Eukaryotic Initiation Factor 2α Kinase and Phosphatase Activity during Postischemic Brain Reperfusion


*Department of Emergency Medicine, †Department of Physiology, and ‡Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan 48201; §Department of Emergency Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104; and ¶Howard Hughes Medical Institute, and ‡Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48109.

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When ischemic brain is reperfused, there is in vulnerable neurons immediate inhibition of protein synthesis associated with a large increase in phosphorylation of the α-subunit of eukaryotic initiation factor 2 [eIF2α, phosphorylated form eIF2α(P)]. We examined eIF2α kinase and eIF2α(P) phosphatase activity in brain homogenate postmitochondrial supernatants obtained from rats after 3 to 30 min of global brain ischemia (cardiac arrest), after 5 min of ischemia and 5 min of reperfusion (5R), and after 10 min of ischemia and 90 min reperfusion (90R). Because it has been suggested that PKR might be specifically responsible for producing eIF2α(P) during reperfusion, we also examined in brain homogenates from wild-type and PKR0/0 C57BL/6J × 129/SV mice the effect of 5 min of ischemia and 5 min of reperfusion on eIF2α(P). Cytosolic brain eIF2α(P) in the 5R and 90R groups was 18- and 23-fold that of nonischemic controls without any change in the rate of eIF2α(P) dephosphorylation. There was no change in eIF2α kinase activity between 3 and 30 min of ischemia, but an 85% decrease in the 5R group; the 90R group was similar to controls. In wild-type and PKR0/0 mice total eIF2α was identical, and there was an identical 16-fold increase in eIF2α(P) at 5 min of reperfusion. Our observations contradict hypotheses that PKR activation, loss of eIF2α(P) phosphatase activity, or any general increase in eIF2α kinase activity are responsible for reperfusion-induced phosphorylation of eIF2α, and we suggest that the mechanism may involve regulation of the availability of eIF2α to a kinase.

Key Words: eIF2α; brain; ischemia; reperfusion; phosphatase; kinase; phosphorylation.

INTRODUCTION

Protein synthesis in selectively vulnerable neurons is inhibited during reperfusion following more than 4 min of global brain ischemia (1, 2, 3, 16, 33, 34), and this is associated in these same cells with a rapid and large increase in phosphorylation of Ser51 on the α-subunit of eukaryotic initiation factor 2 [eIF2α, phosphorylated form eIF2α(P)] (15). It is known that under conditions of cellular stress, the rate of global protein synthesis is downregulated by phosphorylating Ser51 on the α-subunit of eukaryotic initiation factor 2 (23, 26). Burda et al. presented evidence of increased phosphorylation of eIF2α in rats subjected to four-vessel occlusion for 15 or 30 min followed by 30 min of reperfusion (5). After a 10-min cardiac arrest, we found in brain homogenates that eIF2α(P) is increased by ~20-fold after 10 min of reperfusion and by ~24-fold after 90 min of reperfusion (14, 15). More recently we mapped eIF2α(P) to the selectively vulnerable hippocampal and cortical neurons that display markedly inhibited protein synthesis (15).

Two obvious mechanisms that could be involved in phosphorylation of eIF2α during early reperfusion are an increase in eIF2α kinase activity or a loss of eIF2α(P) phosphatase activity (15). Burda et al. recently reported increased in vitro eIF2α kinase activity in ischemic but not reperfused brain homogenates (6). However, Hu and Wieloch did not find any difference in 32P incorporation into eIF2α by brain homogenates prepared after 15 min of ischemia and 30 or 60 min of reperfusion (19). Neither group studied phosphatase activity. Burda et al. suggested that the double-stranded RNA-dependent eIF2α kinase (PKR) was responsible for increased eIF2α(P) during reperfusion and surprisingly reported an approximately 50% increase in the levels of PKR following 30 min of ischemia and after 30 min of ischemia and 30 min of reperfusion (6). It is difficult to understand an increase in PKR levels during ischemia, when in vivo protein synthesis does not occur, or during early reperfusion, when in vivo protein synthesis is substantially inhibited. Thus
we undertook these experiments to examine the effects of brain ischemia and reperfusion on eIF2α kinase and eIF2α(P) phosphatase activity in brain homogenates and whether PKR is responsible for phosphorylating eIF2α following brain ischemia.

MATERIALS AND METHODS

Materials

Pure eIF2 was a gift from Christopher Proud (University of Dundee, UK), and the partially purified eIF2α kinase HRI was a gift from Scot Kimball (Pennsylvania State University). Antibody against PKR (clone M515) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The monoclonal anti-eIF2α that recognizes both phosphorylated and unphosphorylated eIF2α was developed by Dr. Edgar Henshaw (31). We have previously described the production and characterization of the affinity-purified polyclonal antibody that is monospecific against eIF2α(P) (15), and other investigators have also confirmed its specificity for eIF2α(P) (30, 32).

Okadaic acid was purchased from Sigma Chemical Co (St. Louis, MO). All other chemicals were reagent grade.

Wild-type C57BL/6 mice (WT) and Long–Evans rats were purchased from Harlan (Indianapolis, IN). A colony of homozygous PKR-deficient mice was established from homozygous founders (36) kindly provided by Brian R. G. Williams (Cleveland Clinic, Cleveland, OH). The genotype of the founders was confirmed by genomic Southern analysis. Briefly, the PKR-deficient mice were produced by inactivation of an allele of the PKR gene in 129/SV (ev) mouse embryonic stem (ES) cells. The 3′ portion of exon two and the 5′ portion of exon three of the PKR gene and the intervening intron sequence were replaced with a neomycin resistance gene, and they were frozen in dry ice and ethanol and stored at –80°C until used.

Animal Models

All animal experiments were approved by our institutional review board and were conducted following the “Guide for the Care and Use of Laboratory Animal Care” (NIH Publication No. 86-23, revised 1985).

Rat cardiac arrest and resuscitation was performed as previously described (14, 15). Experimental groups for study of the effect of the duration of ischemia on eIF2α kinase activity contained three animals each and were nonischemic controls (NIC), 3 min of ischemia, 7 min of ischemia, 10 min of ischemia, 20 min of ischemia, and 30 min of ischemia. Experimental groups for study of the effect of ischemia and reperfusion on eIF2α(P) phosphatase and eIF2α kinase activity contained three animals each and were NIC, 5 min of ischemia followed by 5 min of reperfusion (5R), and 10 min of ischemia followed by 90 min of reperfusion (90R).

At the appropriate times, the rat brains were rapidly removed and the forebrains were separated from the brain stem and cerebellum. For Western blotting to characterize the effect of ischemia and reperfusion on the level of eIF2α(P), a postmitochondrial supernatant (PMS) of one hemisphere was prepared as we have previously described (14, 15). For the in vitro kinase and phosphatase reactions, a PMS of the other hemisphere was prepared by utilizing a hand Dounce homogenizer containing 2.5 ml/g hemisphere of ice-cold 50 mM Hepes (pH 7.5), 140 mM potassium acetate, 4 mM magnesium acetate, 2.5 mM DTT, 1 µg/ml aprotinin, 10 µg/ml leupeptin, and 7 µg/ml pepstatin and then centrifuging the homogenate at 12,000g for 15 min at 4°C. For both PMS preparations, the protein concentration was determined by the Folin phenol reagent method, and they were frozen in dry ice and ethanol and stored at –80°C until used.

We modified our previous methods for rat cardiac arrest and resuscitation (14) to achieve this in mice. Anesthesia was induced by inhalation of 1–3% halothane/50% NO2 with supplemental oxygen. Femoral artery cannulation and tracheal intubation were performed, and arterial blood pressure and electrocardiogram were continuously monitored. Rectal temperature was maintained between 36.5 and 37.5°C. The mice were then subjected to a 5-min cardiac arrest followed by a maximum of 3 min of resuscitation to achieve resumption of spontaneous circulation followed by 5 min of reperfusion with a minimum mean arterial pressure of 60 mm Hg. Forebrains from nonischemic and reperfused mice were homogenized in 5 ml/g brain ice-cold 50 mM Tris (pH 7.4), 20 mM EDTA, 20 mM EGTA, 1 mM dithiothreitol, 150 mM KCl, 50 mM NaF, 0.23 mM phenylmethylsulfonyl fluoride, 1 µg/ml aprotinin, 10 µg/ml leupeptin, and 7 µg/ml pepstatin A. The homogenates were centrifuged at 12,000g for 15 min at 4°C, and following protein concentration determinations, the PMS preparations were stored at –80°C.

Western Blotting

As previously described (15), after SDS–PAGE on 10% polyacrylamide gels and electroblot transfer of the proteins to nitrocellulose, Western blots were performed utilizing primary antibody concentrations of 40 µg/ml for anti-eIF2α(P) and anti-eIF2α and 1 µg/ml for anti-PKR.
Generation of α-Subunit-Phosphorylated eIF2

We utilized the method of Kimball et al. (20) to prepare eIF2 containing Ser51-phosphorylated α-subunit [eIF2α(P)]. Pure eIF2 (5 µg) was incubated for 40 min at 37°C in a 50-µl reaction containing 2 µl HRI, 200 µM ATP, 0.8 mM Mg acetate, 2 mM DTT, 20 mM Hepes (pH 7.4), and 120 mM KCl, and the reaction was stopped by adding EDTA to a final concentration of 10 mM. Radiolabeled eIF2 was produced by substituting [γ-32P]ATP (sp act 39.5 dpm/nmol) for the unlabeled ATP in the above reaction. We confirmed phosphorylation of eIF2α by subjecting aliquots obtained before and after the labeling reaction to SDS–PAGE followed by electroblot transfer to nitrocellulose and generation of an autoradiograph as well as Western blots for both total eIF2α and eIF2α(P) as previously described (15). We observed a 90–100% radiolabeling efficiency, determined by filter retention assay (14).

eIF2α(P) Phosphatase Assay

Brain homogenates were examined for their ability to dephosphorylate eIF2α(P) by a modification of the eIF2α(P) phosphatase assay of Kimball et al. (20). To minimize the effects of the differing amounts of endogenous eIF2α(P) contained in the reperfused and NIC (Table I), we added eIF2α(P) in 30-fold excess to total endogenous eIF2α before beginning the reactions. The 50-µl reactions were prepared on ice and contained 200 µg PMS protein, 130 mM Mops (pH 7.4), 45 mM KCl, 0.5 mM DTT, 1 mM magnesium acetate, 0.1 µg/ml bovine serum albumin, 1 mM ATP, and 24 ng/µl exogenous eIF2α(P), which was added last. After t0 aliquots were taken, the reactions were incubated at 30°C, and samples were taken at 10, 20, and 30 min. All reaction samples were immediately mixed with an equal volume of 2× SDS–PAGE loading buffer and boiled for 90 s. After electrophoresis and transfer to nitrocellulose, eIF2α(P) was examined by Western blotting. Band densities were examined for significant differences between groups by ANOVA and Sheffé post hoc comparison.

To overcome interference from the ~20-fold greater amount of eIF2α(P) endogenous in the reperfused homogenates (Table I), we utilized a 12-fold greater dilution of PMS and studied the time course of in vitro phosphorylation of eIF2α by NIC and reperfused brain homogenates. We prepared 0.2 µg PMS protein/µl in 125 µl of the above reaction, and samples taken at 0, 5, 10, and 20 min of incubation were electrophoresed, transferred to nitrocellulose, and examined by Western blotting for eIF2α(P) as above. Band densities plotted against time were fit by linear regression, and reaction rates for the experimental groups were compared by ANCOVA of linear regression followed by Tukey post hoc comparison of the regression slopes (37).

eIF2α Kinase Assay

The tissue homogenization and reaction conditions utilized here to examine for in vitro activity of the known mammalian eIF2α kinases were adapted from those developed by other investigators (7, 17, 21, 25). Phosphorylation of eIF2α by brain homogenates obtained from the NIC and ischemia-only experimental groups was determined by immunoblotting for eIF2α(P) before and after a 15-min reaction. The reaction mixtures (50 µl final volume) were prepared on ice and contained 2.4 µg PMS protein/µl, 12 ng pure eIF2α(P), 25 mM Hepes (pH 7.2), 100 mM KCl, 2 mM Mg acetate, 1 mM MnCl2, 1 mM dithiothreitol, 1 µM leupeptin, 150 mM aprotinin, and 5 µM okadaic acid. After a t0 sample was taken, ATP and GTP were added to final concentrations of 100 µM each, and the reactions were incubated at 35°C. Reaction samples were immediately mixed with an equal volume of 2× SDS–PAGE loading buffer and boiled for 90 s. After electrophoresis and transfer to nitrocellulose, eIF2α(P) was examined by Western blotting. Band densities were examined for significant differences between groups by ANOVA and Sheffé post hoc comparison.

RESULTS

Brain eIF2α(P) Levels after Ischemia and Reperfusion

As before (15) there was no significant change from NICs in the level of eIF2α(P) observed in the PMS obtained after any duration of ischemia alone (not shown). In contrast, the 5R group showed an 18-fold increase in eIF2α(P), and the 90R group showed a 23-fold increase in eIF2α(P) (Table I). In vitro translation (14) in PMS from the 5R and 90R groups was inhibited ~70% compared to NIC (data not shown).

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>eIF2α(P) density</th>
<th>Ratio to NIC</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>NIC</td>
<td>0.10 ± 0.04</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>5R</td>
<td>1.81 ± 1.1</td>
<td>17.9</td>
<td>0.05</td>
</tr>
<tr>
<td>90R</td>
<td>2.29 ± 0.14</td>
<td>22.6</td>
<td>1.3 × 10^-5</td>
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Note. Data are shown as means ± standard deviation. NIC, nonischemic controls; 5R, 5 min of cardiac arrest followed by 5 min of reperfusion; and 90R, 10 min of cardiac arrest followed by 90 min of reperfusion. The P values (Scheffé) are in comparison to nonischemic controls.

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These results are consistent with our previous observations (14, 15).

**In Vitro Dephosphorylation of eIF2α(P)**

To assure ourselves that a simultaneous kinase reaction or degradation of eIF2α were not interfering with our assay of eIF2α(P) dephosphorylation, we compared autoradiography to Western blotting for detection of dephosphorylation of eIF2α(P) and examined total eIF2α during the reaction (Fig. 1). During the reaction there was no proteolytic degradation of eIF2α (Fig. 1C). The loss of 32P label on eIF2α (Fig. 1A) was paralleled by loss of eIF2α(P) immunoreactivity (Fig. 1B); this confirms that rephosphorylation of eIF2α did not occur during the reaction.

As expected (12), our phosphatase reactions were linear (mean \( r^2 = 0.93 \)). Ischemia and reperfusion did not induce any loss of eIF2α(P) phosphatase activity (Fig. 2, ANCOVA \( P > 0.25 \)). Mean phosphatase reaction rates (\( \Delta \text{eIF2α(P)} \) density/min ± standard error) were NIC = \(-0.026 ± 0.001\), 5R = \(-0.028 ± 0.002\), and 90R = \(-0.028 ± 0.003\). Inhibitor 2 (Promega, Madison, WI) blocked the PMS eIF2α(P) phosphatase reaction (not shown), consistent with phosphoprotein phosphatase 1 activity (reviewed in 15).

**In Vitro Phosphorylation of eIF2α**

The duration of ischemia had no effect on the ability of the brain homogenate to phosphorylate eIF2α during the 15-min kinase reaction (Fig. 3, ANOVA \( P = 0.25 \)). In our time course studies of phosphorylation of the α-subunit of exogenous eIF2 by NIC, 5R, and 90R brain PMS, the increase in eIF2α(P) was linear (all individual reactions had \( r^2 > 0.92 \)). Mean eIF2α kinase reaction rates (\( \Delta \text{eIF2α(P)} \) density/min ± standard error) were NIC = \(0.021 ± 0.002\), 5R = \(0.003 ± 0.001\), and 90R = \(0.021 ± 0.003\) (Fig. 4). Remarkably, the rate of phosphorylation of eIF2α by 5R PMS was only 15% of that seen with either NIC (\( P < 0.001 \)) or 90 R (\( P < 0.001 \)) PMS. NIC and 90R PMS displayed similar eIF2α kinase reaction rates (\( P > 0.5 \)).

**Role of PKR in Phosphorylation of eIF2α during Early Reperfusion**

Band density of eIF2α was identical between the WT and PKR0/0 mice and was unaffected by ischemia and reperfusion (Fig. 5). In the PMS from control animals there was no difference between eIF2α(P) band densities seen in WT (lanes 1–3) and PKR0/0 (lanes 7–9) mice. The mean band density of eIF2α(P) was increased by 20-fold in both WT (lanes 4–6) and PKR0/0 (lanes 10–12) mice after 5 min of global brain ischemia followed by 5

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**FIG. 1.** Time courses of dephosphorylation of eIF2α(32P) by brain homogenates obtained from nonischemic controls (NIC) and from rats subjected to 10 min of cardiac arrest followed by 90 min of reperfusion (90R). After reaction aliquots were subjected to SDS-PAGE followed by electroblot transfer of the proteins to nitrocellulose, the membrane was examined by (A) autoradiography, (B) Western blotting with the antibody monospecific for Ser51-phosphorylated eIF2α, and (C) Western blotting with the antibody that recognizes both phosphorylated and unphosphorylated eIF2α. Loss of eIF2α(P) immunoreactivity (B) parallels the loss of 32P signal on eIF2α (A) while there is no change in total eIF2α (C).

**FIG. 2.** Effect of ischemia and reperfusion on dephosphorylation of eIF2α(P) by brain homogenates. A large excess of eIF2α(P) (see text) was added to reactions to overcome interference from eIF2α(P) present in reperfused homogenates. (A) Representative Western blots of eIF2α(P) during dephosphorylation by brain homogenates obtained from a nonischemic control (NIC), after 5 min of cardiac arrest and 5 min of reperfusion (5R), and after 10 min of cardiac arrest and 90 min of reperfusion (90R). (B) Dephosphorylation of eIF2α(P) by the experimental groups (mean ± standard deviation, \( n = 3 \) each group). Ischemia and reperfusion had no effect on the rate of eIF2α(P) dephosphorylation by the brain homogenates (ANCOVA \( P > 0.5 \)).
min of reperfusion (in both cases Scheffe P against control < 0.001), and there was no difference between the mean eIF2α(P) band densities from reperfused WT and PKR0/0 mice (Scheffe P = 0.99). An anti-PKR immunoblot of the same membrane identified PKR at Mr , 68 kDa in PMS from the WT but not from the PKR0/0 mice, confirming the knockout (not shown).

**DISCUSSION**

In this study, we observed a rapid and large increase in the amount of eIF2α(P) in the brains of both rats and mice during the first 5 min of reperfusion after 5 min of global brain ischemia. In rats subjected to 10 min of ischemia, the increased eIF2α(P) persisted at 90 min of reperfusion. This is consistent with our previous results obtained after 10 min of ischemia and 10 and 90 min of reperfusion (15) and with evidence that protein synthesis is inhibited in the reperfused brain following as little as 2 min of ischemia (1).

We examined hypotheses that the increase in eIF2α phosphorylation during early reperfusion involved general alterations in the activity of either eIF2α kinases or eIF2α(P) phosphatases and specifically required the activity of PKR. Our results contradict all of these possibilities. At 5 min of reperfusion when eIF2α(P) levels are ~18-fold greater than normal, there was in the PMS of brain homogenates a substantial decrease in general eIF2α kinase activity with no change in eIF2α(P) phosphatase activity. At 90 min of reperfusion eIF2α kinase activity was unchanged from controls. Similarly, Hu and Wieloch (19) reported no change in eIF2α phosphorylation in their two-vessel occlusion model; they did not exam-

**FIG. 3.** Phosphorylation of the α-subunit of exogenous eIF2 by postmitochondrial supernatants (PMS) of rat brain homogenates during a 15-min reaction. Three experimental animals are included in each of the groups, which are nonischemic controls (NIC) and 3, 7, 10, 20, and 30 min of cardiac arrest (I-3, I-7, I-10, I-20, and I-30). The eIF2α(P) in 60-µg PMS protein aliquots obtained before and after the reaction was assayed by Western blotting with the antibody monospecific for Ser51-phosphorylated eIF2α. The increase in eIF2α(P) density (mean ± standard deviation) was determined by subtracting the eIF2α(P) density present before the kinase reaction from that present after the reaction. The duration of ischemia had no significant effect (ANOVA P > 0.25) on the ability of the brain homogenates to phosphorylate eIF2α.

**FIG. 4.** Phosphorylation of eIF2α by brain homogenates obtained after ischemia and reperfusion. To overcome interference from eIF2α(P) present in reperfused homogenates, the reactions contained only 0.2 µg/µl homogenate protein and a large excess of pure unphosphorylated eIF2α (see text). (A) Representative Western blots of the time course of eIF2α phosphorylation by brain homogenates obtained (1) from a nonischemic control (NIC), (2) after 5 min of cardiac arrest and 5 min of reperfusion (5R), and (3) after 10 min of cardiac arrest and 90 min of reperfusion (90R). (B) Phosphorylation of eIF2α by the experimental groups (mean ± standard deviation, n = 3 in each group). The reaction rate (Δ eIF2α(P) density/min) for the 5R homogenate was reduced 85% (Tukey P < 0.001 vs both NIC and 90R).

**FIG. 5.** Effect of brain ischemia and reperfusion on eIF2α(P) in wild-type (WT) and PKR0/0 mice. After SDS–PAGE of postmitochondrial supernatants (PMS, 50 µg protein), the proteins were transferred to nitrocellulose, and the membrane was sequentially immunoblotted with anti-eIF2α(P) and anti-eIF2α. Lanes 1–3, three control WT mice; lanes 4–6, three reperfused WT mice; lanes 7–9, three control PKR0/0 mice; and lanes 10–12, three reperfused PKR0/0 mice. The band density of total eIF2α was identical between the WT and PKR0/0 mice and was unaffected by 5 min of ischemia followed by 5 min of reperfusion. WT and PKR0/0 control mice showed no difference in eIF2α(P) band density. After ischemia and reperfusion, there was an identical 20-fold increase in eIF2α(P) in brain PMS from both WT and PKR0/0 animals.
ine eIF2α phosphatase activity. In PKRKO mice the absence of PKR had no effect on the extent of in vivo phosphorylation of eIF2α during early reperfusion.

These observations suggest involvement of a regulatory mechanism other than simply the balance of kinase and phosphatase activity because the state of this balance we observed at 5 min of reperfusion should favor dephosphorylation. In this regard, alteration of the availability of eIF2 to the kinase(s) could affect the extent of eIF2α phosphorylation during early reperfusion. Gupta’s group (4, 8, 13, 17, 25, 27, 28) has identified a 67-kDa peptide (p67) that when glycosylated binds to eIF2 and protects eIF2α from phosphorylation, and they have shown that preincubation of brain homogenates with antibody against p67 enhances subsequent in vitro phosphorylation of eIF2α. Different cell lines contain different absolute concentrations of p67, and cells with high p67 content exhibit enhanced resistance to viral-induced eIF2α phosphorylation (4). Viral-induced phosphorylation of eIF2α and shutoff of host-cell protein synthesis is associated with rapid activation of a 60-kDa enzyme that deglycosylates p67 (28), which makes p67 unable to prevent phosphorylation of eIF2α but does not lead to p67 degradation (8). These findings suggest that p67 deglycosylation during ischemia and reperfusion and/or the level of expression of p67 by various groups of neurons could affect their susceptibility to eIF2α phosphorylation.

We observed a substantially greater increase in the concentration of eIF2α(P) during our in vitro kinase reactions with brain homogenates than did Burda et al. (6); this may reflect details of the reaction conditions. We included a substantial excess of exogenous eIF2 as concentration and consequently kinase activity (18); we included okadaic acid to inhibit phosphatases (12). Our reactions also included both Mn²⁺, which is necessary for maximal activity of PKR (18), and GTP as well as ATP because we noted that eIF2α phosphorylation was enhanced when both were present.

The PKR knockout mice used in these experiments have previously been shown to appear physically normal and generally healthy, but they have a diminished interferon-γ-induced antiviral response (36), consistent with the known role of PKR during viral infection (11). The fact that exactly the same degree of phosphorylation of brain eIF2α occurred during reperfusion regardless of whether PKR was present argues against a role for PKR in the formation of eIF2α(P) under these circumstances.

The identity of the enzyme that phosphorylates eIF2α during reperfusion following brain ischemia remains unknown. There are three known eIF2α kinases (10); PKR, the heme-regulated inhibitor (HRI) found predominantly in reticulocytes, and GCN2, a yeast eIF2α kinase for which an analogue has been reported in Drosophila (29) but not in mammals. Mellor et al. doted on a rat brain library a cDNA coding for an eIF2α kinase with 82% amino acid sequence homology to HRI (22). However, Pal et al. did not find HRI immunoreactivity in rabbit brain sections (24). Unlike the activation of HRI by oxidized glutathione (GSSG) in the rabbit reticulocyte lysate model (9), we have not found either inhibition of in vitro translation or activation of eIF2α kinase activity to be induced by addition of GSSG to brain homogenates (unpublished data). Resolution of the current uncertainty regarding the identity of the kinase will require the identification and immunohistochemical mapping of the brain eIF2α kinases and further studies of the effects of inactivation of the individual enzymes on eIF2α phosphorylation during brain reperfusion.

This study also does not provide evidence regarding regional eIF2α(P) phosphatase or kinase activity. Resolution of these issues will require immunohistochemical exploration of the effect of brain ischemia and reperfusion protein phosphatase 1 and specific eIF2α kinases.

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