Inhibition of mammalian translation initiation by volatile anesthetics

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Volatile anesthetics are essential for modern medical practice, but sites and mechanisms of action for any of their numerous cellular effects remain largely unknown. Previous studies with yeast showed that volatile anesthetics induce nutrient-dependent inhibition of growth through mechanisms involving inhibition of mRNA translation. Studies herein show that the volatile anesthetic halothane inhibits protein synthesis in perfused rat liver at doses ranging from 2 to 6%. A marked disaggregation of polysomes occurs, indicating that inhibition of translation initiation plays a key role. Dose- and time-dependent alterations that decrease the function of a variety of translation initiation processes are observed. At 6% halothane, a rapid and persistent increase in phosphorylation of the α-subunit of eukaryotic translation initiation factor 2 occurs. This is accompanied by inhibition of activity of the guanine nucleotide exchange factor eIF2B that is responsible for GDP-GTP exchange on eIF2. At lower doses, neither eIF2α phosphorylation nor eIF2B activity is altered. After extended exposure to 6% halothane, alterations in two separate responses regulated by the target of rapamycin pathway occur: 1) redistribution of eIF4E from its translation-stimulatory association with eIF4G to its translation-inactive target of rapamycin pathway occur: phosphorylation nor eIF2B activity is altered. After extended exposure to 6% halothane, alterations in two separate responses regulated by the target of rapamycin pathway occur: 1) redistribution of eIF4E from its translation-stimulatory association with eIF4G to its translation-inactive complex with eIF4E-binding protein-1; and 2) decreased phosphorylation of ribosomal protein S6 (rpS6) with a corresponding decrease in active forms of a kinase that phosphorylates rpS6 (p70S6K). Changes in the association of eIF4E and eIF4G are observed only after extended exposure to low anesthetic doses. Thus dose- and time-dependent alterations in multiple processes permit liver cells to adapt translation to variable degrees and duration of stress imposed by anesthetic exposure.

halothane; eukaryotic translation initiation factor 2α phosphorylation; mammalian target of rapamycin pathway

Although volatile anesthetics revolutionized medical practice when introduced in 1846, the mechanisms of action responsible for the physiological effects of these drugs remain essentially unknown. In addition to affecting cells of the central nervous system, these drugs affect all cells, tissues, and organs examined (4, 26, 36). Molecular genetic studies with the small, relatively simple eukaryote Saccharomyces cerevisiae (yeast) provide an opportunity for gaining insight regarding physiologically relevant effects of these drugs (28) and generating hypotheses that are testable in more complex eukaryotes. Extensive similarities exist between the activity of these drugs in mammals and simpler eukaryotes, such as yeast, suggesting conservation of cellular mechanisms responsible for the responses (28, 34, 45). These similarities include rapid and reversible induction of responses, a sharp dose-response curve, correlation between the lipophilicity of various anesthetics and their potency for inducing responses (termed the Meyer-Overton relationship), additivity of doses of different anesthetics in producing effects, and lack of effect in yeast of volatile lipophilic compounds that do not induce anesthesia in mammals (termed nonimmobilizers).

Results from yeast show that the volatile anesthetic isoflurane affects availability of certain critical amino acids, such as leucine and tryptophan (38), and is accompanied by inhibition of both cell division and translation initiation in strains auxotrophic for these nutrients (28, 37). Results from yeast suggest that a variety of volatile anesthetics, including halothane, enflurane, methoxyflurane, and sevoflurane, act by the same or very similar mechanisms of action being elucidated for isoflurane. Results supporting this statement include finding that all these drugs rapidly and reversibly arrest yeast growth (28) and that all mutants tested respond similarly to all five anesthetics (28, 46).

Volatile anesthetics, including halothane, isoflurane, enflurane, sevoflurane, and desflurane, have also been shown to inhibit protein synthesis in larger eukaryotes. This has been reported in a number of different systems, including intact animals (23–25), perfused organs (10, 40, 41), tissue slices in culture (15–18), and cells in culture (3, 6, 8, 21, 35). Studies regarding effects of volatile anesthetics on protein synthesis in perfused rat liver, the system employed in the work reported here, demonstrate that halothane exposure produces a rapid and dose-dependent inhibition of translation initiation affecting synthesis of both secreted and retained proteins (10, 12). Cellular mechanisms responsible for inhibition of translation induced by volatile anesthetics in any of the larger eukaryotic systems remain unknown. Halothane, the anesthetic whose activity in inhibiting protein synthesis in perfused liver has been best characterized (10, 12), is a halogenated hydrocarbon. Clinically, concentrations of halothane up to 5% are used in children for induction of anesthesia (7, 19) or for endotracheal intubation without muscle relaxants (20), whereas concentrations up to 2% can be used to maintain anesthesia (9).

In the present study, halothane was found to induce post-translational modification of translation initiation factors regulated by multiple signaling pathways. The alterations identified are consistent with the observed decrease in translation initiation. The various modifications occur in a time- and dose-dependent pattern, indicating that liver cells adapt their translational response to the degree and duration of stress imposed.

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MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River Breeding Laboratories) weighing ~125–145 g were maintained on a 12:12-h light-dark cycle and were provided food (Harlan-Teklad Rodent Chow) and water ad libitum. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine.

Liver perfusions. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 μg/g body wt; Abbott Laboratories) and were prepared for in situ liver perfusion as previously described (10), with the following modifications: halothane without thymol as a preservative (Halocarbon Laboratories) was vaporized in a mixture of 95% O2 and 5% CO2 with the use of a Fluotec 3 vaporizer and delivered to the perfusion apparatus at a flow rate of 5.5 l/min; the perfusate was preequilibrated with the halothane gas mixture for ~45 min before the start of the perfusion. In addition, all 20 amino acids were added to the medium at 2.5 times the normal plasma levels in rats (44). This level of amino acids is required to partially activate positive translation regulatory factors (e.g., p70S6K), thus permitting examination of the effect of the translation inhibitory activity of halothane on these factors.

Measurement of liver protein synthesis. After an initial 45-min perfusion with nonradioactive medium, perfusate containing 1-[3H]valine (1.25 × 10−4 μCi/ml; Amersham Biosciences) was delivered for 15 min. A portion of liver was then removed, immediately frozen between aluminum blocks precooled in liquid nitrogen, and stored at −80°C. Incorporation of 1-[3H]valine into perchloric acid-precipitable protein was determined as described previously (32, 43).

Polysome profiles. Polysomes were isolated and analyzed by sucrose density gradient (SDG) centrifugation by slight modification of a previously described procedure (11). Briefly, a freshly excised liver sample was homogenized with a Dounce homogenizer in 3 vol of SDG buffer (20 mM HEPES, pH 7.5, 100 mM KCl, 5 mM MgCl2) per gram of liver. The homogenate was centrifuged at 3,000 g for 10 min at 4°C. One volume of detergent mix (10% Triton X-100 and 10% sodium deoxycholate) was added to 9 vol of supernatant. A 500-μl aliquot of this mixture was layered onto a 20–48% SDG formed by alternately adding and freezing (in liquid nitrogen) 1.45-m1 aliquots of 20, 24, 28, 32, 36, 40, 44, and 48% sucrose in SDG buffer. Before use, gradients were thawed overnight at 4°C. Gradients were centrifuged at 40,000 rpm for 110 min at 4°C using a Beckman SW41 rotor. After centrifugation, gradients were fractionated, and the A260 was continuously recorded using an Isco gradient fractionator (Instrumentation Specialties).

Western blotting. Sample preparation for immunoblot analysis was performed as described previously (33). Briefly, freshly excised liver samples were homogenized in 7 vol of ice-cold homogenization buffer (20 mM HEPES, pH 7.4, 2 mM EDTA, 50 mM NaF, 100 mM KCl, 0.2 mM EDTA, 50 mM β-glycerophosphate, 1 mM DTT, 1 mM benzamidine, 0.5 mM sodium vanadate, 1× protease inhibitor cocktail) per gram of liver with the use of a Polytron homogenizer. Homogenates were centrifuged at 1,000 g for 3 min at 4°C before conducting the assays.

Statistical analysis. Values are presented as means ± SE. The number of rat livers per group (n) is indicated in the figure legends. When appropriate, data were analyzed by a one-way analysis of variance followed by a Tukey-Kramer multiple comparisons test or Student’s t-test using Graphpad Instat 3.0 software with statistical significance set at P < 0.05.

RESULTS

Initially, we established conditions that produced the previously reported inhibition of protein synthesis by halothane in perfused liver (10). On the basis of incorporation of radiolabeled valine, we found significant anesthetic-induced inhibition that was dose dependent, ranging from a reduction to 77% of control at 2% halothane up to a reduction to 36% of control at 6% halothane after 1 h of anesthetic exposure (Fig. 1A).

A hallmark of anesthetic action is the rapidity of induced responses. Examination of the time course for inhibition of protein synthesis during exposure to 6% halothane showed that protein synthesis was significantly decreased to 73% of the control after only 15 min of exposure (Fig. 1B). During longer periods of exposure (30 or 60 min), the rate of synthesis was further reduced to 52 and 38% of control rates, respectively. Thus inhibition occurred rapidly, and the amount of inhibition increased as time of exposure was extended.

Alteration of translation initiation plays a major role in regulation of protein synthesis in response to numerous cellular stresses (27). To examine whether halothane exposure affected initiation, the distribution of ribosomal subunits within polysome profiles was examined. There was a dramatic redistribution from polysomes to monosomes and free ribosomal subunits (i.e., ribosomal subunits not associated with mRNA) after 60 min of exposure to 2, 4, or 6% halothane (Fig. 2, A and B, bottom). During exposure to 6% halothane, the shift was rapid, as it was observed after only a 15-min exposure (Fig. 2B). The
extent of inhibition increased substantially with longer periods of exposure. These findings are consistent with the results obtained when incorporation of radiolabeled valine was examined.

One mechanism that regulates translation initiation in response to environmental stimuli is phosphorylation of the α-subunit of eIF2. Phosphorylated eIF2 bound to GDP forms an inactive complex with its guanine nucleotide exchange factor, eIF2B, inhibiting regeneration of the eIF2-GTP complex required for translation initiation (39). To assess effects of halothane exposure on this translation initiation process, we examined alterations in both phosphorylation of eIF2α and activity of eIF2B. At 6% halothane, phosphorylation of eIF2α increased significantly after only a 15-min exposure. After 60 min of exposure, the level of eIF2α(P) increased to more than twofold above control levels (Fig. 3, A and B). Total eIF2α levels were not altered during halothane exposure (Fig. 3B). In contrast to the rapid increase in phosphorylation of eIF2α observed when perfusate was exposed to 6% halothane, there was no significant increase in eIF2α phosphorylation in rat livers after 60 min of exposure to 2 or 4% halothane (Fig. 3A).

Consistent with the increased phosphorylation of eIF2α observed during exposure to 6% halothane, eIF2B activity rapidly decreased to 70–80% of control values in response to this treatment (Fig. 3C). Thus inhibition of formation of the translationally active eIF2-GTP complex likely plays a role in the reduced translation precipitated by 6% halothane. In contrast, eIF2B activity was not altered by exposure to either 2 or 4% halothane for 60 min (Fig. 3C). Although this lack of effect of lower halothane concentrations on eIF2B activity was different from the inhibition observed during exposure to 6% halothane, it is consistent with the unaltered levels of eIF2α(P) observed during exposure to these lower drug levels (Fig. 3A).

A separate mechanism regulating translation initiation is assembly of the active eIF4F complex (containing eIF4E, eIF4G, and eIF4A) required for several processes, including recognition of the 5′ cap structure of mRNA (22, 42). The eIF4E component of this complex is required for recognition of the mRNA cap but is sequestered in an inactive form when bound to 4E-BP(s). This leads to decreased amounts of active eIF4F complex. In contrast to the alterations of eIF2α phosphorylation and eIF2B activity, which occurred rapidly, alterations in eIF4F association arose only after extended exposure. After 15 min of exposure there was no significant difference in the association of eIF4E with either 4E-BP1 or eIF4G (Fig. 4, A and B). After a 30-min exposure, the association of eIF4E with eIF4G was reduced to 50% of the control, although there was not a corresponding increase in the level of eIF4E associated with 4E-BP1. Binding of eIF4E by another member of the 4E-BP family or an alternative mechanism that regulates the association of eIF4E with eIF4G may be responsible for the dissociation of eIF4E and eIF4G at this time. A 60-min exposure to 6% halothane led to increased levels of eIF4E complexed with 4E-BP1 and a corresponding decrease in eIF4E associated with eIF4G. During exposure to lower concentrations of halothane, there was a decrease in levels of translationally active eIF4E-eIF4G complex, although only at 4% halothane was this statistically significant (Fig. 4B). As observed after a 30-min exposure to 6% halothane, there was no change in the association of eIF4E with 4E-BP1 at these lower concentrations of anesthetic (Fig. 4A), again suggesting that another member of the 4E-BP family or an alternative mechanism plays a role in regulating the association of eIF4E with eIF4G under these conditions.

A third mechanism that affects translation initiation is altered phosphorylation of rpS6 (13). Halothane administered at 6% in the perfusate induced a significant decrease to 60% of control levels of phosphorylated rpS6 after 60 min of perfusion (Fig. 5, A and B). The levels of phosphorylated rpS6 were relatively stable during shorter periods of drug exposure. The levels of phosphorylated rpS6 were not affected by exposure to 2 or 4% halothane for 60 min (Fig. 5A).

![Fig. 1. Halothane (Hal) rapidly inhibits translation in perfused liver. A: after perfusion of rat livers with medium exposed or not exposed to varying concentrations of Hal for 45 min, l-[3H]valine was added to the perfusate, and perfusion was continued for an additional 15 min. Liver samples were harvested and immediately frozen. Incorporation of l-[3H]valine into acid-precipitable complexes was determined, and the rate of protein synthesis was calculated (32, 43). At least 6 livers were used for each condition. Percentages indicate rate of synthesis at the different levels of Hal exposure vs. unexposed (0% Hal) controls. White bar, results from livers that were not exposed to Hal; light gray, medium gray, and black bars, results from perfusions conducted with 2, 4, or 6% Hal, respectively. B: perfusions were conducted for a total of 15, 30, or 60 min with perfusate exposed or not exposed to 6% Hal. During the last 15 min of each perfusion, l-[3H]valine was added, and rates of protein synthesis were determined as described above. At least 8 livers that were exposed to Hal and at least 5 livers that were not exposed to Hal were used at each time point. White bars, results obtained from control livers not exposed to Hal for each of the indicated lengths of time; light gray, medium gray, and black bars, results from livers exposed to 6% Hal for 15, 30, or 60 min, respectively. **P < 0.01 vs. unexposed controls.](http://ajpendo.physiology.org/Downloadedfrom)
Activity of the 70-kDa kinase that phosphorylates rpS6, termed p70S6K1, is regulated by a series of phosphorylations. Dephosphorylation of p70S6K1 decreases its kinase activity. Consistent with the decreased levels of phosphorylated rpS6 observed only after extended exposure to high levels of halothane, we found that levels of phosphorylated p70S6K1 also decreased only under these conditions (Fig. 5, C and D).

**DISCUSSION**

Perfused rat liver provides advantages for studying direct effects of volatile anesthetics on regulation of protein synthesis in mammals. These include maintenance of the intact liver in a relatively normal physiological state during extended exposure, the ability to harvest sufficient tissue for a variety of assays to examine the extent and mechanisms of translational alteration, the ability to administer a wide range of anesthetic...
doses, the ability to precisely regulate the level of amino acids and other metabolites present in the perfusate and isolate the liver from anesthetic-induced alterations in hormones and metabolites, and well-characterized translational responses of the liver to a variety of stresses.

Consistent with findings in yeast (37) and mammalian systems (3, 6, 8, 10, 15–18, 21, 23–25, 35, 40, 41), the volatile anesthetic halothane inhibits protein synthesis in perfused rat liver. Analysis of polysome profiles showed that translation initiation is dramatically inhibited by this drug. Both inhibition of incorporation of radiolabeled amino acid and disaggregation of polysomes are dose- and time-dependent. It is possible that the pentobarbital sodium used to anesthetize the animals (50 μg/g body wt) before the surgery, independently or in combination with halothane, affects protein synthesis. Two lines of reasoning suggest that pentobarbital sodium does not signifi-

Fig. 3. Exposure to high levels of Hal induces hyperphosphorylation of eIF2α and inhibition of eIF2B activity. Perfusion with the indicated concentrations of Hal were conducted for the designated exposure times as described in the legend to Fig. 1 and MATERIALS AND METHODS. Fresh samples of liver were homogenized. A and B: aliquots of 1,000-g supernatants were subjected to SDS-PAGE. After transfer to polyvinylidene difluoride membrane, the separated proteins were probed with an antibody that specifically recognizes eIF2α phosphorylated on Ser51 [designated eIF2α(P); B, top] or an antibody against total eIF2α (B, bottom). Relative amounts of phosphorylated and total eIF2α were determined. Representative blots from livers exposed or not exposed to 6% Hal for 60 min are shown (B). C: aliquots of 10,000-g supernatants were used to determine eIF2B guanine exchange factor activity. At least 8 livers were used for each Hal exposure, and at least 5 were used for each unexposed control. Bars are as described in the legend of Fig. 1B. *P < 0.05; **P < 0.01 vs. unexposed controls.

Fig. 4. Extended exposure to Hal decreases translationally active eIF4E. A microtiter dish assay (29) was used to determine relative levels of eIF4E complexed with 4E-BP1 (A) or eIF4G (B) after perfusions with medium exposed to the specified concentrations of Hal. Perfusion with the indicated times were conducted as described in the legend to Fig. 1 and MATERIALS AND METHODS. At least 8 livers that were exposed to Hal and at least 5 livers that were not exposed to Hal were used at each time point. Bars are as described in the legend of Fig. 1B. **P < 0.01 vs. unexposed controls.
cantly affect protein synthesis in these studies. First, pentobarbital sodium included in perfusate at up to 100 μg/ml does not affect protein synthesis in perfused lung (41). Second, the concentration of pentobarbital sodium in the liver is reduced during perfusion when perfusate void of pentobarbital sodium replaces the blood containing pentobarbital sodium. The injected pentobarbital sodium would have to continue to affect protein synthesis despite this reduced concentration. Regardless of whether pentobarbital sodium affects protein synthesis, it is clear that halothane is essential for the observed effects because both control and experimental animals received equivalent doses of pentobarbital sodium.

An array of findings indicate that inhibition of translation in yeast exposed to volatile anesthetics results from deprivation for nutrients that the cells must obtain from their external environment (growth medium): strains prototrophic for all amino acids are highly resistant to the growth inhibitory effects of anesthetics (37); auxotrophic strains that contain extra copies of genes encoding permeases for critical amino acids that must be obtained from the medium are anesthetic resistant, whereas strains containing deletions of these genes are hypersensitive (38); increasing or decreasing the concentration of critical amino acids in the medium leads to resistance or hypersensitivity, respectively, to these drugs in appropriately auxotrophic strains (38); and uptake of leucine and tryptophan from medium is inhibited by isoflurane (38). The alterations of translation components observed during halothane exposure of rat liver are consistent with characterized changes known to occur during amino acid deprivation in mammalian systems (Fig. 6) (27), suggesting that limitation of nutrients may also play a critical role in the anesthetic response of liver.

Studies in yeast show that inhibition of translation initiation by volatile anesthetics involves two processes: a rapidly induced general amino acid control (GCN)-independent pathway accompanied by a marked decrease in the level of eIF2α(P) and a GCN-dependent phase required to maintain inhibition that occurs only after more than 60 min of exposure and is accompanied by hyperphosphorylation of eIF2α (37). In contrast to our findings in yeast, increased phosphorylation of eIF2α in rat liver during exposure to 6% halothane occurs rapidly (within the first 15 min). Results from both systems indicate that response to anesthetic exposure is multifaceted and complex, involving multiple pathways operating at different times. It is intriguing to speculate that these mechanisms play roles in the activity of these drugs as general anesthetics due to alterations in transport of amino acids and related compounds that act as neurotransmitters (38).

Flaim et al. (10) previously reported a slight inhibition of initiation in liver when perfusate was exposed to 4% halothane for 45 min. The inhibition we observed at 4% halothane after 60 min of exposure is much more dramatic. This difference may be due to the fact that the perfusate in the previous studies (10) contained amino acids at 5 times rat arterial plasma

Fig. 5. A and B: decreased phosphorylation of rpS6 occurs during extended exposure to 6% Hal. Perusfates with medium exposed to the indicated concentrations of Hal were conducted for the specified times as described in the legend to Fig. 1 and MATERIALS AND METHODS. Aliquots of 1,000-g supernatants were subjected to SDS-PAGE, and the separated proteins were probed with an antibody that specifically recognizes phosphorylated rpS6 after transfer to membrane. Relative levels of phosphorylated rpS6 were determined from at least 8 livers that were exposed to Hal and at least 5 livers that were not exposed to Hal at each time point. Relative levels of phosphorylated rpS6 were determined by quantitation of the levels of chemiluminescence in the β- and γ-bands vs. total chemiluminescence in the α-, β-, and γ-bands. Bars are as described in the legend of Fig. 1B. *P < 0.05; **P < 0.01 vs. unexposed controls.
concentrations, whereas the perfusate used in our studies contained amino acids at only 2.5 times these concentrations. Finding less inhibition of translation initiation when amino acid concentrations are higher is consistent with findings in yeast that increased levels of amino acids reverse the growth inhibitory effects of volatile anesthetics (38). The increased exposure time used in our studies is another potential factor in the observed difference.

The dissimilar alterations of translation initiation factors observed at 2, 4, or 6% halothane suggest that various mechanisms mediate inhibition at these different concentrations. For example, at 6% halothane the rapid and persistent increase in phosphorylation of eIF2α and the concurrent decrease in eIF2B activity suggest that these alterations are critical for reduced initiation at this anesthetic concentration. However, at 2 or 4% halothane, neither eIF2α phosphorylation nor eIF2B activity is affected even after extended exposure. One possible explanation for the failure to observe effects on eIF2α or eIF2B is that the extent of alterations induced by 2 or 4% halothane is low and fails to reach statistical significance under the experimental conditions. On the basis of our findings with 6% halothane, this seems unlikely. Significant changes in both eIF2α phosphorylation and eIF2B activity are observed when there are modest effects on protein synthesis during exposure to 6% halothane (e.g., 73% of control after 15 min). The level of inhibition of translation observed after a 60-min exposure to 4% halothane (61% of control) is greater than that observed after 15 min at 6% (Fig. 1, A and B, respectively). Thus relatively low levels of inhibition of translation by 6% halothane are accompanied by significant changes in both eIF2α and eIF2B, whereas neither factor is altered during exposure to 2 or 4% halothane even when inhibition of translation is greater. These results suggest that altered activity of eIF2 does not play a role in reducing translation at low halothane concentrations. This dramatic difference produced by a small increase in halothane concentration is similar to the sharp dose-response curve observed for other effects of volatile anesthetics.

Another feature of the effects of halothane is the time-dependent occurrence of alterations to translation factors. For example, phosphorylation of eIF2α increases rapidly after halothane administration, whereas altered phosphorylation of rpS6 and p70S6K1 occurs only after extended exposure. It is intriguing to speculate that rapid inhibition of translation at 6% is initially dependent on altered eIF2α phosphorylation, whereas the more dramatic inhibition of translation after extended exposure requires additional pathways, including decreased levels of phosphorylated rpS6 and redistribution of eIF4E out of its translationally active association with eIF4G. Other mechanisms affecting initiation may also participate in this anesthetic-induced inhibition of protein synthesis. Examination of effects on translation in perfused livers of mice mutant for various eIF2α kinases or 4E-BPs will provide additional insight regarding this possibility.

The dissociation of eIF4E and eIF4G observed at all concentrations of halothane appears to occur partly or wholly independently of eIF4E binding to 4E-BP1 regardless of the concentration of anesthetic. At 6% halothane, the decreased association of eIF4G with eIF4E is observed after 30 min of exposure, although increased association of eIF4E with 4E-BP1 is not observed until after 60 min. At both 2 and 4% halothane, the observed decrease in eIF4E-eIF4G complex is not accompanied by increased levels of eIF4E bound to 4E-BP1. In addition, this dissociation appears to initially occur independently of mTOR signaling, because alterations of the eIF4E-eIF4G complex occur in the absence of altered phosphorylation of p70S6K1 or rpS6 as well as in the absence of increased association of eIF4E with 4E-BP1. mTOR-independent regulation of the association of eIF4E with eIF4G has been observed in skeletal muscle (1, 31) but not in liver (31). Further mechanisms responsible for inhibition of translation at 2 and 4% halothane may remain to be identified. It is not clear whether the decreased association of eIF4G with eIF4E, the only statistically significant change observed at 4% halothane, is sufficient to induce the substantial decline in translation observed (61% of control). Additional mechanisms that regulate translation initiation that were not uncovered in these experiments may be necessary for this inhibition. Inhibition of translation elongation may also play a role, although phosphorylation of elongation factor 2, which is regulated by mTOR (for a review see Ref. 5), is not altered after exposure to 6% halothane for 60 min (data not shown). Studies with mice containing mutations in various translation factors will provide additional insight.

In conclusion, the results of these studies indicate that the volatile anesthetic halothane inhibits translation initiation in mammalian liver in a dose- and time-dependent manner. Reg-
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ulation of both eIF2α kinase activity and the mTOR response pathway plays a role in this inhibition. Volatile anesthetics may provide new tools for investigating cellular responses to altered amino acid nutrition in mammalian cells as well as in yeast.

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REFERENCES


