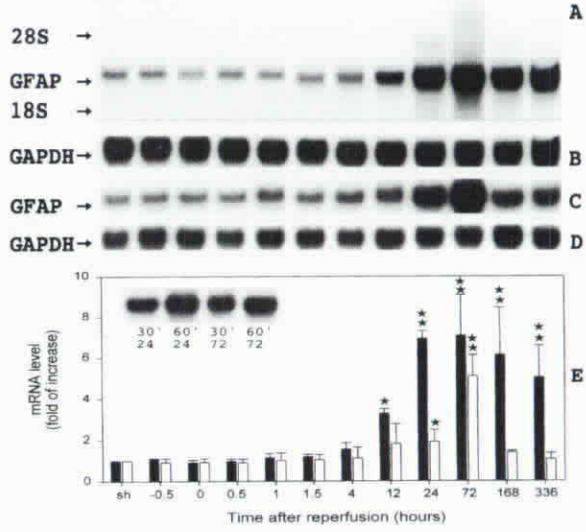

Fig. 2. Time-course of GFAP mRNA expression in the cerebral cortex following 60-min ischemia. Experiments were otherwise as described in Fig. 1. Data are mean ± SD from 3 animals. –0.5 and 0 denote 30- and 60-min ischemia with no reperfusion. The upper left insertion (E) shows the relative intensity of GFAP mRNA in the ipsilateral cortex of rats subjected to 30- (30') or 60-min (60') ischemia followed by 24- and 72-hr reperfusion.

as compared to 30-min ischemia in both ipsilateral and contralateral cerebral cortex.

Localization of GFAP mRNA by in Situ Hybridization

Results from in situ hybridization confirmed those noted in the Northern blot analysis and revealed characteristic spatial distribution of GFAP mRNA expression after ischemic insult (Fig. 3). In rats subjected to 30-min ischemia, following 24-hr reperfusion, an increase in GFAP mRNA expression was observed mainly within the MCA territory of the ipsilateral cerebral cortex (demarcated region). Substantial amount of the signal was also noted in the adjacent cerebral cortex, in particular, the amygdaloid nucleus region. GFAP signal was not increase in the contralateral side (Fig. 3A). Following 72-hr reperfusion, increased GFAP mRNA was observed in virtually the entire ipsilateral cerebral cortex, in particular the amygdaloid nucleus and areas surrounding the MCA territory. Substantial amount of the signal was also noted in the hippocampal and subcortical thalamic nucleus, as well as the contralateral cerebral cortex (Fig. 3C). Following 2-week reperfusion, GFAP mRNA was induced exclusively within the ipsilateral MCA territory and subcortical thalamic nucleus. No signal was detected in the adjacent and contralateral cerebral cortex (Fig. 3E).

In rats subjected to 60-min ischemia, increased GFAP mRNA was observed in area surrounding the right MCA territory (infarct region) and the outer cortical layers within the right MCA territory, following 24-hr reperfusion. GFAP signal was lower than basal level in cortical layers 4 to 6 within the right MCA territory. Following 72-hr reperfusion, no signal was detected within the ipsilateral MCA territory. However, increased GFAP signal were noted in the remaining region of the cerebral cortex (peri-infarct region) and subcortical thalamic nucleus, as well as the contralateral cerebral cortex (Fig. 3D). Following 2-week reperfusion, GFAP signal was located exclusively in area immediately surrounding

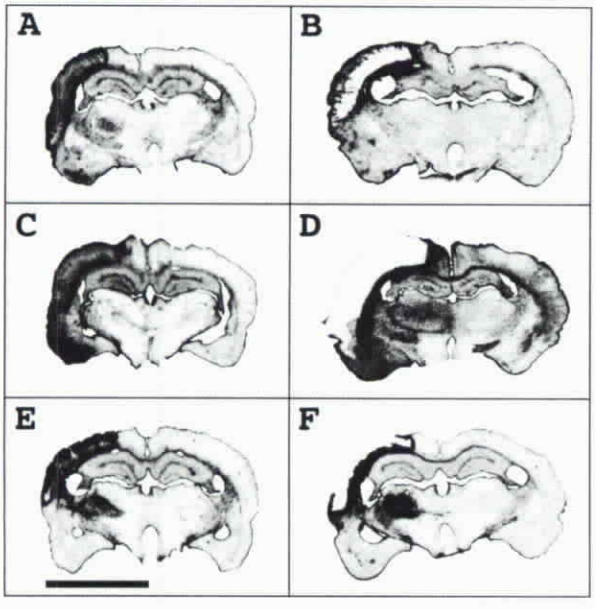

Fig. 3. In situ hybridization of GFAP mRNA in the rat brain. The regional distribution of GFAP mRNA is shown in rats subjected to 30-min (left panel) and 60-min (right panel) ischemia followed by 24-hr (A&B), 72-hr (C&D) and 2-wk (E&F) reperfusion. Brain slices (25 μm) were hybridized with ^{35}S-labelled GFAP probe as described in the text. Following 24-hr reperfusion, GFAP message increased mainly within the ischemic MCA territory (demarcated region) after 30-min ischemia (A). GFAP mRNA increased in the outer cortical layers but decreased in the inner cortical layers within the MCA territory (infarct region) and area surrounding the infarct after 60-min ischemia (B). Following 72-hr reperfusion, GFAP mRNA expression extended to the entire ipsilateral cerebral cortex after 30-min ischemia, particularly the amygdala region (C). No GFAP signal was detected within the infarct area in rats subjected to 60-min ischemia. However, GFAP signal was heavily expressed in the remaining ipsilateral cerebral cortex (D). Substantial amount of GFAP signal was also observed in the ipsilateral subcortical thalamic region and contralateral cerebral cortex (D). Following 2-wk reperfusion, GFAP message increased exclusively within the ischemic MCA territory after 30-min ischemia (E) or in areas immediately surrounding the necrosis tissue after 60-min ischemia (F), as well as the ipsilateral subcortical region (E&F). Scale bar=7mm.

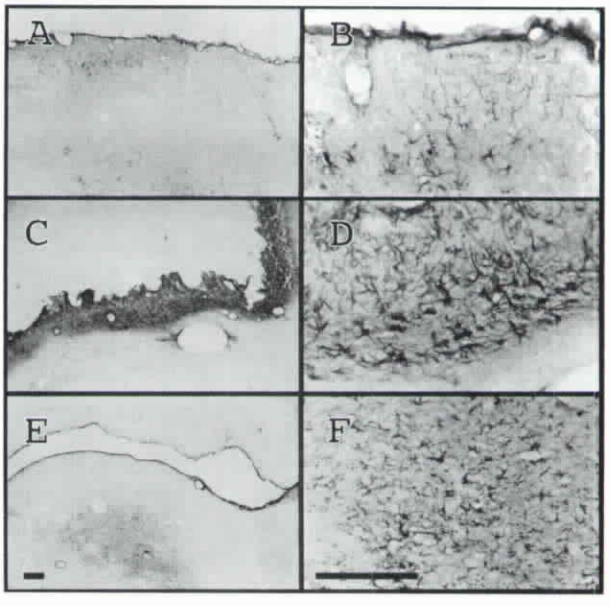

Fig. 4. Immunohistochemical studies of GFAP in the rat brain. Brain slices (25 μm) were obtained from rats subjected to 60-min ischemia. Following 24-hr reperfusion, GFAP positive cells were located in the outer cortical layers within the MCA territory as well as the surrounding area (A). The characteristic appearance of GFAP positive cells in this region is shown in B. On the other hand, following 2-week reperfusion, GFAP immunoreactivity was heavily expressed in area surrounding the necrosis region (C). GFAP-labeled cells in this region are shown in D. GFAP immunoreactivity was also observed in the ipsilateral subcortical thalamic nucleus region (E). The morphology of GFAP-positive cells in this region is shown in F. Scale bar= 200 μm.

the necrosis region and subcortical thalamic region. No signal was detected in the contralateral cerebral cortex (Fig. 3F).

Identification of GFAP-like Immunoreactivity

We further examine whether increase in GFAP mRNA resulted in an increase in the protein level. GFAP-like immunoreactivity was examined in rats subjected to 60-min ischemia followed by 24-hr (Figs. 4A and B) and 2-week (Fig. 4C-F) reperfusion (Fig. 4). Following 24-hr reperfusion, significant increase in GFAP positive cells were noted in the outer cortical layers of the infarct and peri-infarct regions (Fig. 4A). The characteristic appearance of GFAP positive cells in this region was shown in Figure 4B. Following 2-week reperfusion, GFAP immunoreactivity was heavily expressed in area surrounding the necrosis region (Fig. 4C). The morphology of GFAP-positive cells in these regions is shown in Figure 4D, which was different from Figure 4B. GFAP immunoreactivity was also observed in the ipsilateral subcortical thalamic nucleus region (Fig. 4E). The typical appearance of GFAP-positive cells in this region is shown in Figure 4F, which was different from Figs. 4B and 4D.

Discussion

Despite the notion that neuron-glial and glial-glial interactions are thought to play a critical role in the fate of neurons after brain injury, the underlying mechanism is still not completely understood (1). In this transient MCAO model, we observe that infarction volumes correlated with the duration of vessel occlusion (data not shown), which is comparable to the previous report by Liu et al (28).

Both mild (30-min) and severe (60-min)

ischemia led to an increase in GFAP mRNA in the ipsilateral cerebral cortex (Figs. 1 and 2). Although the infarct volumes following severe ischemia were twice as large as those derived from mild ischemia, the degree of GFAP mRNA induction was only slightly more intense following severe than mild ischemia. Interestingly, increased GFAP mRNA was also noted in the contralateral cerebral cortex following both mild and severe ischemia, where no visible tissue damage was noted. However, the degree of induction in the contralateral side was much lower as compared to the ipsilateral counterpart. To our knowledge, we are the first group to demonstrate the extent of GFAP mRNA induction in the contralateral cerebral cortex with this MCAO model. Interestingly, the amount of induction in the contralateral side is much higher following severe than mild ischemia. It is very likely that there is a threshold for GFAP induction and this threshold is relatively low and/or sensitive to external stimuli.

Results from in situ hybridization studies reveal characteristic regional distribution of GFAP induction following severe vs. mild ischemia. In rats subjected to severe ischemia, increased GFAP mRNA signal was noted in tissue surrounding the right MCA territory (infarct region) and outer cortical layers within the right (ischemic) MCA territory following 24-hr reperfusion. Surprisingly, GFAP signal within the remaining right MCA territory was either lower than basal level or not detectable at all. There were no visible changes in the GFAP mRNA signal in the ipsilateral cerebral cortex following 4-hr reperfusion. And after 12-hr reperfusion, the GFAP signal was similar in regional distribution as that following 24-hr reperfusion, though to a lesser extent (data not shown). The reason for this opposite response between outer cortical and inner cortical layers within the right MCA territory is not at present clear. Nevertheless, we have reported previously that within the right MCA territory the expression of bFGF mRNA was increased in cortical layers 1 and 2 (25) and CNTFR α mRNA was up regulated in cortical layer 2 but down regulated in layer 5 (26). It is very likely that trophic factors may play a role, at least in part, for this differential response. Following 72-hr reperfusion, no signal was detected within the right MCA territory. However, increased GFAP signal was detected in the remaining ipsilateral cerebral cortex. Following 2-week reperfusion, GFAP signal was noted only in area surrounding the necrosis. On the other hand, in rats subjected to mild ischemia, GFAP mRNA was initially expressed mainly within the ipsilateral MCA territory following 24-hr reperfusion. It then spread to virtually the entire ipsilateral cerebral cortex following 72-hr reperfusion. Following 2-week reperfusion, GFAP signal was localized exclusively within the ipsilateral MCA territory. To our knowledge, we are the first group to report these intriguing temporal and spatial induction profiles that are distinct from those following severe ischemia using the same model. These findings may explain, at least in part, the discrepancy in the induction profiles of GFAP in different models with different severities. Nevertheless, the mechanism for this differential GFAP expression pattern between mild and severe ischemia is, at present, not clear. It is very likely that there are different population of astrocytes or different biological mechanisms for induction and maintenance of reactive gliosis, dependent upon the degree of injury (34, 39). For example, Valentim et al (43) reported that significant increases in GFAP phosphorylation were noted in the vulnerable CA1 region after global ischemia. Different GFAP sib genes may also be responsible for different profiles of induction. For instance, a new GFAP mRNA isomer with a 7-bp insertion in the 3'UTR region is associated with memory retention in rat (13).

Petito et al (37) reported that reactive astrocytosis develops in undamaged brain, but is reversible with prolonged survival. In the present study, both mild and severe ischemia, GFAP signal was detected transiently in the peri-infarct (undamaged) area following 72-hr reperfusion. The mechanism of this transient induction most likely is through spreading depression (17). It is very likely that upon stimuli astrocyte may first expresses GFAP. Then GFAP was either returned to basal level if no injury was detected or maintained elevated by some local unknown factors, such as c-fos, to signal the existence of tissue injury. Indeed, it has been shown that GFAP contains AP-1 sites in its promoter region (31, 42). Others have shown that c-fos and other IEGs were induced in the similar regions as GFAP mRNA was noted (2, 3). An increase in the binding activity of AP-1 was also observed in the region surrounding the ischemic cortex (40). Furthermore, we have reported previously that fasting not only reduce the infarct volume but also shift c-fos mRNA from peri-infarct (undamaged) region into right MCA territory (24). This findings is similar to the spatial distribution of GFAP mRNA in severe vs. mild ischemia. Nevertheless, demonstrating that this increase in GFAP expression can be blocked by an antisense c-fos oligonucleotide would provide direct evidence that ischemic induction of c-fos mediates the induction of GFAP gene. At 72-hr, increased GFAP signal was also noted in the contralateral cerebral cortex (undamaged area). Similarly, an astrocytic activation was also detected in the contralateral hemisphere following unilateral mechanical lesion in the rat cerebral cortex (8). These results suggest that GFAP is very sensitive to external stimuli, since the

contralateral side also sustained very mild ischemia due to the occlusion of left CCA. It is very likely that the distribution and degree of induction are dependent on the severity of insult. This is the probable cause why the GFAP signal in the contralateral cerebral cortex was much higher in rats subjected to severe than mild ischemia. Since no c-fos was induced in the contralateral side in our previous report (3, 24); the transient induction of GFAP in contralateral side further supports the local factor hypothesis. Along this line, mice lacking GFAP are expected to sustain more severe injury in autoimmune encephalomyelitis (21) and be hypersensitive to traumatic cerebrospinal injury (33). Lastly, the activated astrocytes may then release trophic factors, cytokines, and neuropeptides to reduce injury and/or promote recovery (39, 46). This may partially explain why GFAP-null mice displayed post-traumatic hypertrophy, which suggests that GFAP up-regulation is not an obligatory requirement for reactive gliosis (35).

Furthermore, with mild ischemia, we have demonstrated for the first time that a marked increase of GFAP signal was noted in the amygdaloid nuclear complex, which has the highest intensity within ischemic cortex. The reason for this induction is not at present clear. Iizuka et al (14) have reported an increase in GFAP immunoreactivity in the thalamic region in a permanent MCAO model. Here we reported both mild and severe ischemia led to an increase in both GFAP mRNA and immunoreactivity in the ipsilateral subcortical thalamic nucleus. The extent of induction was proportional to the severity of insult. The cortical regions that become infarcted contain the primary somatosensory cortex and receive thalamocortical fibers from specific thalamic nuclei, that is, the ventroposteromedial and ventroposterolateral nuclei. These findings are consistent with the notion of retrograde neuronal degeneration due to thalamo-cortical fiber damage in the ischemic cortical regions. Since it was long accepted that the amygdala was primarily associated with sub-cortical structures and has widespread projects to multiple regions of the cerebral cortex (44). It is very likely that amygdala may play a role in this retrograde damage. Nevertheless, the regulatory mechanisms underlying GFAP expression accompanying retrograde damage remain to be studied. Interestingly, Du et al. have reported that both mild and severe ischemia led to same degree of cortical infarction but through different cell death mechanisms, apoptosis vs. necrosis, in this model (10). It would be interesting to know whether the same or different cell death mechanism is involved in this retrograde damage following mild vs. severe ischemia. Although some global ischemia also showed similar induction of GFAP in this region, our results suggest that survival of some thalamic neurons depend on their projections to target neurons in the cortex.

Increased GFAP immunoreactivity was observed in the same area where increased GFAP mRNA was observed. Several mechanisms have been suggested. For example, increase in antigenic epitopes caused by post-translational modifications, increased accessibility of antibodies to the glial filaments, accumulation of GFAP due to a decreased degradation rate, increased translation of preexisting GFAP mRNA, increased transcription of GFAP gene, or a stabilization of GFAP mRNA (8, 11). Our data suggested, at least in part, that increase in de novo synthesis of GFAP is responsible for its increased expression after brain ischemia.

In conclusion, GFAP signal were increase in both ipsilateral and contralateral hemisphere following both mild and severe ischemia in a transient MCAO model in the rat. Although the temporal induction profiles were similar between mild and severe ischemia, the spatial induction profiles were different. A dramatic increase in GFAP signal was noted in the amygdaloid nuclear complex following mild but not severe ischemia. It is possible that different mechanisms were involved or different pools of astrocytes were activated. Furthermore, GFAP was transiently expressed in tissue without visible damage and prolonged induction was associated with structural damage. It is very likely that GFAP may serve as a probe to detect and then signal tissue injury, with the help of some local factors. Reactive astrocytes then subsequently release trophic factors to reduce injury and/or promote recovery. Nevertheless, the mechanisms leading to the induction of GFAP and differential induction profiles between mild and severe ischemia remain to be studied.

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