

## Mini-Review

# Acute and Persistent Protein Synthesis Inhibition Following Cerebral Reperfusion

Donald J. DeGracia

Department of Physiology, and the Center for Molecular Medicine and Genetics, Wayne State University, Detroit, Michigan

Lack of recovery from protein synthesis inhibition (PSI) closely correlates with neuronal death following brain ischemia and reperfusion. It has therefore been suggested that understanding the mechanisms of PSI will shed light on the mechanisms of selective neuronal death following ischemia and reperfusion. It is now known that the PKR-like ER kinase (PERK)-mediated phosphorylation of eukaryotic initiation factor 2 $\alpha$  (eIF2 $\alpha$ ) causes translation inhibition at initial reperfusion. Activation of PERK, in turn, indicates endoplasmic reticulum stress and activation of the unfolded protein response. However, phosphorylation of eIF2 $\alpha$  is a transient event and can account for PSI only in the initial hours of reperfusion. Although a number of other regulators of protein synthesis, such as eIF4F, 4EBP-1, eEF-2, and S6 kinase, have been assessed following cerebral ischemia and reperfusion, the causes of prolonged PSI have yet to be fully elucidated. The purpose of the present article is to bring together the evidence indicating that, at minimum, postischemic PSI should be conceptualized as consisting of two components: an acute, transient component mediated by unfolded protein response-induced eIF2 $\alpha$  phosphorylation and a longer term component that correlates with neuronal death. Ischemic tolerance appears to separate the acute and persistent components of reperfusion-induced translation inhibition. Specific models of the relationship among acute PSI, persistent PSI, and neuronal death are presented to clarify issues that have emerged from ongoing work in this area.

© 2004 Wiley-Liss, Inc.

**Key words:** brain ischemia and reperfusion; eIF2 $\alpha$ ; eIF4E; eIF4G; ischemic tolerance; PERK; protein synthesis inhibition; unfolded protein response

Lack of recovery from protein synthesis inhibition (PSI) closely correlates with neuronal death following brain ischemia and reperfusion (I/R), and this has led to the suggestion that understanding the mechanisms of PSI will shed light on the mechanism of selective neuronal

death following I/R (Hossmann, 1993). The past decade has seen a flurry of activity in this area, in which the phosphorylation of the alpha subunit of eukaryotic initiation factor 2 [eIF2 $\alpha$ ; phospho-form eIF2 $\alpha$ (P)] was identified as an important mechanism of postischemic PSI (for review see DeGracia et al., 2002). Furthermore, reperfusion-induced eIF2 $\alpha$  phosphorylation is mediated by the PKR-like eIF2 $\alpha$  kinase (PERK), indicative of endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR). However, it is now clear that phosphorylation of eIF2 $\alpha$  is a transient event that can account for PSI only in the initial hours of reperfusion. Although several regulators of protein synthesis other than eIF2 $\alpha$  phosphorylation have been assessed following cerebral I/R, the causes of sustained PSI have yet to be fully elucidated. The purpose of this Mini-Review is to bring together the evidence indicating that, at minimum, postischemic PSI should be conceptualized as consisting of two components: 1) an acute, transient component, unrelated to selective vulnerability and mediated by UPR-induced eIF2 $\alpha$  phosphorylation, and 2) a longer-term component that correlates with cell death. Furthermore, we discuss how ischemic tolerance appears to separate these components. Here we develop models that clarify the relationship between acute and persistent PSI and cell death in hopes of guiding further work in this area.

### PSI FOLLOWING CEREBRAL I/R

All forms of brain ischemia, including transient global, permanent focal, and transient focal ischemia lead to PSI during reperfusion (Hossmann, 1993). PSI follow-

Contract grant sponsor: National Institutes of Health; Contract grant number: NS044100.

\*Correspondence to: Dr. D.J. DeGracia, Department of Physiology, Wayne State University, 3125 Scott Hall, 540 East Canfield Ave., Detroit, MI 48201. E-mail: ddegraci@med.wayne.edu

Received 9 March 2004; Accepted 24 May 2004

Published online 13 July 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.20225

ing transient global ischemia provides a prototypical picture. Upon reperfusion following global ischemia, there is brain-wide PSI, which gradually recovers over several hours in the majority of brain regions, where the rate of translational recovery is dependent on the duration of ischemia (Bodsch et al., 1986). However, in brain regions where protein synthesis fails to recover, cell death ensues. With relatively short global ischemic durations, this effect is localized to the CA1 sector of the hippocampus (Dienel et al., 1980). With focal ischemia models, the pattern of PSI is more complex because of variations in regional cerebral blood flow caused by focal occlusion and collateral circulation. What is clear from focal models is that there is a dissociation of PSI and energy metabolism. Regions of PSI can extend beyond those showing depletion of ATP (Hossmann, 1993).

### TRANSLATION INITIATION

Kleihues and Hossmann (1971) observed polysome disaggregation during postischemic PSI, leading them to suggest that PSI was due to inhibited translation initiation, a suggestion that has been borne out by many studies (for review see Hossmann, 1993). Because translation initiation regulation plays such an important role in postischemic PSI mechanisms, this section provides a brief review of this process. The function of translation initiation is to bring together four components: 1) the 40S ribosomal subunit, 2) the 60S ribosomal subunit, 3) the mRNA to be translated, and 4) the methionyl-containing initiator tRNA (Met-tRNA<sub>i</sub><sup>Met</sup>). Translation initiation is orchestrated by at least a dozen eukaryotic initiation factors, two of which mediate rate-limiting steps.

The delivery of the Met-tRNA<sub>i</sub><sup>Met</sup> is mediated by eIF2 · GTP. At the end of initiation, eIF2 · GDP is released and requires the activity of eIF2B to exchange GDP for GTP and thereby recycle eIF2 · GTP for another round of initiation. Phosphorylation of the alpha subunit of eIF2 [eIF2(αP)] leads to a decrease in the rate of Met-tRNA<sub>i</sub><sup>Met</sup> delivery and, hence, a slowing of protein synthesis because eIF2(αP) is a competitive inhibitor of eIF2B. The molar ratio of eIF2 to eIF2B in brain is ~5:1 (Oldfield et al., 1994), meaning that phosphorylation of >20% of eIF2α can lead to an almost complete inhibition of translation initiation.

The mRNA is delivered to the 40S subunit by eIF4F. eIF4F consists of the subunits eIF4E, eIF4A, and eIF4G. eIF4E binds the m<sup>7</sup>-GTP cap of the mRNA, and eIF4A, along with eIF4B, unwinds mRNA secondary structure and mediates ATP-dependent scanning to the AUG start codon. eIF4G serves as a scaffolding protein, linking to the 40S subunit via eIF3 binding and linking the mRNA via eIF4E (Sonenberg and Dever, 2003). eIF2 regulation increases or decreases global rates of protein synthesis, and regulation of eIF4 controls the repertoire of mRNAs delivered to the ribosomes. Low levels of eIF4F will favor translation of more abundant mRNAs, and, as levels of eIF4F increase, less abundant mRNAs will be recruited into translation (Gingras et al., 1999).

The regulation of eIF4F is complex. Both eIF4E and eIF4G are phosphoproteins, but the functional significance of their phosphorylation remains unclear (Gingras et al., 1999; Scheper and Proud, 2002). The 4EBPs, of which 4EBP-1 is the most well studied, are a class of eIF4E binding proteins that sequester eIF4E and prevent formation of eIF4F (Gingras et al., 1999). When the 4EBPs become phosphorylated in response to amino acids, insulin, or growth factors, eIF4E is released and is capable of entering into the eIF4F complex (Scheper and Proud, 2002). eIF4G can undergo proteolytic fragmentation via calpains (Neumar et al., 1998) or caspase 3 (Marissen and Lloyd, 1998). Such fragmentation can cleave the eIF4E binding domain from the eIF3 binding domain, allowing eIF4E-independent initiation of uncapped mRNAs or of mRNAs containing internal ribosome entry sites (IRES sequences; Hellen and Sarnow, 2001).

### POSTISCHEMIC PSI AND eIF2α

It is now established that brain I/R induces a PERK-mediated phosphorylation of at least 30% of eIF2α almost immediately after the onset of reperfusion (for review see DeGracia et al., 2002). However, several recent studies have demonstrated that eIF2α phosphorylation is a transient event and does not correlate with persistent PSI. Althausen et al. (2001) showed, after 1 hr of middle cerebral artery occlusion (MCAO), a transient ~20-fold increase in eIF2α phosphorylation that returned almost to control levels by 6 hr of reperfusion, but protein synthesis remained inhibited even at 24 hr of reperfusion. Martin de la Vega et al. (2001) reported a similar result in which eIF2α(P) levels returned to control values by 4–6 hr of reperfusion following 30 min of four-vessel occlusion (4VO) ischemia, whereas polysomes remained disaggregated, and no difference was observed in this pattern between vulnerable hippocampal CA1 and resistant cerebral cortex. We observed eIF2α(P) to return to control levels in unfractionated brainstem homogenates at 4 hr of reperfusion following 10 min of global brain ischemia, whereas in vivo brainstem protein synthesis remained at 56% of control values (Kumar et al., 2003). At 4 hr of reperfusion in the cerebral cortex, although protein synthesis was only 41% of control values, eIF2α(P) levels decreased from a maximum of 20-fold to 3-fold vs. control (Kumar et al., 2003), in line with our previous observation that eIF2α(P) was cleared substantially from the cytosol by this time (DeGracia et al., 1997). In whole hippocampus, eIF2α(P) levels decreased from a maximum of 12-fold to ~5-fold vs. control, but protein synthesis was 25% of the control value (Kumar et al., 2003). Thus, these data suggest that there is no correlation among eIF2α(P) levels, selectively vulnerable brain regions, and persistent PSI following brain reperfusion.

Dephosphorylation of eIF2α(P) at >4 hr of reperfusion is consistent with the report of Martin de la Vega et al. (2001) showing a progressive increase in eIF2α(P) phosphatase activity at 2 and 4 hr of reperfusion. Recently, this group reported an ~2-fold increase in the protein GADD34 at 4 hr of reperfusion in cortex and hippocam-

pus (Garcia et al., 2004). GADD34 is a stress-induced regulatory subunit of a phosphatase complex that dephosphorylates eIF2 $\alpha$  (Novoa et al., 2003). The report of Garcia et al. (2004) correlates with that of Doutheil et al. (1999), who showed an ~5-fold increase in GADD34 mRNA at 2 hr of reperfusion in cortex and hippocampus. Thus, current evidence supports the idea that the GADD34-mediated dephosphorylation of eIF2 $\alpha$ (P) is well underway by 4 hr of reperfusion.

#### OTHER REGULATORS OF PROTEIN SYNTHESIS FOLLOWING CEREBRAL I/R

Studies of eIF2 following brain I/R have been performed, along with studies of other regulators of protein synthesis. The eIF4 components have received significant attention, as have S6 kinase and eukaryotic elongation factor 2 (eEF-2).

Although the phosphorylation of both eIF4E (Martin de la Vega et al., 2001) and eIF4G (Garcia et al., 2004) is decreased during ischemia, eIF4E phosphorylation is normalized by 30 min of reperfusion (DeGracia et al., 1996; Martin de la Vega et al., 2001), and eIF4G phosphorylation increases about 50% over control levels by 4 hr of reperfusion (Garcia et al., 2004). Given that the function of eIF4G phosphorylation is not understood, the function of increased eIF4G phosphorylation during reperfusion is likewise not clear. In cortex and hippocampus, 4E-BP1 was dephosphorylated during ischemia but had returned to control levels of phosphorylation by 4 hr of reperfusion following 30 min of 4VO, and there was no change in the levels of 4E-BP1 (Martin de la Vega et al., 2001).

The levels of both eIF4E and eIF4G decrease as a function of ischemic duration, and we have shown both to be calpain substrates (Neumar et al., 1995, 1998). DeGracia et al. (1996) observed fragmentation of eIF4G at 90 min of reperfusion, a result confirmed by Martin de la Vega et al. (2001). Garcia et al. (2004) also provided evidence to support calpain-mediated, but not caspase 3-mediated, eIF4G proteolysis. The one study that evaluated the functional significance of changes in levels of eIF4E and eIF4G showed a decrease in the eIF4F complex isolated from hippocampus at 4 hr of reperfusion, which paralleled decreases in both eIF4E and eIF4G (Martin de la Vega et al., 2001).

S6 kinase is activated by hyperphosphorylation in progrowth conditions (e.g., serum administration) and phosphorylates the ribosomal protein S6, thereby stimulating translation of mRNAs coding for ribosomal proteins and elongation factors (Meyhaus, 2000). Martin de la Vega et al. (2001) measured S6 kinase activity by an immune complex kinase activity assay and showed a transient decrease in S6 kinase activity that returned to control values by 4 hr of reperfusion following 30 min of 4VO in rats. Althusen et al. (2001) observed, after 1 hr of MCAO, an almost complete dephosphorylation of S6 kinase subunits that returned close to control levels by 6 hr of reperfusion, but there was again complete dephosphorylation by 24 hr of reperfusion. After 2 hr of MCAO in rats, Janelidze et al.

(2001) reported a persistent decrease in phosphorylation of S6 kinase over 24 hr reperfusion, and a decrease in the level of S6 kinase at 24 hr reperfusion.

In the only report to measure eEF-2, Althusen et al. (2001) showed no change in levels of eEF-2 for as long as 6 hr of reperfusion. However, ischemia induced dephosphorylation of eEF-2 that was normalized by 1 hr of reperfusion but then decreased significantly below control by 6 hr of reperfusion. Insofar as phosphorylation of eEF-2 inhibits its activity (Ryazanov, 2002), this result indicates that regulation of eEF-2 does not contribute to persistent PSI in reperfusion.

Thus, to summarize, the levels of eIF4E and eIF4G decrease as a function of ischemic duration, and dephosphorylation of S6 kinase occurs at later reperfusion durations. It is not clear how these changes would contribute to the acute phase of PSI, in that the >20-fold increase in eIF2 $\alpha$ (P) would be expected to inhibit *in vivo* translation fully during early reperfusion. However, it has been suggested (Martin de la Vega et al., 2001) that changes in other translation regulators, such as eIF4F and S6 kinase, may contribute to persistent PSI, implying that prolonged PSI is mediated by multiple causes.

The issue, however, is the functional significance of changes in translation regulators other than eIF2 $\alpha$  and by what means they would contribute to persistent PSI. For example, activation of S6 kinase leads to increased phosphorylation of ribosomal protein S6, which, in turn, enhances translation of mRNAs coding for ribosomal proteins, thereby increasing the number of ribosomes in the cell (Meyhaus, 2000). However, it is not at all apparent that there is a requirement for an increased amount of ribosomes at later reperfusion. For example, we have shown that there is no evidence of oxidative damage to ribosomes at 8 hr of reperfusion (Krause et al., 1992) and that ribosomes isolated from 8-hr-reperfused cortex, when given cofactors from controls, are competent at *in vitro* protein synthesis (DeGracia et al., 1993). In fact, to our knowledge, no measurement of the phosphorylation level of ribosomal protein S6 itself has been made in reperfused brain, making the functional significance of changes in S6 kinase activity unclear.

The decrease in eIF4G could be of particular functional significance. As elaborated by DeGracia et al. (2002), fragments of eIF4G could serve to inhibit eIF4E and suppress translation of capped mRNAs. One means to test this hypothesis would involve isolating mRNA-bound ribosomes (e.g., polysomes) from brain regions showing persistent PSI at long reperfusion durations and determining the relative proportion of capped and uncapped mRNAs.

In general, only *in vitro* translation rates or *in situ* autoradiography studies have been performed on brain following longer reperfusion durations. Studies addressing mechanisms of prolonged PSI at reperfusion durations >12 hr are clearly needed to clarify the relative roles of the variety of possible translation inhibition mechanisms.

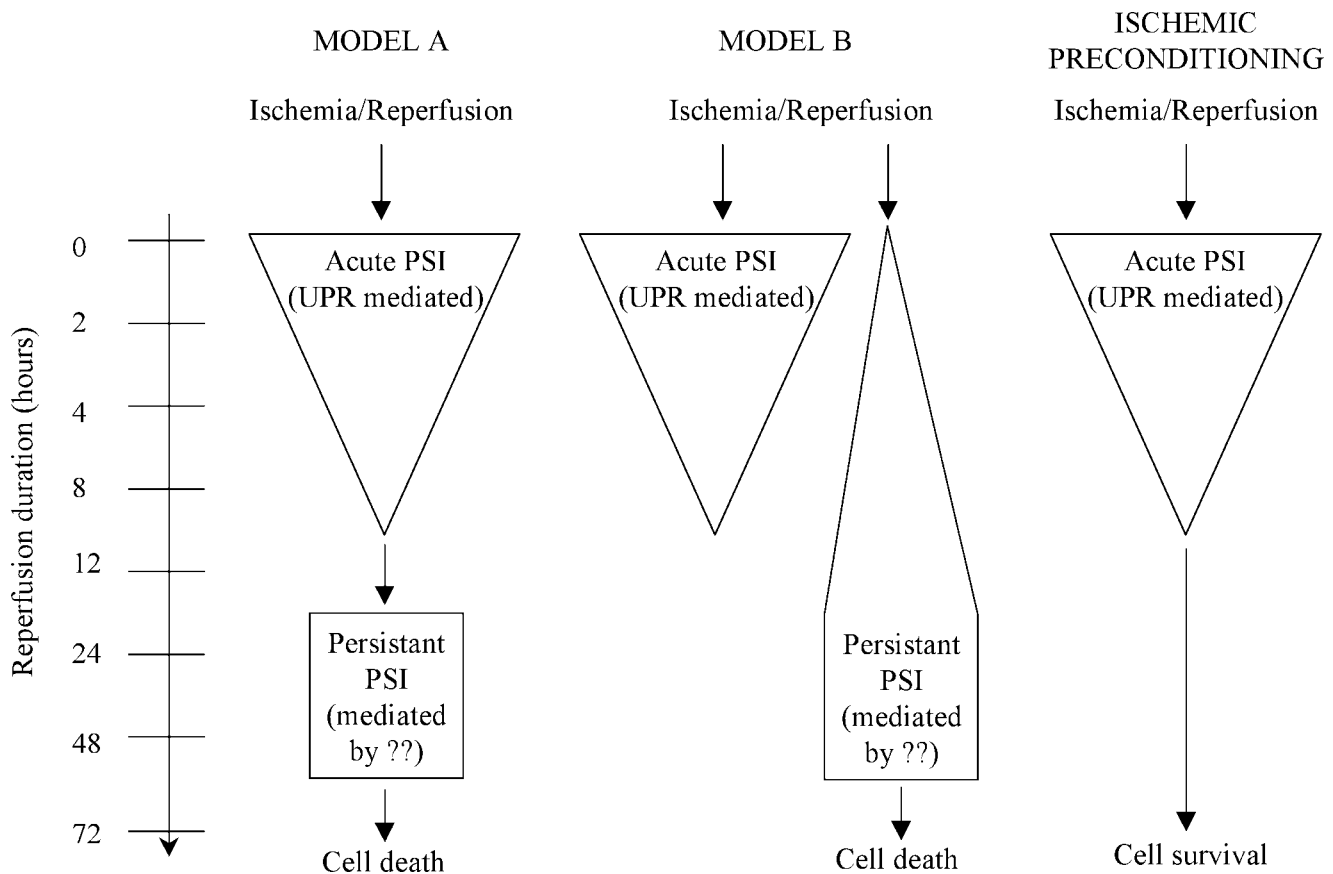


Fig. 1. Two different models of acute and persistent protein synthesis inhibition (PSI) in the brain following ischemia and reperfusion, contrasted with the effects of ischemic tolerance on PSI. Acute PSI is caused by UPR-induced eIF2 $\alpha$  phosphorylation, an event that peaks between 10 and 90 min of reperfusion and abates between 4 and 6 hr of reperfusion. The cause(s) of persistent PSI has not yet been definitively established. Model A posits a direct causality between acute and persistent PSI. That is, persistent PSI represents a lack of recovery from acute PSI. In model B, acute and persistent PSI occur by separate and parallel pathways, an example of which would be the UPR-mediated phosphorylation of eIF2 $\alpha$  and the calpain-mediated degradation of

eIF4G. In model B, therefore, persistent PSI is not due to a lack of recovery from acute PSI, and persistent PSI is masked by the massive phosphorylation of eIF2 $\alpha$  in acute PSI. Models A and B are contrasted with ischemic tolerance (IT), which eliminates persistent, but not acute, PSI. A causal relationship between persistent PSI and cell death is indicated in both model A and model B. However, not shown is the possibility that UPR-mediated acute PSI also contributes to cell death (e.g., by CHOP synthesis). Models A and B are not necessarily mutually exclusive and are offered to clarify our current understanding of reperfusion-induced PSI and to help focus future research efforts.

### EFFECTS OF ISCHEMIC TOLERANCE ON PSI

Ischemic tolerance (IT) in the brain is the phenomenon in which exposure to a brief, nonlethal ischemic insult produces a delayed-type tolerance to cell death following a second exposure to a lethal insult (for review see Kirino, 2002). IT enhances recovery of protein synthesis following the lethal insult. In gerbils exposed to two sublethal 2-min ischemic episodes 1 day apart, followed by a lethal 5-min exposure 48 hr later, there was complete recovery of protein synthesis in hippocampal CA1 and a prevention of cell death (Furuta et al., 1993). It is significant that PSI and polysome disaggregation occurred during early reperfusion in both IT and non-IT animals in this study. Thus, in the

study by Furuta et al. (1993), IT prevented the persistent, but not the acute, component of PSI.

The study by Garcia et al. (2004) evaluated translation regulators following IT. Consistent with the findings of Furuta et al. (1993), eIF2 $\alpha$  phosphorylation in the first 4 hr of reperfusion rose and declined identically in animals with and without IT. IT induced other modest changes in translation regulators during reperfusion, including enhanced phosphorylation of eIF4E and eIF4G and no loss of eIF4E, although eIF4G levels declined below controls. The most dramatic change induced by IT was a pronounced increase in GADD34 protein levels. GADD34 increased twofold over control levels in animals without IT and increased fourfold over control levels in animals with IT. How-

ever, given that the time course of eIF2 $\alpha$  phosphorylation was similar in animals with and without IT, the function of the IT-induced increase in GADD34 is presently unclear.

In sum, PSI, polysome disaggregation, and eIF2 $\alpha$ (P) occur during early reperfusion in animals with and without IT, but prolonged PSI is prevented in animals with IT. Together, these data serve as perhaps the strongest evidence to date indicating that reperfusion-induced PSI can be divided into acute and persistent phases.

### MODELS OF ACUTE AND PERSISTENT PSI

Figure 1 provides two possible, though not necessarily mutually exclusive, models of acute and persistent PSI following brain reperfusion. In model A, acute PSI is caused by the UPR-mediated inhibition of protein synthesis. By a means not yet determined, the acute PSI may be causally related to persistent PSI. That is, persistent PSI represents a *lack of recovery* from acute PSI. This would not directly involve eIF2 $\alpha$  phosphorylation, insofar as this abates after 4 hr of reperfusion, but may involve an as yet undetermined causal relationship. Model B indicates that the causes of acute and persistent PSI occur simultaneously with the onset of reperfusion but that the factors causing persistent PSI are masked by the UPR-mediated translation inhibition. In this case, abatement of eIF2 $\alpha$ (P) allows unmasking of the other mechanisms contributing to persistent PSI. In model B, then, persistent PSI is due not to a lack of recovery from acute PSI but to mechanisms operating in parallel. For example, the parallel pathways of UPR-mediated eIF2 $\alpha$  phosphorylation and the calpain-mediated fragmentation of eIF4G fit with model B. However, until the causal role of eIF4G degradation in persistent PSI is established, such a model must remain tentative.

The relationship between persistent PSI and cell death shown in models A and B is based on the correlation of these two phenomena. Again, however, until the mechanisms of persistent PSI are determined, the positing of causality between persistent PSI and cell death must also remain tentative. One complexity not illustrated in Figure 1 is that processes activated by the UPR in the acute phase of PSI, such as CHOP synthesis, could also contribute to cell death (DeGracia et al., 2002; Tajiri et al., 2004). However, given that the UPR occurs brain-wide following global ischemia (Kumar et al., 2003), it will be imperative to demonstrate a specific contribution by the UPR to selective vulnerability. Of course, factors not related to PSI, such as caspase activation (Liou et al., 2003) or free radical production (White et al., 2001), also contribute to cell death following brain I/R.

The effect of ischemic preconditioning is also shown in Figure 1, in which IT eliminates the persistent, but not the acute, phase of PSI. Inclusion of ischemic preconditioning suggests that up-regulation of genes induced by IT (Kirino, 2002) plays a role in buffering vulnerable cells against the mechanisms inducing persistent PSI but that these genetic changes do not affect the acute, UPR-mediated phase of PSI.

### CONCLUSIONS

Our understanding of the molecular causes of PSI following brain I/R has advanced significantly in the past decade. The result of this deeper understanding is the realization that postischemic PSI involves a complex set of processes and does not have a single underlying cause. Acknowledgment of such complexity is a significant step forward. Given the current evidence, it seems reasonable to posit the models described above that subdivide postischemic PSI into at least an acute phase and a persistent phase. These models make clear the need to 1) determine the mechanisms of persistent PSI; 2) determine the relationship, if any, between acute and persistent PSI; and 3) determine the relationship of both acute and persistent PSI to cell death processes. Given the central importance of protein synthesis in neuronal viability and repair following ischemia and reperfusion, it is expected that a causal relationship between PSI and cell death will emerge. Clearly, understanding this relationship will be important to the development of therapies to halt neuronal death after postischemic brain reperfusion.

### ACKNOWLEDGMENTS

The author thanks his colleagues in Germany and Spain for their stimulating collegiality and important work in moving this area forward.

### REFERENCES

- Althausen S, Mengesdorf T, Mies G, Olah L, Naim AC, Proud CG, Paschen W. 2001. Changes in the phosphorylation of initiation factor eIF-2 $\alpha$ , elongation factor eEF-2 and p70 S6 kinase after transient focal cerebral ischaemia in mice. *J Neurochem* 78:779–787.
- Bodsch W, Barbier A, Oehmichen M, Grosse Ophoff B, Hossmann KA. 1986. Recovery of monkey brain after prolonged ischemia. II. Protein synthesis and morphological alterations. *J Cereb Blood Flow Metab* 6:22–33.
- DeGracia DJ, O'Neil BJ, Frisch C, Krause GS, Skjaerlund JM, White BC, Grossman LI. 1993. Studies of the protein synthesis system in the brain cortex during global ischemia and reperfusion. *Resuscitation* 25:161–170.
- DeGracia DJ, Neumar RW, White BC, Krause GS. 1996. Global brain ischemia and reperfusion: modifications in eukaryotic initiation factors associated with inhibition of translation initiation. *J Neurochem* 67:2005–2012.
- DeGracia DJ, Sullivan JM, Neumar RW, Alousi SS, Hikade KR, Pittman JE, White BC, Rafols JA, Krause GS. 1997. Effect of brain ischemia and reperfusion on the localization of phosphorylated eukaryotic initiation factor 2 $\alpha$ . *J Cereb Blood Flow Metab* 17:1291–1302.
- DeGracia DJ, Kumar R, Owen CR, Krause GS, White BC. 2002. Molecular pathways of protein synthesis inhibition during brain reperfusion: implications for neuronal survival or death. *J Cereb Blood Flow Metab* 22:127–141.
- Dienel GA, Pulsinelli WA, Duffy TE. 1980. Regional protein synthesis in rat brain following acute hemispheric ischemia. *J Neurochem* 35:1216–1226.
- Douthel J, Althausen S, Gissel C, Paschen W. 1999. Activation of MYD116 (gadd34) expression following transient forebrain ischemia of rat: implications for a role of disturbances of endoplasmic reticulum calcium homeostasis. *Brain Res Mol Brain Res* 8:63:225–232.
- Furuta S, Ohta S, Hatakeyama T, Nakamura K, Sakaki S. 1993. Recovery of protein synthesis in tolerance-induced hippocampal CA1 neurons after transient forebrain ischemia. *Acta Neuropathol* 86:329–336.

- Garcia L, Burda J, Hrehorovska M, Burda R, Martin ME, Salinas M. 2004. Ischaemic preconditioning in the rat brain: effect on the activity of several initiation factors, Akt and extracellular signal-regulated protein kinase phosphorylation, and GRP78 and GADD34 expression. *J Neurochem* 88:136–147.
- Gingras AC, Raught B, Sonenberg N. 1999. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem* 68:913–963.
- Hellen CUT, Sarnow P. 2001. Internal ribosome entry sites in eukaryotic mRNA molecules. *Genes Dev* 15:1593–1612.
- Hossmann KA. 1993. Disturbances of cerebral protein synthesis and ischemic cell death. *Prog Brain Res* 96:161–177.
- Janelidze S, Hu BR, Siesjo P, Siesjo BK. 2001. Alterations of Akt1 (PK-Balpa) and p70(S6K) in transient focal ischemia. *Neurobiol Dis* 8:147–154.
- Kirino T. 2002. Ischemic tolerance. *J Cereb Blood Flow Metab* 22:1283–1296.
- Kleihues P, Hossmann KA. 1971. Protein synthesis in the cat brain after prolonged cerebral ischemia. *Brain Res* 35:409–418.
- Krause GS, DeGracia DJ, Skjaerlund JM, O'Neil BJ. 1992. Assessment of free radical-induced damage in brain proteins after ischemia and reperfusion. *Resuscitation* 23:59–69.
- Kumar R, Krause GS, Yoshida H, Mori K, DeGracia DJ. 2003. Dysfunction of the unfolded protein response during global brain ischemia and reperfusion. *J Cereb Blood Flow Metab* 23:462–471.
- Liou AK, Clark RS, Henshall DC, Yin XM, Chen J. 2003. To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: a review on the stress-activated signaling pathways and apoptotic pathways. *Prog Neurobiol* 69:103–142.
- Marissen WE, Lloyd RE. 1998. Eukaryotic translation initiation factor 4G is targeted for proteolytic cleavage by caspase 3 during inhibition of translation in apoptotic cells. *Mol Cell Biol* 18:7565–7574.
- Martin de la Vega C, Burda J, Nemethova M, Quevedo C, Alcazar A, Martin ME, Danielisova V, Fando JL, Salinas M. 2001. Possible mechanisms involved in the down-regulation of translation during transient global ischaemia in the rat brain. *Biochem J* 357:819–826.
- Meyuhas O. 2000. Synthesis of the translational apparatus is regulated at the translational level. *Eur J Biochem* 267:6321–6330.
- Neumar RW, DeGracia DJ, White BC, McDermott PJ, Evans DR, Krause GS. 1995. Eukaryotic initiation factor 4E degradation during brain ischemia. *J Neurochem* 65:1391–1394.
- Neumar RW, DeGracia DJ, Konkoly LL, Khoury JI, White BC, Krause GS. 1998. Calpain mediates eukaryotic initiation factor 4G degradation during global brain ischemia. *J Cereb Blood Flow Metab* 18:876–881.
- Novoa I, Zhang Y, Zeng H, Jungreis R, Harding HP, Ron D. 2003. Stress-induced gene expression requires programmed recovery from translational repression. *EMBO J* 22:1180–1187.
- Oldfield S, Jones BL, Tanton D, Proud CG. 1994. Use of monoclonal antibodies to study the structure and function of eukaryotic protein synthesis initiation factor eIF-2B. *Eur J Biochem* 221:399–410.
- Ryazanov AG. 2002. Elongation factor-2 kinase and its newly discovered relatives. *FEBS Lett* 514:26–29.
- Scheper GC, Proud CG. 2002. Does phosphorylation of the cap-binding protein eIF4E play a role in translation initiation? *Eur J Biochem* 269:5350–5359.
- Sonenberg N, Dever TE. 2003. Eukaryotic translation initiation factors and regulators. *Curr Opin Struct Biol* 13:56–63.
- Tajiri S, Oyadomari S, Yano S, Morioka M, Gotoh T, Hamada JI, Ushio Y, Mori M. 2004. Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP. *Cell Death Differ* [advance online publication doi: 10.1038/sj.cdd.4401365].
- White BC, Sullivan JM, DeGracia DJ, O'Neil BJ, Neumar RW, Grossman LI, Rafols JA, Krause GS. 2001. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. *J Neurol Sci* 179:1–33.