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Long-term alcohol administration inhibits synthesis of both myofibrillar and sarcoplasmic proteins in heart

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Abstract

Alcohol decreases the rate of protein synthesis in cardiac muscle. We investigated the effects of feeding rats a diet containing alcohol for 16 weeks on the myocardial synthesis of myofibrillar and sarcoplasmic (non-myofibrillar) proteins. Alcohol administration decreased the overall rate of protein synthesis in cardiac muscle by 22% compared with controls ($P < .05$). The rate of synthesis of proteins in the myofibrillar and sarcoplasmic fractions was diminished proportionately after feeding a diet containing alcohol ($P < .05$). We examined the effects of diminished rates of protein synthesis on the expression of myofibrillar and non-myofibrillar proteins. The cellular content of actin and α-myosin heavy chain isoform was significantly reduced and there was an increase in the β-myosin heavy chain isoform after feeding rats a diet containing alcohol. The reduced expression of myosin heavy chain isoform and actin did not result from a decreased abundance of messenger RNA for either of these proteins. The myocardial content of troponin C and T was unchanged whereas that of troponin I was increased. Ethanol administration reduced the expression of eEF2 and the inducible form of the 70-kDa heat shock protein, whereas the cognate form of the 70-kDa heat shock protein was unaffected in a non–myofibrillar-enriched fraction of cardiac muscle. These results suggest that (1) the reduced protein content observed in the heart after feeding a diet containing alcohol is a consequence of reduced synthesis of both myofibrillar and sarcoplasmic proteins, and (2) the expression of both actin and α-myosin heavy chain isoform is affected independently of the messenger RNA content of the proteins. We conclude that translational control mechanisms appear to be important in regulating the expression of myocardial proteins during long-term ethanol intoxication.

1. Introduction

Heart disease, as well as cirrhosis, represents an important etiology of mortality in long-term alcoholics. Excessive ethanol consumption can result in a syndrome referred to as alcoholic heart muscle disease. Alcoholic heart muscle disease is rarely produced by short-term ethanol administration. However, it is observed in those patients who excessively consume alcohol for prolonged periods (>80 g of ethanol a day for >10 years). The clinical feature of this syndrome is a defect in myocardial contractility as assessed by a reduction in ejection fraction, with the degree of cardiac dysfunction proportional to the duration and severity of alcohol consumption [1].

The major pathological features revealed through biopsy or postmortem examination include dilation of both ventricles of the heart, thinning of the ventricular wall with fibrosis and endocardial fibroelastic thickening, interstitial edema, and focal areas of necrosis within the ventricular wall [2-4]. Microscopic examination of biopsy specimens obtained from human beings reveals myocyte degeneration, loss of striations, and myofilament dissolution, consistent with alterations in structural and myofibrillar proteins [2-6]. Addition of ethanol to the medium reduces the number and uniformity of the myofibrils in myocytes in culture [7]. It therefore appears that in alcoholic heart disease the mechanical performance of the heart as a pump is seriously compromised by loss of contractile elements or their functional impairment by fragmentation and disarrangement. However, there are exceedingly few reports concerning the synthesis or expression [8,9] of myofibrillar proteins after extended periods of ethanol administration.

Numerous hypotheses have been put forth to explain the development of the alcoholic heart muscle disease, suggesting that the molecular basis for this disease is probably

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multifactorial. As alcoholic heart muscle disease is associated with reduced contractility and derangements in myofibrillar architecture, it follows that one explanation for these changes is that the integrity of cellular proteins may be compromised by prolonged ethanol intake. Early work indicated that long-term ethanol consumption led to a decreased association of actin and myosin heavy chain isoform in vitro [10]. It was suggested that persistent changes in some proteins may have occurred and may have been related to an inhibition of protein synthesis. We have provided evidence that long-term exposure of rats to a diet containing ethanol results in an inhibition of protein synthesis through a decreased efficiency of messenger RNA (mRNA) translation [11].

Most of the studies outlined above measure the rate of incorporation of radioactivity into mixed proteins within the cell. They do not differentiate between protein synthesis in different subcellular fractions or expression of individual proteins. Indeed studies investigating the effect of ethanol on subcellular fractions or individual proteins are scant. Preedy and Peter [12,13] reported that the synthesis of myofibrillar and sarcoplasmic proteins are either unaffected or slightly increased by providing alcohol in the diet for 6 weeks. Likewise, Tierman et al [14] reported that the synthesis of myofibrillar or non-myofibrillar proteins was unaffected despite a 30% decrease in the overall rate of protein synthesis after 3 weeks of consuming a diet containing alcohol. In contrast, Schreiber et al [15] reported a decrease in the synthesis of actin with more prolonged alcohol ingestion.

It remains unclear the consequences of prolonged reductions in rates of protein synthesis after long-term alcohol abuse on myocardial proteins. We would predict that such a prolonged diminished rate of protein synthesis would potentially affect the expression of myocardial proteins. The purpose of these investigations was to determine the synthesis of proteins in the myofibrillar and sarcoplasmic (non-myofibrillar) fractions of hearts fed a diet containing alcohol for 16 weeks. We further evaluated the expression of several proteins involved in the control of contractile function and cellular homeostasis.

2. Materials and methods

2.1. Long-term alcohol feeding of animals

Pathogen-free, male Sprague-Dawley rats (Charles River Breeding Laboratories, Cambridge, Mass) were maintained for 16 weeks on an ethanol-containing diet in which alcohol was provided both in drinking water and previously described agar blocks [11,16-19]. Initially, all rats were provided the agar block without ethanol for 2 days. Thereafter, the animals were randomly assigned to either an alcohol or control group. Animals in the alcohol group were given free access to ethanol-containing agar blocks. The concentration of ethanol in the agar blocks was increased in 10% increments from 10% to 40% over the first 4 weeks [11,17-19]. Ethanol-fed rats remained on the 40% ethanol-agar block diet for the remainder of the experimental protocol. Ethanol consumption averages 17 ± 2 g/kg body weight per day and the plasma ethanol concentrations at time of heart excision averaged 21 ± 3 mmol/L in this model. Control agar blocks contained an equal caloric amount of dextrin maltose. Nutrient intake in both groups was furnished by standard rat chow (Harlan Teklad no. 8604, Madison, Mich). Control rats were provided the same amount of solid food as consumed by the alcohol-fed group [11,17-19]. Total energy consumption was the same in both groups [20].

2.2. Protein synthesis

The rate of protein synthesis in vivo was determined using the flooding-dose technique described by Garlick et al [21] and modified in our laboratory [11,18,19,22-27]. Animals were anesthetized (Nembutal; 100 mg/kg body weight) and a polyethylene catheter (PE 50 tubing) was surgically placed in the carotid artery. Then, a bolus infusion of [^{3}H]-L-phenylalanine (150 mmol/L, 30 μCi/mL; 1 mL/100 g body weight) was given via the jugular vein over a 10- to 15-second interval. At 2, 6, and 10 minutes after injection of the radioisotope, blood samples (1 mL) were drawn for measurement of phenylalanine concentrations and radioactivity. Immediately after the removal of the 10-minute blood sample, the myocardium was excised, frozen between aluminum blocks cooled to the temperature of liquid nitrogen, and the frozen heart weighed.

2.3. Total mixed protein synthesis

All hearts were powdered under liquid nitrogen with a mortar and pestle and a portion was used to estimate the rate of incorporation of [^{3}H]phenylalanine into total mixed proteins. Approximately 0.2 g of powdered tissue was homogenized in 2 mL of ice-cold 3.6% (wt/vol) $HClO_4$ and centrifuged at 10 000 × g for 11 minutes at 4°C. The supernatant was decanted, and the pellet was washed a minimum of 5 times with 3.6% $HClO_4$ to remove any acid-soluble radioactivity. The pellet was sequentially washed with acetone, chloroform/methanol (1:1, vol/vol), and water. The pellet was dissolved in 0.1 mol/L NaOH, and samples were assayed for protein, with crystalline bovine serum albumin as a standard. Another sample was assayed for radioactivity by liquid-scintillation spectrometry with corrections for quenching (disintegrations per minute).

2.4. Myofibrillar and sarcoplasmic protein synthesis

Another portion (1 g) of the frozen, powdered heart was used to separate the myofibrillar and sarcoplasmic proteins according to the procedures used previously in our laboratory [27] adapted from Hoover-Plow and Clifford [28]. Cardiac muscle samples were homogenized in 7.5 mL of ice-cold buffer consisting of 10 mmol/L KH_2PO_4 (pH 7.4) using a motor-driven glass-on-glass homogenizer. The samples

were centrifuged at 3000 × *g* at 4°C for 20 minutes. The pellet contains the myofibrillar proteins and the supernatant fraction contains the sarcoplasmic proteins. The pellet containing the myofibrillar proteins was washed twice with 2 mL of buffer containing 0.1 mmol/L KH_2PO_4 (pH 7.4). The supernatant fractions from the washes were pooled and added to the non-myofibrillar fraction. The myofibrillar pellet was dissolved in 2 mL of 0.6 mol/L NaCl, centrifuged for 30 seconds at 500 × *g* at 4°C. The supernatant was saved for measurement of radioactivity in myofibrillar proteins and the pellet discarded. Both the myofibrillar and sarcoplasmic protein fractions were treated with an equal volume of 10% (wt/vol) trichloroacetic acid. The supernatants were discarded and the precipitated protein fractions were processed as described above for measurement of incorporation of radioactivity in total protein.

Rates of protein synthesis (nanomoles of Phe incorporated per hour per milligram of protein) were calculated as described earlier using the mean specific radioactivity of the plasma phenylalanine as the precursor pool [11,18,19,22-27]. The specific radioactivity of the plasma phenylalanine was measured by high-performance liquid chromatography analysis of supernatants from trichloroacetic acid extracts of plasma as previously described [29]. The specific radioactivities from the 3 time points were averaged. The assumption in using this technique to measure rates of protein synthesis in vivo is that the intracellular concentration (>1 mmol/L) of phenylalanine is elevated to such an extent that any dilution effect of nonradioactive phenylalanine derived from the proteolysis on the specific radioactivity would be negligible. Under the condition of elevated plasma phenylalanine concentrations, the specific radioactivity of the intracellular phenylalanine is assumed to be equal to the specific radioactivity of the transfer RNA–bound phenylalanine. These assumptions have been verified in isolated perfused hearts [30,31] and under similar conditions in vivo in the dog [32].

2.5. Western blot analysis

Heart muscle was homogenized in 7 volumes of buffer A (20 mmol/L HEPES, pH 7.4, 100 mmol/L KCl, 0.2 mmol/L EDTA, 2 mmol/L ethyleneglycotetraacetic acid, 1 mmol/L dithiothreitol, 50 mmol/L NaF, 50 mmol/L β-glycerolphosphate, 0.1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L benzamidine, 0.5 mmol/L sodium vanadate, and 1 mmol/L microcystin LR) using a Polytron homogenizer set at 60% of its maximum speed. The samples were mixed with equal volumes of 2× Laemmli sodium dodecyl sulfate sample buffer (60°C). The samples were mixed, boiled for 3 minutes, and centrifuged. Equal amounts of protein were subjected to sodium dodecyl sulfate polyacrylamide slab gel electrophoresis using 10% acrylamide gel followed by transfer of proteins to polyvinylidene fluoride (BioTrace, Pall Gelman, Pensacola, Fla) as described previously [18,19,22,23,33-36]. After blocking with milk, the membranes were incubated with antibodies specific for α-myosin heavy chain isoform, β-myosin heavy chain isoform, actin, troponin C, troponin I , troponin T, eEF2 [34,37,38], 70-kDa heat shock protein in cognate form (HSC70) and inducible form (HSP70), and Grp78. The blots were then developed using ECL (Amersham Pharmacia Biotech Inc, Piscataway, NJ). The blots were exposed to x-ray film in a cassette. After development, the film was scanned (Microtek ScanMaker IV) and quantified using National Institutes of Health Image 1.6 software. Commercially available antibodies were obtained from the following sources: Santa Cruz Biotechnology Inc, Santa Cruz, Calif (actin, troponin I, troponin C, troponin T, HSC70, Grp78); Sigma-Aldrich, St Louis, Mo (α-myosin heavy chain isoform); Chemicon International, Temecula, Calif (β-myosin heavy chain isoform); and Stressgen Biotechnologies Corp, Victoria, BC, Canada (HSP70).

2.6. RNA extraction and Northern blot analysis of myosin heavy chain isoform and actin mRNA

The relative abundance of myosin heavy chain isoform and actin mRNA was determined by Northern blot analysis. Total RNA was isolated using TotallyRNA as outlined by the manufacturer (Ambion, Inc, Austin, Tex). Samples (25 μg) of total RNA were electrophoresed under denaturing conditions using 1.0% agarose/2.2 mol/L formaldehyde gels. After electrophoresis, RNA was transferred to a Genescreen Plus membrane (DuPont NEN, Boston, Mass) using Scheicher & Schuell's Turboblotter. The membranes were checked under a UV transilluminator to verify efficiency of transfer. The RNAs were probed with oligonucleotides for rat myosin heavy chain isoforms, actin, eEF2, HSP70, or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA that had been radioactively labeled using [^{32}P]ATP terminal deoxynucleotide transferase (Promega, Madison, Wis). After hybridization, the blots were exposed at −80°C for 17 to 48 hours to Blue Sensitive Autoradiographic Film (Marsh Biomedical Products, Rochester, NY) in a cassette equipped with a DuPont Lightning Plus intensifying screen. The autoradiograms were scanned and analyzed as described above for Western blot analysis. The amount of GAPDH mRNA in individual samples was used to normalize myosin heavy chain isoform and actin mRNA values on the same membrane.

2.7. Statistical analysis

Results are expressed as means ± SE for 4 to 8 animals in each group. The statistical evaluation of the data was performed using a Student *t* test to determine significance between means. Differences among means were considered significant when $P < .05$.

3. Results

3.1. Protein synthesis

The effect of long-term alcohol administration on the rate of total protein synthesis is presented in Fig. 1. Feeding rats

a diet containing ethanol caused a 22% decrease in the rate of synthesis of total mixed proteins in cardiac muscle compared with values from pair-fed control rats. In cardiac muscle, the total mixed proteins is the average of the synthesis of myofibrillar proteins and non-myofibrillar (sarcoplasmic) proteins. The contribution of the myofibrillar and sarcoplasmic proteins to the rate of total mixed protein synthesis was investigated in myocardial proteins of each fraction from control rats and rats fed a diet containing alcohol (Fig. 1). The rate of synthesis of sarcoplasmic proteins was approximately 2-fold higher than that of myofibrillar proteins in hearts from control rats.

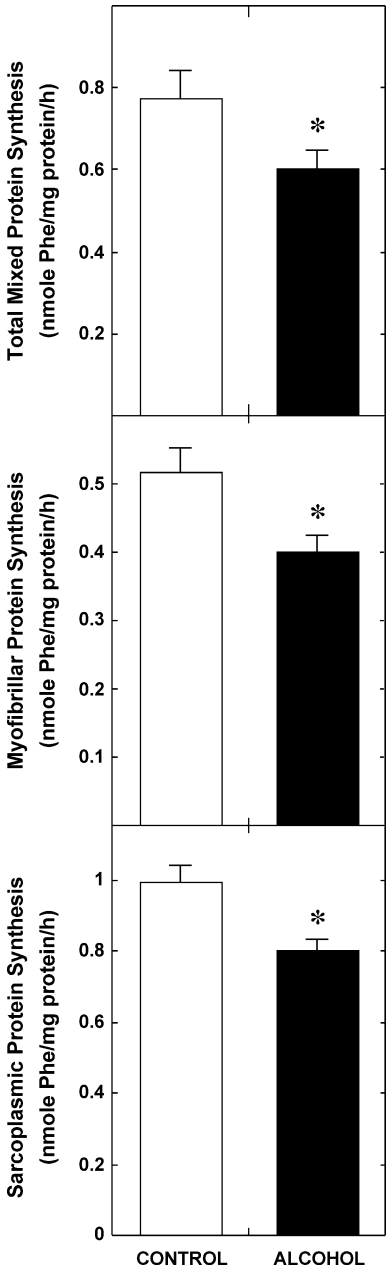

Fig. 1. The rate of synthesis of total mixed proteins (top), myofibrillar (middle), and sarcoplasmic (bottom) proteins in hearts of rats fed a diet containing ethanol for 16 weeks (alcohol) or rats pair-fed a diet that was isonitogenous and isoenergetic with respect to the alcohol group. Rates of protein synthesis were measured in vivo after intravenous injection with saline containing [^{3}H]-L-phenylalanine as described in Materials and methods. Sarcoplasmic and myofibrillar proteins were separated as described in Materials and methods. Values shown are means ± SE for 4 to 10 animals in each group. The asterisk indicates significance at $P < .01$ vs control.

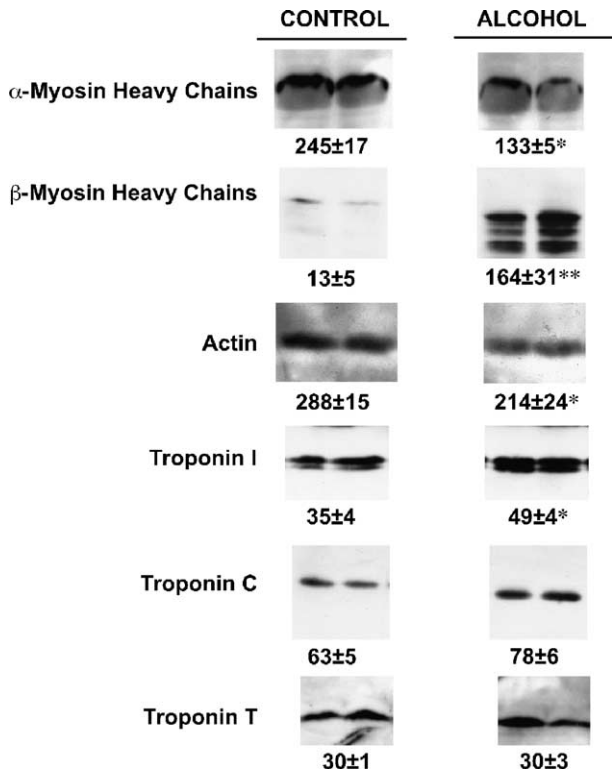

Fig. 2. Effect of long-term alcohol feeding on relative amount of α-myosin heavy chain isoform, β-myosin heavy chain isoform, actin, troponin I, troponin C, and troponin T in the myofibrillar fraction. Equal amounts of protein from the myofibrillar fraction described in Fig. 1 were electrophoresed and transferred to polyvinylidene fluoride membranes. The figure shows a representative immunoblot and desitometric analysis of several immunoblots for α- and β-myosin heavy chain isoforms, actin, troponin I, troponin C, or troponin T in myofibrillar fractions of hearts obtained from animals described in Fig. 1. Values shown are means ± SE for 6 to 10 animals in each group. The asterisk indicates significance at $P < .05$ vs control; double asterisks, $P < .001$ vs control.

As was observed in hearts from control rats, the rate of synthesis of sarcoplasmic proteins remained approximately 2-fold more than myofibrillar proteins after feeding rats a diet containing alcohol (Fig. 1). Feeding the alcohol-containing diet reduced the synthesis of proteins in both fractions. The rate of synthesis of both myofibrillar and sarcoplasmic proteins was significantly decreased by 23% and 20%, respectively, in animals fed a diet containing alcohol compared with controls (Fig. 1).

3.2. Expression of myofibrillar proteins

The myofibrillar fraction of the myocardium contains proteins associated with the contractile apparatus including actin, myosin heavy chain isoforms, and regulatory proteins, such as troponin C, I, and T. The effect of feeding rats a diet

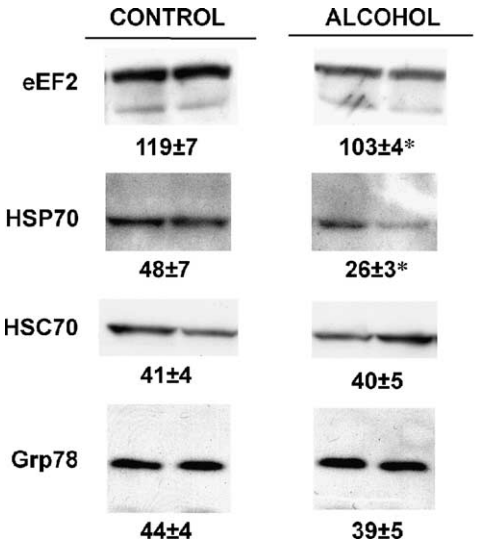

Fig. 3. Effect of long-term alcohol feeding on relative amount of eEF2, inducible HSP70, cognate HSC70, and Grp78 in the sarcoplasmic (non-myofibrillar) fraction. Equal amounts of protein from the sarcoplasmic fraction described in Fig. 1 were electrophoresed and transferred to polyvinylidene fluoride membranes. The figure shows a representative immunoblot and desitometric analysis of several immunoblots for eEF2, inducible HSP70, cognate HSC70, and Grp78 in sarcoplasmic fractions of hearts obtained from animals described in Fig. 1. Values shown are means ± SE for 6 to 12 animals in each group. The asterisk indicates significance at $P < .05$ vs control.

containing alcohol on the myofibrillar content of these proteins is shown in Fig. 2. Densitometric analysis of several immunoblots revealed the relative amount of α-myosin heavy chain isoform was reduced by approximately 45% in hearts from rats fed a diet containing alcohol for 16 weeks compared with pair-fed controls (Fig. 2). In contrast, the relative abundance of β-myosin heavy chain isoform was increased 11-fold in hearts from rats fed a diet containing alcohol for 16 weeks compared with pair-fed controls (Fig. 2). The relative amount of actin was reduced by approximately 25% in the myocardium from rats fed a diet containing alcohol for 16 weeks compared with pair-fed controls (Fig. 2).

In contrast to actin and α-myosin heavy chain isoform, the expression of other protein involved in the contractile apparatus was not decreased by long-term alcohol consumption. The relative abundance of troponin C was actually increased by approximately 40% and the relative amount of troponin T and I remained unchanged in hearts from rats fed a diet containing alcohol for 16 weeks compared with pair-fed controls (Fig. 2).

3.3. Expression of non-myofibrillar proteins

We have previously reported that long-term alcohol consumption induces a defect in the elongation phase of protein synthesis [11,19]. eEF2 is an essential component of the process of peptide-chain elongation. Densitometric analysis of several immunoblots revealed the relative amount of eEF2 was reduced by approximately 10% in hearts from rats fed a diet containing alcohol for 16 weeks compared with pair-fed controls (Fig. 3).

Members of the HSP70 family (the consecutive cognate HSC70 and inducible HSP70) play a role in a number of cellular processes including peptide-chain elongation [39]. The 70-kDa heat shock protein is present both in a cognate form (HSC70) and an inducible form (HSP70). We assessed the effects of long-term ethanol feeding on both HSC70 and HSP70 proteins in cardiac muscle. Densitometric analysis of several immunoblots revealed the relative amount of HSC70 was not significantly changed in hearts from alcohol-fed rats compared with pair-fed controls (Fig. 3). In contrast to HSC70, the relative abundance of HSP70 was reduced by approximately 50% in animals consuming alcohol for 16 weeks compared with pair-fed controls (Fig. 3).

Grp78 is a molecular chaperone whose function is to assure proper folding of newly synthesized proteins in the endoplasmic reticulum. In liver and brain, ethanol enhances its expression and Grp78 is up-regulated at doses of alcohol above 5 g/kg [40]. There are no reports of effect of ethanol on Grp78 in heart. Densitometric analysis of several immunoblots revealed the relative abundance of Grp78 was not significantly changed in hearts from alcohol-fed rats compared with pair-fed controls (Fig. 3).

3.4. Northern blot analysis of mRNA

The reduction of α-myosin heavy chain isoform or actin protein and the increase in β-myosin heavy chain isoform could result from either reduced abundance of myosin heavy chain isoform or actin mRNA for translation or diminished efficiency of myosin mRNA translation during long-term feeding of rats with a diet containing ethanol. To assess which of these mechanisms occurred in alcohol-fed rats, the steady-state abundance of myocardial myosin heavy chain isoform,

Table 1
Effect of long-term alcohol administration on expression of myosin heavy chain isoforms, actin, eEF2 and HSP70 mRNA

	α-Myosin heavy chain (Arb unit/18S RNA)	β-Myosin heavy chain (Arb unit/18S RNA)	Actin (Arb unit/ GAPDH RNA)	eEF2 (Arb unit/ 18S RNA)	HSP70 (Arb unit/ GAPDH RNA)
Control	7.0 ± 0.6	3.7 ± 0.7	3.1 ± 0.2	3.4 ± 0.4	8.0 ± 0.4
Alcohol	8.4 ± 0.7	8.3 ± 1.1*	2.8 ± 0.3	3.8 ± 0.4	10.5 ± 1

Values shown are means ± SE for n = 8 to 11 in each group. The abundance of α-myosin heavy chain isoform, β-myosin heavy chain isoform, actin, eEF2, and HSP70 mRNA was measured by Northern blot analysis as described in Materials and methods. The α-myosin heavy chain isoform, β-myosin heavy chain isoform, and eEF2 mRNA were normalized by dividing by the abundance of 18S RNA on the same membrane to correct for any differences in loading. Actin and HSP70 mRNA were normalized by dividing by the abundance of GAPDH mRNA on the same membrane to correct for any differences in loading.

* $P < .01$ vs control.

actin, eEF2, and HSP70 mRNA content was analyzed by Northern blot analysis (Table 1). Long-term alcohol consumption significantly increased the myocardial content of β-myosin heavy chain isoform mRNA compared with values obtained in cardiac muscle from pair-fed controls, whereas α-myosin heavy chain isoform mRNA was unchanged. The mRNA content for actin, eEF2, and HSP70 was unchanged in rats fed a diet containing alcohol compared with values obtained in cardiac muscle from pair-fed controls.

4. Discussion

Long-term alcohol feeding resulted in a lower heart weight compared with pair-fed controls, partially because of a 25% loss in cardiac protein per heart [11]. The loss of protein mass results, in part, from a diminished (25%) rate of synthesis of total mixed myocardial proteins. The rate of synthesis of total mixed proteins is the average of the synthesis of proteins contained in the myofibrillar structures (myofibrillar) and non-myofibrillar (sarcoplasmic) proteins. The rate of synthesis of proteins in the myofibrillar and sarcoplasmic fractions were reduced by approximately 20% and 23%, respectively. Moreover, the rate of synthesis of sarcoplasmic proteins remained 2 times that of myofibrillar proteins after feeding rats a diet containing alcohol. Thus, the relationships between the synthesis of total, myofibrillar, and sarcoplasmic proteins in cardiac muscle from rats fed a diet containing alcohol indicate that alcohol administration most likely affected the synthesis of both myofibrillar and sarcoplasmic proteins similarly.

The mechanism(s) by which long-term alcohol intoxication causes diminished rates of protein synthesis have not been completely elucidated. Regulation of protein synthesis occurs through changes in the abundance of ribosomes, translational efficiency, and/or concentration of translatable mRNA. Because approximately 80% of the RNA in muscle is ribosomal RNA, changes in total RNA content presumably reflect changes in the number of ribosomes. The RNA content of hearts from alcohol-fed rats was not different compared with controls [19]. Therefore, alterations in the relative abundance of ribosomes were not responsible for inhibition in myocardial protein synthesis from rats fed alcohol. The efficiency of translation, calculated by dividing the protein synthesis rates by the total RNA content, provides an index of how rapidly the existing ribosomes synthesize protein. The translational efficiency was diminished by approximately 50% in animals fed a diet containing alcohol [19]. Thus, alcohol feeding induces an inhibition of protein synthesis by reducing the mRNA translational efficiency.

The mRNA translational efficiency is controlled by both the ability to initiate peptide-chain translation (translation initiation) and the ability to elongate growing peptide chains. Long-term alcohol ingestion results in a inhibition of both translation initiation and elongation [11,19]. The response to an inhibition of translation initiation to long-term alcohol administration can be general, that is, affecting the translation of most if not all mRNAs and/or specific, that is, the translation of a single class or subset of proteins is reduced. In the present report, we have evaluated the effect of long-term alcohol administration on both the general and the specific regulation of protein expression in heart.

A fall in the rate of synthesis of proteins contained in the myofibrillar fraction may be expected to alter the steady-state expression of proteins found within this fraction. Of the proteins with potential significance for cardiac performance, the myosin heavy chains and actin are prominent because of their role in the process of cardiac muscle contraction. Two types of myosin heavy chains (α and β) are expressed in the mammalian myocardium. We observed a reduction in the relative abundance of α-myosin heavy chain isoform and actin in the myofibrillar fraction of rats fed a diet containing ethanol compared with pair-fed control rats. It is not clear as to the functional consequences of such a loss of α-myosin heavy chain isoform and actin in hearts from rats fed a diet containing ethanol.

There are several potential mechanisms that may account for a reduction in content of α-myosin heavy chain isoform and actin in the myofibrillar fraction via a diminished rate of synthesis of protein. A reduced abundance of mRNA encoding for contractile proteins would be consistent with transcriptional or mRNA stability in the control of contractile protein synthesis. In the present study, neither α-myosin heavy chain isoform nor actin mRNA content was diminished in hearts from rats fed a diet containing ethanol compared with pair-fed control rats. Our results are in contrast to those of Meehan et al [8] in that we did not observe an increase in the relative abundance of α-myosin heavy chain isoform mRNA after feeding rats a diet containing ethanol for 90 days. The difference in the abundance of α-myosin heavy chain mRNA between the 2 studies may relate to the duration of the ethanol feeding. Regardless, the decline in the myocardial content of the α-myosin heavy chain isoform and actin protein was not associated with a diminished abundance of mRNA for these 2 contractile proteins suggesting that control of the expression of these proteins is dependent upon the control of protein synthesis or degradation after long-term alcohol intake. In this regard, the myocardial expression of α-myosin heavy chain isoform protein is depressed because of inhibition of peptide-chain elongation, a process also diminished by long-term ethanol intake [11,41], rather than a diminished mRNA content during contractile arrest [42].

In contrast, the relative abundance of the β-myosin heavy chain isoform was actually increased 11-fold in hearts from rats fed a diet containing ethanol compared with pair-fed control rats. Such a change in the relative abundance of the β-myosin heavy chain isoform expression in our studies is consistent with previous reports examining the ratio of α- to β-myosin heavy chain isoforms in hearts from rats fed a diet containing ethanol using silver staining techniques [8,43]. The abundance of β-myosin heavy chain isoform mRNA was actually increased after long-term exposure to ethanol.

This finding is consistent with a reported alcohol-induced up-regulation of the β-myosin heavy chain isoform mRNA using an entirely different method of long-term feeding of ethanol to rats [8].

An alternative explanation to account for the fall in cellular abundance of myofibrillar proteins is translational control, whereby the abundance of mRNA is not diminished but the ability of the protein synthetic machinery to translate mRNA into protein is impaired. A decrease in the expression of α-myosin heavy chain isoform and actin without a corresponding reduction in the abundance of their respective mRNAs suggests that these proteins are controlled through translational regulation rather than transcriptional control. This conclusion is consistent with reports demonstrating that α-myosin heavy chain isoform protein expression can be regulated at the translational level in other conditions [42,44]. Likewise, the β-myosin heavy chain expression may also be under translational control. In our study, the abundance of β-myosin mRNA doubled, but the expression of β-myosin protein expression increased approximately 10-fold, suggesting evidence of translational control.

Translational control may also account for changes in the abundance of proteins in the non-myofibrillar fraction. eEF2 is a sarcoplasmic protein involved in the process of peptide-chain elongation whose expression is regulated by translational control mechanisms. As was observed for actin and the α-myosin heavy chain isoform, the myocardial content of eEF2 was significantly decreased. The mRNA of eEF2 possesses a 5′-terminal tract of oligopyrimidines (5′-TOPs). The expression of mRNAs containing 5′-TOPs motif appears regulated in part through the phosphorylation of ribosomal protein S6. Although the biochemical mechanisms involved in the regulation of expression of mRNAs containing 5′-TOPs motif in response to S6 phosphorylation remain unknown, reductions in the phosphorylation of the ribosomal protein S6 may lead to alterations in the cellular content of proteins encoded by these types of mRNAs [45]. We have previously established that long-term alcohol feeding inhibits the phosphorylation of ribosomal protein S6 kinase in heart, the kinase responsible for phosphorylating the ribosomal S6 protein [11]. As observed with actin and α-myosin heavy chain isoform, the abundance of mRNA for eEF2 remains unchanged in hearts from rats fed a diet containing alcohol compared with control animals. Our findings are consistent with the observation that the decreased eEF2 protein content occurred secondary to a diminished translation of eEF2 rather than transcriptional control mechanisms.

In addition to eEF2, we analyzed the effects of feeding rat a diet containing ethanol on HSP70. The 70-kDa heat shock protein is representative of a class of proteins that facilitate protein-protein interactions and act as a molecular chaperone. Its function is important for cell growth even under basal conditions. In this regard, HSP70 appears important in peptide-chain elongation by guiding growing nascent polypeptide through the channel on the 80S ribosome [39]. Furthermore, mutants of HSP70 lead to a slow growing phenotype with a reduced proportion of actively translating ribosomes [46]. Furthermore, decreased polysomal HSP70 may slow peptide-chain elongation during skeletal muscle atrophy [47]. Members of the HSP70 family are composed of the constitutive HSC70 and inducible HSP70. In the present set of experiments, HSC70 was unaffected by long-term alcohol feeding. In contrast, HSP70 was reduced approximately 50% in rats fed a diet containing ethanol for 16 weeks compared with pair-fed controls. In the present study, there were no significant decreases in the abundance of HSP70 mRNA after feeding rats with a diet containing ethanol. The decrease in HSP70 is also consistent with the fall in global mixed protein synthesis observed in rats consuming an alcohol-containing diet for 16 weeks.

In summary, the results of the present investigations provide evidence that the synthesis of both myofibrillar and sarcoplasmic proteins in cardiac muscle are reduced after long-term administration of ethanol. Such a finding is consistent with the general reduction in protein synthesis observed in animals maintained on a diet containing alcohol for 14 to 16 weeks [11,19,24,41]. We further provide evidence that the changes in protein expression of myosin heavy chain isoform and actin are not the result of altered expression of mRNA encoding these proteins indicating the block in protein synthesis occurs at the level of mRNA translation. Long-term alcohol abuse reduces the translation of mRNA of myocardial proteins by inhibiting 3 sites, namely, (1) the formation 48S preinitiation complex, (2) diminished ribosomal protein S6 kinase activity, and (3) reduced elongation because of diminished expression of eEF2 [11,19,24,41]. The consequences of these alterations are a loss of myocardial proteins with a reduction in the expression of specific myofibrillar and non-myofibrillar proteins over the period of providing alcohol in the diet.

Acknowledgments

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