Cellular energy status modulates translational control mechanisms in ischemic-reperfused rat hearts


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Submitted 23 August 2004; accepted in final form 11 May 2005

Cellular energy status modulates translational control mechanisms in ischemic-reperfused rat hearts. Am J Physiol Heart Circ Physiol 289: H1242–H1250, 2005. First published May 13, 2005; doi:10.1152/ajpheart.00859.2004.—Mechanisms regulating ischemia and reperfusion (I/R)-induced changes in mRNA translation in the heart are poorly defined, as are the factors that initiate these changes. Because cellular energy status affects mRNA translation under physiological conditions, it is plausible that I/R-induced changes in translation may in part be a result of altered cellular energy status. Therefore, the purpose of the studies described herein was to compare the effects of I/R with those of altered energy substrate availability on biomarkers of mRNA translation in the heart. Isolated adult rat hearts were perfused with glucose or a combination of glucose plus palmitate, and effects of I/R on various biomarkers of translation were subsequently analyzed. When compared with hearts perfused with glucose plus palmitate, hearts perfused with glucose alone exhibited increased phosphorylation of eukaryotic elongation factor 2 (eEF2), the α-subunit of eukaryotic initiation factor 2 (eIF2), and AMP-activated protein kinase (AMPK), and these hearts also exhibited enhanced association of eIF4E with eIF4E binding protein 1 (4E-BP1). Regardless of the energy substrate composition of the buffer, phosphorylation of eEF2 and AMPK was greater than control values after ischemia. Phosphorylation of eEF2 and eIF4E and the association of eIF4E with 4E-BP1 were also greater than control values after ischemia but only in hearts perfused with glucose plus palmitate. Reperfusion reversed the ischemia-induced increase in eEF2 phosphorylation in hearts perfused with glucose and reversed ischemia-induced changes in eIF4E, eEF2, and AMPK phosphorylation in hearts perfused with glucose plus palmitate. Because many ischemia-induced changes in mRNA translation are mimicked by the removal of a metabolic substrate under normal perfusion conditions, the results suggest that cellular energy status represents an important modulator of I/R-induced changes in mRNA translation.

Free fatty acids; cellular stress; adenosine 5′-monophosphate-activated protein kinase

Various signal transduction pathways are activated in cardiomyocytes in response to ischemia and reperfusion (I/R). These pathways culminate in changes that lead to apoptosis or recovery from the I/R-induced injury. Alterations in protein expression are required for either of these processes, and, recently, a considerable amount of effort has been vested in elucidating how protein expression is regulated in response to I/R in cardiac muscle.

Modulation of protein expression can be mediated, in part, through changes in gene transcription. Indeed, the majority of studies on I/R-induced protein expression have to date focused on this regulatory step. Several transcription factors are induced during myocardial I/R, including the heat shock transcription factor (HSF) and the redox-regulated transcription factors NF-κB and activator protein-1 (AP-1). Although the induction and activation of transcription factors undoubtedly contribute to the changes in protein expression observed in the heart with I/R, less emphasis has been paid to the potential roles of mRNA stability and mRNA translation.

Several of the proteins, referred to as translation factors, regulating mRNA translation are influenced by I/R-associated phenomena, such as changes in cellular energy status, redox status, and MAPK signaling (3, 11, 43). This suggests that I/R-induced changes in protein expression may be mediated in part by altered mRNA translation. These translation factors include, but are not limited to, the eukaryotic initiation factors (eIF)4E and eIF4G and the eIF4E binding protein 1 (4E-BP1), which regulate the delivery of 7-methyl-GTP capped mRNAs to the 40S ribosomal subunit during the initiation phase of translation (56); the initiator methionyl tRNA (met-tRNAi)-binding protein eIF2 and its regulatory protein, the guanine nucleotide exchange factor eIF2B, which regulate the delivery of met-tRNAi to the 40S ribosomal subunit during translation initiation (21); and finally, eukaryotic elongation factor-2 (eEF2), which controls polypeptide-chain translocation during the elongation phase of translation (40). The function of many of these translation factors is regulated through their phosphorylation and dephosphorylation (54). Indeed, the phosphorylation status of several translation factors is affected by cerebral I/R (8, 49). Pertinently, the studies on cerebral I/R have led to the suggestion that translation and transcription may be uncoupled during I/R. Therefore, to fully define altered protein expression patterns induced by I/R, an assessment of mRNA translation is required (15).

The role of energy substrate availability, particularly that of free fatty acids (FFAs), in modulating heart function during I/R has been investigated extensively, and the majority of the findings suggests that FFAs are detrimental to the heart following I/R (9, 34, 51). Recent studies, however, indicate that the presence of FFAs immediately following an ischemic episode helps the heart overcome the ischemia-induced cellular stress and thus propagates the return of normal cardiac function. In this regard, activation of the peroxisome proliferator-activated receptor-α transcription factor, which stimulates the expression of genes encoding enzymes necessary for fatty acid metabolism, is associated with improved contractile function and decreased infarct size in mouse hearts 24 h after I/R (63).
In addition, mouse hearts lacking the membrane fatty acid translocase CD36, which are unable to metabolize long-chain fatty acids, have low cellular ATP levels and poor ischemic tolerance. However, the recovery of cellular ATP and cardiac efficiency acutely following ischemia (8 min) is similar to controls when the hearts are perfused with medium chain fatty acids that do not require CD36 activity for their uptake (24).

Because prior reports (6, 7) indicate that several translation factors in the rat heart are responsive to energetic stress, we hypothesize that many I/R-induced changes in the translational control of protein expression in the heart will be mimicked by limiting substrate availability under normal perfusion conditions through alterations in cellular energy status. Therefore, the objective of the study described herein was to investigate the effects of I/R on several translation factors in an isolated perfused rat heart model and to determine whether any of these changes are mimicked by altering the energy substrate composition of the perfusate.

MATERIALS AND METHODS

Animal care. The animal facilities and experimental protocol used for the studies described herein were reviewed and approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine. Adult male Sprague-Dawley rats (~400 g) were maintained on a 12:12-h light-dark cycle with a standard diet (Harlan-Tekland Rodent Chow, Madison, WI), and water was provided ad libitum.

Experimental protocol. Rats were deprived of food overnight and anesthetized via intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt; Abbott Laboratories, Chicago, IL). The heart was excised, cannulated, and perfused through the aorta by using a modified Langendorff technique as described previously (55). Briefly, Krebs-Henseleit bicarbonate buffer (37°C) gassed with humidified 95% O2-5% CO2 and containing 11 mM glucose was retrogradely perfused through the heart with a hydrostatic pressure of 60 mmHg for 10 min. After the initial perfusion, buffer containing either 11 mM glucose or 11 mM glucose and 11 mM palmitate and dialyzed 1.5 mM palmitate bound to 3% BSA (Fraction V, MP Biomedicals, Aurora, OH) was then recirculated through the heart at a flow rate of 15 ml/min for 20 min. Global ischemia was initiated by reducing the flow rate to ~1 ml/min for 25 min. Subsequently, hearts were either frozen between a tissue clamp precooled to the temperature of liquid N2 or reperfused at a flow rate of 15 ml/min for an additional 15 min before being frozen. Control nonischemic hearts were perfused continuously at 15 ml/min for 60 min before being frozen.

Sample preparation. Frozen tissue was powdered in a container precooled to the temperature of liquid N2 and collected in a cryogenic vial before further utilization. A portion (~0.4 g) of powdered tissue was homogenized in seven volumes of homogenization buffer (10 mM 20 HEPES (pH 7.4), 100 KCl, 0.2 EDTA, 2 mM EGTA, 1 diethiothreitol, 50 sodium fluoride, 50 β-glycerophosphate, 0.1 PMSF, 1 benzamidine, and 0.5 sodium vanadate, including 10 μl/ml protease inhibitor cocktail (Sigma, St. Louis, MO)). The homogenate was immediately centrifuged at 1,500 g for 10 min at 4°C. An aliquot of the resultant supernatant was saved and later analyzed for protein by using a commercially available assay kit (Bio-Rad, Hercules, CA).

Immunoblot analysis. The tissue content of specific proteins was evaluated in 1,500 g supernatants by protein immunoblot analysis. Antibodies were purchased from Cell Signaling (Beverly, MA) unless otherwise stated. Equal concentrations of protein were subjected to SDS-PAGE. After separation, proteins were transferred to polyvinylidene difluoride membranes, and the membranes were incubated with antibodies that recognize 4E-BP1 (Bethyl, Montgomery, TX), eIF4E (26), p38 MAPK, AMP-activated protein kinase (AMPK), eEF2, or eIF2α (27). Proteins were visualized by enhanced chemiluminescence (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). When subjected to SDS-PAGE, 4E-BP1 is resolved into multiple electrophoretic forms whereby the most highly phosphorylated form exhibits the slowest mobility, thus allowing for an assessment of both protein content and phosphorylation status. Changes in 4E-BP1 phosphorylation were assessed as described previously (16). Changes in the phosphorylation status of the remaining proteins were assessed by first stripping the membranes of antibodies and reanalyzing them with antibodies that specifically recognize the phosphorylated forms of eEF2 (Thr56), AMPK (Thr172), eEF2 (Thr56), p38 MAPK (Thr180/Tyr182), or eIF2α (Ser51; Biosource, Camarillo, CA), respectively. The amount of protein in the phosphorylated form was normalized to the total amount of the respective protein before data transformation.

Analysis of 4E-BP1 association with eIF4E. eIF4E was immunoprecipitated from 1,500 g supernatants by using a monoclonal eIF4E antibody. Equal volumes of sample were subjected to immunoblot analysis by using an antibody against 4E-BP1 (Bethyl Laboratories). Subsequently, membranes were stripped of antibody and reanalyzed by using an antibody that recognizes eIF4E to normalize the data to the amount of eIF4E in the immunoprecipitate before data transformation.

Statistical analysis. Data are expressed as means ± SE. All data were analyzed by the InStat Version 3 statistical software package (GraphPad Software, San Diego, CA) using separate ANOVA for glucose or glucose plus palmitate. When ANOVA indicated a significant overall effect, differences among individual means were assessed by using the Sidak test for multiple comparisons as described previously (60). P values <0.05 were considered statistically significant.

RESULTS

Cellular energetic stress is associated with the activation of AMPK (19), an event that is often demarcated by an increase in phosphorylation of AMPK at Ser172 (20). In the present study, there were no significant differences in AMPK content between hearts subjected to either ischemia or reperfusion and their respective controls (Fig. 1). Moreover, there was a significant increase in AMPK phosphorylation with ischemia in both glucose-perfused and glucose plus palmitate-perfused hearts compared with their respective controls. AMPK phosphorylation continued to be significantly greater than control values with reperfusion in glucose-perfused hearts but not in hearts reperfused with glucose plus palmitate.

Activation of the AMPK signaling pathway is associated with inhibition of translation initiation via phosphorylation of eEF2 at Thr56 (5, 23). As with phosphorylation of AMPK, phosphorylation of eEF2 in glucose-perfused controls was significantly greater than in glucose plus palmitate-perfused controls (Fig. 2), and there was a significant increase in eEF2 phosphorylation with ischemia in both glucose-perfused and glucose plus palmitate-perfused hearts compared with their respective controls. The increase in eEF2 phosphorylation observed with ischemia in both glucose-perfused and glucose plus palmitate-perfused hearts was reversed with reperfusion, because values in reperfused hearts did not differ significantly from their respective controls. Reperfusion did not appear to completely reverse ischemia-induced eEF2 phosphorylation in glucose-perfused hearts because there was a trend (P = 0.08).
for eEF2 phosphorylation to remain elevated compared with glucose-perfused control values. In contrast to the aforementioned changes in eEF2 phosphorylation, there were no significant differences in eEF2 content.

A second signaling pathway that regulates phosphorylation of eEF2 is the MAPK pathway. For example, enhanced signaling through the p38 MAPK results in the inhibition of eEF2 kinase and the subsequent dephosphorylation of eEF2 (29). Thus an alternative explanation for the changes in eEF2 phosphorylation observed in the present study could involve changes in p38 MAPK activity. To assess potential alterations in signaling through this pathway, the effect of oxidative substrate availability and I/R on the phosphorylation state of p38 MAPK was examined by Western blot analysis using phospho-specific antibodies. As shown in Fig. 3, ischemia significantly increased p38 MAPK phosphorylation in glucose-perfused hearts, and reperfusion reversed the effect. In contrast, there was no significant difference in p38 MAPK phosphorylation ischemic hearts compared with controls perfused with glucose plus palmitate. Moreover, there was no significant difference between control hearts perfused with glucose plus palmitate compared with hearts perfused with glucose alone.

Although less well-defined than its association with translation elongation, activation of AMPK is also associated with the inhibition of translation initiation (3, 23), a process that requires binding of initiator met-tRNAi to the 40S ribosomal subunit and recruitment of mRNA to the 40S ribosomal subunit.
Binding of mRNA to the 40S ribosomal subunit is mediated by the protein complex eIF4F, consisting of an RNA helicase, eIF4A, a protein that binds the 7-methyl-GTP cap structure at the 5’-end of most mRNAs, eIF4E, and eIF4G, a scaffolding protein that binds eIF4A and eIF4E (56). Under some translationally repressive conditions, eIF4F assembly is inhibited due to eIF4E being sequestered by 4E-BP1 (18). 4E-BP1 is unable to sequester eIF4E when it is itself highly phosphorylated (17), and, therefore, 4E-BP1 phosphorylation status was assessed in hearts subjected to I/R. As shown in Fig. 4A, there were no significant differences in 4E-BP1 phosphorylation between hearts subjected to either ischemia or reperfusion and their respective controls nor between the respective controls under basal conditions. However, the amount of 4E-BP1 coimmunoprecipitating with eIF4E under basal conditions was greater in glucose-perfused hearts than in glucose plus palmitate-perfused hearts (Fig. 4B). Moreover, the amount of 4E-BP1 bound to eIF4E was significantly increased with ischemia in hearts perfused with glucose plus palmitate, but there was no difference in the association of 4E-BP1 with eIF4E between ischemic and control hearts perfused with glucose. Reperfusion had no effect on the association of 4E-BP1 with eIF4E in glucose-perfused hearts. In contrast, the increased association of 4E-BP1 with eIF4E observed with ischemia in glucose plus palmitate-perfused hearts was abrogated following reperfusion as these values did not differ significantly from control values.

The amount of eIF4E in the cell is thought to be limiting for eIF4F assembly (14), and, as such, decreased eIF4E content may limit eIF4F complex assembly. However, there were no significant differences in eIF4E content between hearts subjected to either ischemia or reperfusion and their respective controls nor between the respective controls under basal conditions. Changes in eIF4E phosphorylation do not appear to modulate eIF4F complex assembly; however, eIF4E activity may be regulated through phosphorylation of eIF4E at Ser209 (43) because phosphorylation of this residue is associated with alterations in the ability of eIF4E to bind mRNA (41, 58). As shown in Fig. 5, there was no difference in phosphorylation of eIF4E at Ser209 between control hearts perfused with either glucose or glucose plus palmitate under basal conditions. Although eIF4E phosphorylation was unaltered by I/R in hearts perfused with glucose, it was significantly greater than control values in glucose plus palmitate-perfused hearts subjected to ischemia. Reperfusion appeared to abate the ischemia-induced increase in eIF4E phosphorylation in glucose plus palmitate-perfused hearts because values in reperfused hearts did not differ significantly from control values.

The other basic step of translation initiation, binding of met-tRNAi to the 40S ribosomal subunit, is dependent on the guanine nucleotide exchange activity of eIF2B (12, 39). Because eIF2B may be directly inhibited through phosphorylation of its ε-subunit (62), both the content and phosphorylation of eIF2Be were assessed in hearts subjected to I/R. As shown in Fig. 6A, there were no significant differences in eIF2Be content or phosphorylation between hearts subjected to either ischemia or reperfusion and their respective controls nor between the respective controls under basal conditions. eIF2B may also be inhibited through phosphorylation of its substrate, eIF2, on its α-subunit at Ser51 (59). As shown in Fig. 6B, phosphorylation of eIF2α was significantly greater in control hearts perfused with glucose than in glucose plus palmitate-perfused control hearts. However, the amount of eIF2B bound to eIF2α was significantly lower in glucose plus palmitate-perfused control hearts than in glucose-perfused control hearts. As shown in Fig. 6A, there was no difference in phosphorylation of eIF2B between hearts subjected to either ischemia or reperfusion and their respective controls nor between the respective controls under basal conditions.

The amount of eIF2B1i associated with eIF2B1i under basal conditions was greater in glucose-perfused hearts than in glucose plus palmitate-perfused hearts (Fig. 4B). Moreover, the amount of eIF2B1i bound to eIF2B1i was significantly increased with ischemia in hearts perfused with glucose plus palmitate, but there was no difference in the association of eIF2B1i with eIF2B1i between ischemic and control hearts perfused with glucose. Reperfusion had no effect on the association of eIF2B1i with eIF2B1i in glucose-perfused hearts. In contrast, the increased association of eIF2B1i with eIF2B1i observed with ischemia in glucose plus palmitate-perfused hearts was abrogated following reperfusion as these values did not differ significantly from control values.

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The amount of eIF2B1i associated with eIF2B1i under basal conditions was greater in glucose-perfused hearts than in glucose plus palmitate-perfused hearts (Fig. 4B). Moreover, the amount of eIF2B1i bound to eIF2B1i was significantly increased with ischemia in hearts perfused with glucose plus palmitate, but there was no difference in the association of eIF2B1i with eIF2B1i between ischemic and control hearts perfused with glucose. Reperfusion had no effect on the association of eIF2B1i with eIF2B1i in glucose-perfused hearts. In contrast, the increased association of eIF2B1i with eIF2B1i observed with ischemia in glucose plus palmitate-perfused hearts was abrogated following reperfusion as these values did not differ significantly from control values.

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The amount of eIF2B1i associated with eIF2B1i under basal conditions was greater in glucose-perfused hearts than in glucose plus palmitate-perfused hearts (Fig. 4B). Moreover, the amount of eIF2B1i bound to eIF2B1i was significantly increased with ischemia in hearts perfused with glucose plus palmitate, but there was no difference in the association of eIF2B1i with eIF2B1i between ischemic and control hearts perfused with glucose. Reperfusion had no effect on the association of eIF2B1i with eIF2B1i in glucose-perfused hearts. In contrast, the increased association of eIF2B1i with eIF2B1i observed with ischemia in glucose plus palmitate-perfused hearts was abrogated following reperfusion as these values did not differ significantly from control values.
with glucose compared with glucose plus palmitate-perfused controls. Although eIF2\alpha phosphorylation appeared to increase with I/R in hearts perfused with glucose, these changes were not statistically significant. In contrast, phosphorylation of eIF2\alpha was significantly greater than control values in glucose plus palmitate-perfused hearts subjected to ischemia. Phosphorylation of eIF2\alpha appeared to be less than ischemic values in glucose plus palmitate-perfused hearts subjected to reperfusion; however, these values were still greater than those in controls. In contrast to the aforementioned changes in eIF2\alpha phosphorylation, there were no significant differences in eIF2\alpha content.

**DISCUSSION**

During an ischemic episode, the delivery of oxygen and oxidative substrates to the affected tissue is compromised, leading to a potential deficit in the energy status of the tissue if sufficiently severe and/or prolonged. One potential mechanism through which the affected tissue may compensate for the reduced energy status is to repress energy-consuming metabolic processes that are not immediately required for cell survival. In this regard, one of the most energy-intensive metabolic processes occurring in most cells is protein synthesis. Because many proteins are relatively long-lived, a temporary reduction in their synthesis, especially if coupled with a decreased rate of protein degradation, should have little effect on cell viability.

In the present study, the finding that AMPK phosphorylation was increased in nonischemic hearts perfused with glucose alone compared with perfusion with glucose plus palmitate suggests that the AMP-to-ATP ratio was elevated under such conditions. However, previous studies (46–48) have shown that, under the conditions used in the present study, the AMP and ATP concentrations are the same in glucose-perfused hearts compared with hearts perfused with glucose plus palmitate.
tate. Instead, the concentration of phosphocreatine is reduced in hearts perfused with glucose alone compared with glucose plus palmitate. Because AMPK is allosterically inhibited by phosphocreatine (52), a reduction in phosphocreatine concentration in hearts perfused with glucose alone may explain the activation of AMPK observed in the present study. Moreover, with the exception of eIF4E, the same biomarkers altered in response to changes in substrate availability were changed during a transient ischemic episode. Thus it appears that cellular energy status represents an important modulator of I/R-induced changes in mRNA translation. As such, the observation that I/R had little effect on several biomarkers of translation in hearts perfused with glucose alone, whereas I/R had opposing effects on these biomarkers in hearts perfused with glucose plus palmitate, may be explained by differences in cellular energy status. For example, the observed changes in AMPK phosphorylation suggest that an energetic stress exists within glucose perfused hearts under basal, ischemic, and reperfused conditions relative to glucose plus palmitate-perfused hearts. In contrast, although ischemia culminates in energetic stress within glucose plus palmitate-perfused hearts, this stress is alleviated by reperfusion.

Although AMPK activity was not measured in the present study, the observed changes in AMPK phosphorylation suggest that AMPK activity returns to control levels in glucose plus palmitate-perfused hearts after reperfusion. This is in contrast to previously published results (32) wherein AMPK activity was elevated in the isolated heart following 1 h of reperfusion with glucose and palmitate. These differences are likely model specific because the previous study utilized no-flow ischemia and employed a working heart model. In the present study, hearts did not contract against a pressure gradient after ischemia. The lack of necessity to expend energy contracting against a pressure gradient may explain why an energetic stress, as demarcated by AMPK phosphorylation, did not persist during reperfusion.

In accordance with results from a recent study (22) employing the isolated perfused heart model, there was a significant increase in both AMPK and eEF2 phosphorylation with ischemia in glucose-perfused hearts. The phosphorylation of these proteins was also increased in glucose plus palmitate-perfused hearts subjected to ischemia. However, comparison of nonischemic controls revealed that phosphorylation of both AMPK and eEF2 was significantly greater in glucose-perfused hearts than in glucose plus palmitate-perfused hearts. These findings indicate that energetic stress, whether through removal of FFAs from the perfusate or through ischemia, modulates phosphorylation of eEF2 in the isolated perfused heart.

The increase in eEF2 and AMPK phosphorylation observed with ischemia in glucose plus palmitate-perfused hearts was abated with reperfusion. Interestingly, however, in glucose-perfused hearts subjected to reperfusion, eEF2 phosphorylation returned to basal levels despite the fact that AMPK phosphorylation remained elevated. This result indicates that the reperfusion-induced decrease in eEF2 phosphorylation observed in the present study is not due solely to inhibited AMPK signaling. Alternative means of decreasing eEF2 phosphorylation include direct dephosphorylation of eEF2 by protein phosphatase 2A or phosphorylation of eEF2 kinase on inhibitory serine residues (53). For example, the SAPK and MAPK have been implicated in the regulation of eEF2 activity. These proteins are activated in hearts subjected to I/R (30) and have recently been shown to phosphorylate eEF2 kinase (28, 29).

However, in contrast to phosphorylation by AMPK (4), phosphorylation by MAPK inhibits eEF2 kinase activity. In the present study, no changes in phosphorylation of the extracellular-regulated protein kinases (ERK)1 or ERK2 were observed (results not shown). However, p38 MAPK phosphorylation was increased by ischemia in hearts perfused with glucose alone, an effect that should inhibit, rather than stimulate, eEF2 kinase. Thus changes in p38 MAPK phosphorylation do not appear to correlate with the observed alterations in eEF2 phosphorylation. Future studies aimed at elucidating how SAPK and MAPK specifically affect mRNA translation during I/R are therefore necessary.

Although less dramatic than the observed changes in eEF2, biomarkers of translation initiation were also altered in the present study (summarized in Fig. 7). It has been demonstrated previously that activation of AMPK correlates with dissociation of 4E-BP1 from eIF4E (3). The best characterized mechanism through which the association of eIF4E with 4E-BP1 is regulated involves phosphorylation of 4E-BP1, whereby hyperphosphorylated 4E-BP1 does not bind to eIF4E but unphosphorylated and hypophosphorylated forms do. For example, phosphorylation of 4E-BP1 is increased, indicative of enhanced translation initiation rates, in primary rat neonatal cardiomyocytes exposed to simulated ischemia for 1 h followed by 30 min of reperfusion (42). However, in the present study, the observed changes in 4E-BP1 binding to eIF4E occurred without a significant change in the proportion of 4E-BP1 present in the hyperphosphorylated γ-form. A similar finding was reported in a study examining the effect of ischemia on PC12 cells in culture (35). In that study, it was suggested that 4E-BP1 phosphorylation does not fully account for the observed changes in 4E-BP1-eIF4E complex formation. A caveat to the conclusion that 4E-BP1 phosphorylation was not altered in the present study is that 4E-BP1 can be phosphorylated on a multitude of sites, and phosphorylation of some does not lead to a change in migration during SDS-PAGE (45). Thus it is possible that phosphorylation of 4E-BP1 on specific residues was altered by perfusion with glucose alone or ischemia but that such changes were not detected as a change in migration during electrophoresis.

The mRNA binding step in mRNA translation is also regulated through changes in eIF4E phosphorylation (Fig. 7). Although there is no known correlation between AMPK and phosphorylation of eIF4E, activation of the MAPK signaling pathway, which has been shown to affect eIF4E phosphorylation (38), has been observed in glucose-perfused hearts subjected to ischemia (2, 30). Therefore, ischemia-induced MAPK activation likely contributes to the changes in eIF4E phosphorylation observed in the present study. Unresolved, however, is why changes in eIF4E phosphorylation were not observed in glucose-perfused hearts. A possible explanation is that the eIF4E kinase, MAPK-integrating kinase (MNK), binds to eIF4G, and phosphorylation of eIF4E is thought to occur in a eIF4E-eIF4G-MNK complex (57). Because eIF4E binding to 4E-BP1 was unaffected by either ischemia or reperfusion, it is likely that formation of the eIF4E-eIF4G-MNK complex was also unaltered and might therefore account for the lack of change in eIF4E phosphorylation.
Another biomarker of translation initiation that was altered in the present study was eIF2. In hearts perfused with glucose alone, phosphorylation of the α-subunit of eIF2 was greater than in hearts perfused with glucose plus palmitate. Phosphorylation of eIF2α was also increased during ischemia in hearts perfused with glucose plus palmitate but not in hearts perfused with glucose alone. The lack of change in response to ischemia in hearts perfused with glucose alone may have been a result of eIF2α phosphorylation already being elevated due to lack of FFA. Thus, although there was a tendency for eIF2α phosphorylation to increase in ischemic hearts perfused with glucose alone, the change was not statistically significant. Interestingly, previous studies (6, 7) have found no correlation between the activation of AMPK and phosphorylation of eIF2α; however, a possible mechanism through which eIF2α phosphorylation might increase both in hearts perfused with glucose alone and in ischemic hearts perfused with glucose plus palmitate is activation of the eIF2α kinase protein kinase R-like endoplasmic reticulum-associated protein kinase (PERK). PERK is responsive to changes in cellular redox status (1), and changes in fat oxidation influence cellular redox status in the heart (31). Moreover, PERK is activated and eIF2α phosphorylation is increased in brain in response to I/R (33).

Many of the changes in biomarkers of mRNA translation observed in the present study would be expected to limit global rates of protein synthesis. Indeed, rates of protein synthesis are...
diminished in hearts subjected to ischemia (25). Based on the larger increase in eEF2 phosphorylation observed in ischemic hearts, it might be expected that mRNA translation would be inhibited at the elongation step by ischemia. In addition, because in many cell types phosphorylation of only a small proportion of eIF2 results in a large reduction in translation initiation (10), a second potential mechanism through which protein synthesis might be regulated by I/R involves phosphorylation of eIF2α (21). However, phosphorylation of eIF2α also promotes the preferential translation of a subset of mRNAs that contain multiple upstream open reading frames in their 5′-noncoding regions (38, 44). Such mRNAs include certain transcription factors and proteins involved in recovery from cell stress. In addition, recent studies (13, 37) indicate that translation of mRNAs through a 7-methyl-GTP cap-independent process, as well as the translation of highly structured mRNAs, increases parallel to the phosphorylation of eIF4E. Interestingly, these mRNAs often encode growth-promoting and stress-response proteins, suggesting that phosphorylation of eIF4E may represent a mechanism whereby cells respond to I/R-induced cell damage. Overall, the changes in translational control reported herein are predicted to limit protein synthetic rates so as to minimize energy expenditure while maintaining the synthesis of proteins required to maintain cellular homeostasis during times of energetic insufficiency. It should be noted, however, that some of the observed changes, e.g., changes in the association of 4E-BP1 with eIF4E, were moderate, and given the complexity of the regulation of mRNA translation, it is not possible at this time to determine how great of an effect the observed changes had on protein expression in this model. Future studies will be required to elucidate whether the observed changes in translational control have a significant effect on protein expression and to assess whether these changes have a functional consequence in the reperfused heart.

In conclusion, the results presented herein demonstrate that several regulatory mechanisms of mRNA translation in the isolated perfused heart are affected by I/R (Fig. 7). Thus we hypothesize that mRNA translation plays an important role in I/R-induced alterations in protein expression in the heart. Moreover, we hypothesize that I/R-induced changes in mRNA and protein expression will be affected by substrate availability at the time of I/R due to their effects on cellular energy status as well as the activation of MAPK- and PERK-dependent signaling pathways. Testing these hypotheses in vivo will be an important area of future research as I/R-induced changes in protein expression can affect the heart’s recovery from I/R-induced injury.

ACKNOWLEDGMENTS

The authors thank Sharon Rannels, Gina Deiter, and Heather Hubler for technical assistance.

GRANTS

This research was supported by National Institutes of Health Grants DK-15658 (to L. S. Jefferson), AA-12814 (to T.C. Vary), and GM-39277 (to T. C. Vary). S. J. Crozier was the recipient of an American Heart Association Pennsylvania-affiliate predoctoral fellowship.

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