Effect of sepsis on eIF4E availability in skeletal muscle

THOMAS C. VARY AND SCOT R. KIMBALL
Department of Cellular and Molecular Physiology, The Pennsylvania State University
College of Medicine, Hershey, Pennsylvania 17033

Received 9 February 2000; accepted in final form 8 June 2000

Vary, Thomas C., and Scot R. Kimball. Effect of sepsis on eIF4E availability in skeletal muscle. Am J Physiol Endocrinol Metab 279: E1178–E1184, 2000.—Chronic septic abscess formation causes an inhibition of protein synthesis in gastrocnemius that is not observed in rats with a sterile abscess. The inhibition is associated with an impaired translation initiation. The present study was designed to investigate the effects of sepsis on phosphorylation and availability of eukaryotic initiation factor (eIF4E) in gastrocnemius 5 days after induction of a sterile or septic abscess. Neither sepsis nor sterile inflammation altered the extent of eIF4E phosphorylation. Moreover, no changes in the amount of the binding protein 4E-BP1 associated with eIF4E or in the phosphorylation of 4E-BP1 were observed during sepsis or sterile inflammation. In contrast, sepsis and sterile inflammation caused a reduction in the relative amount of eIF4G bound to eIF4E compared with controls. The diminished amount of eIF4G bound to eIF4E was not the result of a reduced abundance of eIF4E. Sepsis, but not sterile inflammation, caused an increase in the cellular abundance of eIF4E. The results provide evidence that alterations in the eIF4E system are probably not rate controlling for the synthesis of total, mixed proteins in gastrocnemius during sepsis. Instead, on the basis of our previous studies, changes in eIF2B appear to be responsible for limiting protein synthesis in skeletal muscle during sepsis.

SEPSIS INDUCES PROFOUND ALTERATIONS in whole body protein metabolism. Marked weight loss and accelerated nitrogen excretion characterize the host’s response to infection. Nitrogen losses equivalent to 5–17% of body protein are commonly observed in septic patients despite adequate nutritional support. Much of this nitrogen loss occurs secondary to net catabolism of skeletal muscle protein. Muscle protein wasting in sepsis results from a global decrease in protein synthesis and an increase in protein degradation (for review see Refs. 1 and 29). In contrast, chronic (5 days) inflammation induced by a sterile intra-abdominal abscess does not result in diminished protein content (36) or inhibition of protein synthesis in skeletal muscle (2, 32).

The sepsis-induced inhibition of protein synthesis results from a defect in translational efficiency (7, 30, 32), rather than changes in total mRNA (14, 33) or the number of ribosomes (32). The translational efficiency reflects how well the existing protein synthesis machinery functions and is related to the activity and/or amount of the components involved in the process of protein synthesis. Translational efficiency during sepsis is diminished secondary to a defect in translation initiation rather than reductions in elongation (32). The process of peptide-chain initiation involves essentially four major steps (for review see Refs. 12 and 29): 1) dissociation of the 80S ribosome into 40S and 60S ribosomal subunits, 2) formation of the 43S preinitiation complex with binding of initiator met-tRNA\textsuperscript{met} to the 40S subunit, 3) binding of mRNA to the 43S preinitiation complex, and 4) association of the 60S ribosomal subunit to form an active 80S ribosome.

Two of the steps involved in translation initiation appear important as major regulatory points in the overall control of protein synthesis in vivo. The first step controlling initiation is the binding of met-tRNA\textsuperscript{met} to the 40S ribosomal subunit to form the 43S preinitiation complex. This reaction is mediated by eukaryotic initiation factor (eIF) 2 and is regulated by the activity of another initiation factor, eIF2B. We previously reported that the formation of the 43S preinitiation complex is markedly reduced in skeletal muscle during sepsis (31). Furthermore, we showed that decreased formation of the 43S preinitiation complex is associated with a diminished activity and expression of eIF2B (30, 31, 38), suggesting that reductions in eIF2B function may cause this effect.

The second regulatory step in translation initiation involves the binding of mRNA to the 43S preinitiation complex, which is mediated by eIF4F, a complex of several subunits. One of the subunits, eIF4E, binds the 7-methylguanosine 5’-triphosphate (m\textsuperscript{7}GTP) cap structure present at the 5’-end of eukaryotic mRNAs to form an eIF4E-mRNA complex (22). During translation initiation, the eIF4E-mRNA complex binds to eIF4G and eIF4A to form the active eIF4F complex (22, 23, 26). Formation of the active eIF4F complex allows initia-
tion to proceed. The binding of eIF4E to eIF4G is controlled in part by the translation repressor proteins 4E-BP1, 4E-BP2, and 4E-BP3. Of these proteins, the binding protein 4E-BP1 is the predominant form in skeletal muscle. Binding of 4E-BP1 to eIF4E is hypothesized to limit eIF4E availability for formation of the active eIF4E-eIF4G complex. The binding of 4E-BP1 to eIF4E is regulated, in part, by phosphorylation of 4E-BP1 (for review see Ref. 4).

Despite the importance of eIF4E in controlling translation initiation, there is no information regarding the effects of sepsis on the regulation of eIF4E phosphorylation or availability. The purpose of the present set of experiments was to ascertain whether the eIF4E system is affected in gastrocnemius from septic rats. Studies described here also compare and contrast the effects of a nonseptic vs. a septic abscess of 5 days duration on the regulation of eIF4E to establish the differences between inflammatory and bacterial insults. By comparing the changes in eIF4E between sterile and abscess conditions, the effects resulting from abscess formation per se can be separated from those in chronic infection.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats weighing 150–225 g were maintained on a 12:12-h light-dark cycle and were fed ad libitum. Chronic abdominal sepsis was created by implantation of a fecal-agar pellet (1.5 ml) inoculated with 10⁹ colony-forming units of Escherichia coli and 10⁷ colony-forming units of Bacteroides fragilis into the peritoneal cavity, as previously described (7, 29–33, 38). Replacing the bacterial inoculum with an equal volume of sterile saline (2, 7, 29, 32) generated the nonseptic inflammation abscess. After recovery from the surgical procedures, the intra-abdominal abscess was allowed to develop for 5 days.

Septic and nonseptic inflammatory animals develop an abdominal abscess and show leukocytosis; however, the magnitude of the leukocytosis is greater in the septic animals (35). Bacteremia is not observed in nonseptic abscess or control rats (35). Introduction of the infected fecal-agar pellet results in a hyperdynamic, hypermetabolic septic condition, which is not observed in rats with the nonseptic abscess (6, 29–33, 38). Laparotomy was not performed in control rats, because laparotomy itself does not reduce protein content or synthesis in gastrocnemius after 48 h (20) or 5 days (7, 32). We previously established that nonseptic and septic rats have reduced food intake in the first 2 days after surgery compared with rats fed ad libitum (2, 34). Thereafter, sterile inflammatory and septic rats consume the same amount of food on a daily basis as rats fed ad libitum. Furthermore, there were no significant differences in food intake between the sterile inflammatory and septic rats over the course of abscess formation. Control animals were pair fed to match food intake in nonseptic and septic rats to account for any differences due to reduced food intake during the first 48 h after implantation of the fecal-agar pellet.

Five days after implantation of the fecal-agar pellet, animals were anesthetized with pentobarbital sodium and the gastrocnemius was excised, weighed, and homogenized in seven volumes of buffer A (20 mM HEPES, pH 7.4, 100 mM KCl, 0.2 mM EDTA, 2 mM EGTA, 1 mM dithiothreitol, 50 mM NaF, 50 mM β-glycerophosphate, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 0.5 mM sodium vanadate, and 1 μM microcystin LR) using a Polytron homogenizer. The homogenate was centrifuged at 10,000 g for 10 min at 4°C, and the pellet was discarded. The supernatant was used to evaluate the regulation of the eIF4E system (see below).

Quantification of 4E-BP1-eIF4E and eIF4G-eIF4E complexes. The association of eIF4E with 4E-BP1 and eIF4E was determined in gastrocnemius with the use of immunoblot techniques, as previously described in our laboratory (11, 27). eIF4E, as well as 4E-BP1-eIF4E and eIF4G-eIF4E complexes, was immunoprecipitated from aliquots of 10,000-g supernatants by the use of an anti-eIF4E monoclonal antibody. The antibody-antigen complex was collected by incubation for 1 h with BioMag goat anti-mouse IgG beads (PerSeptive Biosystems, Framingham, MA). Before use, the beads were washed in 1% nonfat dry milk in buffer B (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.1% β-mercaptoethanol, 0.5% Triton X-100, 50 mM NaF, 50 mM β-glycerophosphate, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 1 mM sodium vanadate). The beads were captured using a magnetic sample rack and washed twice with buffer B and once with buffer B containing 500 mM, rather than 150 mM, NaCl. Resuspending in SDS sample buffer and boiling for 5 min eluted protein bound to the beads. The beads were collected by centrifugation, and the supernatants were subjected to electrophoresis on a 7.5% polyacrylamide gel for quantitation of eIF4E or on a 15% polyacrylamide gel for quantitation of 4E-BP1 and eIF4E. Proteins were then electrophoretically transferred to a nitrocellulose membrane. The membranes were incubated with a mouse anti-human eIF4E antibody, a rabbit anti-rat 4E-BP1 antibody, or a rabbit anti-eIF4G antibody for 1 h at room temperature. The blots were then developed using enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ). The blots were exposed to X-ray film in a cassette equipped with Du Pont Lightning Plus intensifying screen. After development, the film was scanned (Microtek Scanner TW-9400, 600 dpi, biplane Maker IV) and quantitated using NIH Image 1.6 software.

Determination of the phosphorylation state of eIF4E. Phosphorylated and unphosphorylated forms of eIF4E in the 10,000-g supernatant of gastrocnemius were separated by isoelectric focusing on a slab gel and quantitated by protein immunoblot analysis, as previously described (11, 27). The proportion of eIF4E present in the phosphorylated state was measured by densitometric scanning of the films and is expressed as a percentage of the total eIF4E content (i.e., phosphorylated + unphosphorylated).

Determination of phosphorylation state of 4E-BP1. The phosphorylated forms of 4E-BP1 were measured after immunoprecipitation of 4E-BP1 from muscle homogenates after centrifugation at 10,000 g (11). 4E-BP1 was immunoprecipitated as described above for immunoprecipitation of eIF4E. The various phosphorylated forms of 4E-BP1 (designated α, β, and γ) were separated by SDS-PAGE and quantitated by protein immunoblot analysis, as described previously (9–11).

Statistical analysis. Values are means ± SE. Statistical evaluation of the data was performed using ANOVA to test for overall differences among groups, followed by the Bonferroni test for multiple comparisons to determine significance between means when ANOVA indicated a significant difference among the group means. Differences among the means were considered significant at P < 0.05.

RESULTS

In the present study, the possible role of the eIF4E system in limiting protein synthesis during sepsis was...
evaluated. One mechanism for modulating eIF4E function involves its relative distribution between active (eIF4G-eIF4E) and inactive (4E-BP1-eIF4E) complexes. To investigate the effects of sepsis on the association of eIF4G with eIF4E, eIF4E immunoprecipitates were used to measure the amount of eIF4G that coprecipitates with eIF4E (Fig. 1). Development of a sterile inflammatory abscess resulted in an 80% decrease in the amount of eIF4G bound to eIF4E. Similarly, sepsis also significantly decreased the amount of eIF4G associated with eIF4E compared with control rats. There were no significant differences in the abundance of eIF4G bound to eIF4E between the sterile inflammatory and septic conditions.

It has been postulated that availability of eIF4E for binding to eIF4G can be modulated by its association with the translation repressor, 4E-BP1. The binding of 4E-BP1 to eIF4E is controlled by its phosphorylation state. Under a variety of conditions, 4E-BP1 undergoes multiple phosphorylations, which are characterized by reduced mobility during electrophoresis. 4E-BP1 is resolved into multiple electrophoretic forms, termed α, β, and γ, representing differentially phosphorylated forms of the protein (13, 19). The most highly phosphorylated form of the protein, 4E-BP1γ, does not bind eIF4E (13, 19). Neither sterile inflammation nor sepsis affected the abundance of 4E-BP1 in the γ-form in skeletal muscle (Fig. 2).

Even though phosphorylation of 4E-BP1 was unaffected in any of the conditions examined, it remained a possibility that sterile inflammation or sepsis modulated the association of 4E-BP1 with eIF4E. To investigate this possibility, eIF4E immunoprecipitates were analyzed for 4E-BP1 content (Fig. 3). The relative amount of 4E-BP1 was normalized by dividing the values for 4E-BP1 by the amount of eIF4E present in the immunoprecipitate. Neither sterile inflammation nor sepsis affected the abundance of 4E-BP1 in the γ-form in skeletal muscle (Fig. 2).

To further define potential mechanisms responsible for the sepsis-induced inhibition of protein synthesis, the effect of sepsis on phosphorylation of eIF4E was examined (Fig. 4). In these studies, slab gel isoelectric focusing was used to separate phosphorylated and non-phosphorylated forms of eIF4E followed by immuno-
blot analysis to quantitate the amount of eIF4E in the two forms. In muscles from control rats, ~60% of eIF4E was in the phosphorylated form. Neither sterile abscess formation nor sepsis significantly affected the proportion of eIF4E in the phosphorylated form.

Finally, reductions in cellular content of eIF4E can lead to an inhibition of protein synthesis. Therefore, we examined whether sepsis altered the muscle content of eIF4E (Fig. 5). No significant differences between control and sterile inflammation were observed. Unexpectedly, muscle eIF4E content was significantly increased approximately twofold during sepsis.

DISCUSSION

We previously established that chronic (5 days) sepsis causes a reduction in global rates of protein synthesis in muscles composed primarily of fast-twitch fibers (e.g., gastrocnemius or psoas), but not soleus (7, 29, 32, 33). However, in contrast to sepsis, chronic (5 days) inflammation induced by sterile abscess formation does not result in any diminution in protein synthesis in skeletal muscle (2, 32). The inhibition in skeletal muscle protein synthesis observed during sepsis is associated with a decreased translation initiation. We have already established that a diminished eIF2B activity and expression is in part responsible for the reduced translation initiation during sepsis (30, 31, 37, 38).

In the present study, we examine the possibility that protein synthesis in skeletal muscle of septic rats is subject to additional regulatory control through modulation of eIF4F function. In cells in culture, overexpression of eIF4E results in a stimulation of global protein synthesis and also causes transformation (reviewed in Refs. 5 and 21). Coexpression of 4E-BP1 reverses the transformed phenotype (24), providing evidence that eIF4E is directly involved in the transformation process. In the present study, muscle eIF4E content was found to be twofold greater in septic animals than in controls. However, in contrast to cells in culture overexpressing eIF4E, global protein synthesis was not increased but instead was decreased. One possible explanation for this apparent discrepancy is that eIF4E is the least abundant initiation factor, and a twofold increase in eIF4E expression may be insufficient to foster an increase in global rates of synthesis; i.e., the content of eIF4E binding proteins in skeletal muscle may be adequate to dampen the effect of the
increase in eIF4E expression. This possibility is supported by the observation that the relative amount of 4E-BP1 bound to eIF4E (i.e., the 4E-BP1-to-eIF4E ratio) is the same in muscle of septic and control rats (Fig. 3). A second possible explanation is that the amount of eIF4G may be limiting in skeletal muscle. Because it is the eIF4G·eIF4E complex that is active in binding mRNA to the 40S ribosomal subunit, there may be insufficient eIF4G to bind to the increased eIF4E present in muscle of septic rats.

In addition to absolute changes in eIF4E content, functionally, eIF4E availability can be regulated through its association with a family of small, acid- and heat-stable proteins, termed 4E-BP1, 4E-BP2, and 4E-BP3, which results in the formation of an inactive complex (13, 19). In rat skeletal muscle, the predominant form of these translation repressor proteins is 4E-BP1, which undergoes reversible phosphorylation. The interaction between 4E-BP1 and eIF4E is regulated by the extent of 4E-BP1 phosphorylation. Phosphorylation of 4E-BP1 releases eIF4E from the 4E-BP1·eIF4E complex and allows the eIF4E·mRNA complex to bind to eIF4G and then to the 40S ribosome (13). Refeeding of starved rats or insulin treatment of diabetic rats increases the phosphorylation of 4E-BP1, causing a dissociation of the 4E-BP1·eIF4E complex, thereby promoting translation initiation (10, 11, 27). In perfused skeletal muscle, stimulation of protein synthesis in response to acute insulin and insulin-like growth factor I administration is associated with a 12- and a 3-fold increase in the amount of eIF4G bound to eIF4E, respectively (11, 28). In the present set of experiments, neither sterile inflammation nor sepsis influenced the phosphorylation of 4E-BP1. In agreement with this observation, neither sterile inflammation nor sepsis altered the relative abundance of 4E-BP1 associated with eIF4E. However, because eIF4E content doubles during sepsis, the absolute amount of 4E-BP1 bound to eIF4E is higher in muscle from septic animals than from controls (see below).

In contrast to the binding of 4E-BP1 to eIF4E, the relative abundance of the eIF4G·eIF4E complex was significantly diminished in skeletal muscle from septic rats. However, because eIF4E content was increased twofold during sepsis, the actual amount of eIF4G present in the eIF4G·eIF4E complex was greater in muscles from septic rats than in muscles from with sterile inflammatory animals and was not appreciably different from controls. In defining the functional consequences of the changes in eIF4E content and distribution observed in muscle from septic animals, it is important to remember that not only does the eIF4G·eIF4E complex bind to the m7GTP cap on mRNA, but so does free eIF4E and the 4E-BP1·eIF4E complex. mRNA associated with free eIF4E or the 4E-BP1·eIF4E complex cannot bind to the 40S ribosome and is not actively translated. Hence, it is the relative amount of eIF4E bound to eIF4G that is important in controlling mRNA binding to the 40S ribosomal subunit. Because the ratio of eIF4G to eIF4E (Fig. 1) and the ratio of 4E-BP1 to eIF4E (Fig. 3) were not significantly different between sterile inflammatory and septic conditions, the distribution of eIF4E between active and inactive forms is similar in both conditions and significantly less than control values. However, rates of protein synthesis are only reduced in gastrocnemius from septic rats compared with sterile inflammatory animals, yet the proportion of eIF4E in the active eIF4G·eIF4E complex is reduced to a similar extent in both conditions. Thus it can be concluded that reduced eIF4E availability does not necessarily account for the inhibition of global rates of protein synthesis during sepsis.

At least two other studies have reported dissociation between eIF4E binding to eIF4G and global rates of protein synthesis. In L6 myoblasts deprived of amino acids, changes in the amount of eIF4E bound to eIF4G were not related to the diminished rates of global protein synthesis (8). Likewise, heat shock may or may not limit global protein synthesis through changes in eIF4E binding to eIF4G, depending on the particular cells examined (25). On the basis of these previous studies in cell culture systems (8, 25) and the present set of experiments conducted with skeletal muscle in vivo, it would appear that decreasing eIF4E availability does not seem to be a general mechanism to limit the overall global synthesis of cellular proteins during
sepsis. Instead, an enhanced formation of the eIF4G- eIF4E complex in cells in culture results in the preferential stimulation in the synthesis of specific proteins (e.g., ornithine decarboxylase, Myc) encoded by mRNAs containing a highly structured 5'-untranslated region. Hence, the changes in eIF4E binding to eIF4G may be associated with altered translation of specific mRNAs during sepsis and inflammation rather than changes in the global rate of protein synthesis.

Phosphorylation of eIF4E enhances the affinity of the factor for m7GTP cap analogs of mRNA (15) and for eIF4G and eIF4A (16). These effects may contribute to activation of mRNA translation initiation. In this regard, increased phosphorylation of eIF4E correlates with enhanced rates of protein synthesis in cells in culture stimulated with mitogens, growth factors, or serum (17, 18, 22) or transformed with ras or src oncogenes (3, 26). However, increased phosphorylation of eIF4E does not always correlate with enhanced rates of protein synthesis (11, 23). In the present study, phosphorylation of eIF4E was not significantly reduced during sepsis. Therefore, it is unlikely that the inhibition of protein synthesis during sepsis occurred through decreased phosphorylation of eIF4E.

In summary, the results of the present experiments provide evidence that sepsis modulates the eIF4E system in gastrocnenmsus. The primary effects of sepsis on the eIF4E system are a diminished relative binding of eIF4E to eIF4G and an increased muscle content of eIF4E protein. The effect of sepsis on eIF4E binding to eIF4G was most likely caused by the abscess formation per se, inasmuch as a similar reduction in this parameter was observed in animals with a sterile abscess. The reduced binding of eIF4E to eIF4G was not accompanied by a corresponding increase in the amount of eIF4E bound to the translation initiation repressor protein 4E-BP1 in sterile inflammation or sepsis. In agreement with this observation, the phosphorylation state of 4E-BP1 was not altered during sepsis. Because global rates of protein synthesis in sterile inflammation and sepsis differ, it is unlikely that changes in eIF4E binding to eIF4G represent the primary derangement leading to the inhibition of protein synthesis in skeletal muscle during sepsis, but these changes may be important in modulating the translation of individual proteins. Studies are underway to assess the role of altered eIF4E binding to eIF4G on the translation of specific proteins during sterile inflammation and sepsis.

The authors are grateful for the outstanding technical support provided by Rebecca Eckman, Sharon Rannels, and Gina Deiter.

This work was supported by National Institutes of Health Grants GM-39277 (T. C. Vary) and DK-15658 (S. R. Kimball) and by a Research Grant (S. R. Kimball) awarded by the American Diabetes Association.

REFERENCES

2. Cooney R, Owens E, Jurasinski C, Gray K, Vannice J, and Vary T. Interleukin-1 receptor antagonist prevents sepsis-in-


