Postprandial Induction of Chaperone Gene Expression Is Rapid in Mice

Joseph M. Dhahbi, Shelley X. Cao, Patricia L. Mote, Brian C. Rowley, John E. Wingo and Stephen R. Spindler

Department of Biochemistry, University of California, Riverside, Riverside, CA 92521

ABSTRACT  Molecular chaperones assist in the biosynthesis and processing of proteins. Most chaperones are induced by physiological stresses. We have shown that dietary energy restriction decreases the mRNA and protein levels of many endoplasmic reticulum chaperones in the livers of mice. Here, we have investigated the response of chaperone mRNA to feeding. Control and 50% energy-restricted C3B10RF1 mice were deprived of food for 24 h, fed, and killed 0, 1.5, 5 or 12 h after feeding. Chaperone mRNAs were strongly induced as early as 1.5 h after feeding in control and energy-restricted mice. The integrated levels of these mRNA over 24 h were significantly lower in energy-restricted mice. The mRNA response to energy intake was mirrored over the course of days in the level of chaperone protein. A similar but smaller response to feeding was found in kidney and muscle. Puromycin and cycloheximide failed to inhibit the feeding response, suggesting that feeding releases chaperone expression from an unstable inhibitor. Studies with dibutyryl-cAMP- and glucagon-supplemented, normal and streptozotocin-diabetic mice suggest that glucagon and insulin may be mediators of the feeding response. Adrenalectomy enhanced the feeding induction, but dexamethasone administration had no effect. Thus, postprandial changes in insulin and glucagon may link chaperone gene expression to feeding, possibly in several tissues including liver. J. Nutr. 132: 31-37, 2002.

KEY WORDS: • feeding • mice • energy restriction • chaperones • glucose-regulated proteins

Many proteins have been shown to require interactions with molecular chaperones for their biosynthesis, maturation, processing and intracellular localization (1). Chaperones also interact with improperly folded, denatured, and aggregated proteins to stimulate their correct refolding or degradation (2). Stress-inducible chaperones respond to a diverse group of stimuli including heat, oxidative and ischemic stress, inflammation, hemodynamics and exposure to toxic chemicals (3–5). Under such conditions, chaperones associate with abnormal proteins to promote their renaturation, prevent their aggregation and promote their degradation when they cannot be properly refolded.

We have previously reported another means of regulating chaperone gene expression. Energy restriction (ER)2, which is the only means of reducing the mean age of onset of most age-related diseases and tumors while extending maximum lifespan in homeothermic vertebrates, significantly and consistently reduced the mRNA and protein levels of many endoplasmic reticulum chaperones in the liver of mice (6–9). Reduced chaperone gene expression may be important to the health benefits of ER (10–13).

The reduction in chaperone expression is proportional to the reduction in calorie consumption (14). In ER mice shifted to control feeding, the transition to higher chaperone gene expression required only 2 wk (8). These data suggest that chaperone mRNA levels adapt relatively rapidly to calorie consumption. In more recent studies, we found that only a few weeks of ER were required to reproduce a majority of the effects of long-term ER on global patterns of gene expression (15). Thus, studies of chaperone gene expression may reveal the mechanisms by which ER produces many of its beneficial physiological effects.

Although only a few weeks are required for a substantial transition from the control to the ER gene expression phenotype, ER produces adaptive changes in gene expression that persist for at least 24 h after feeding (10). For these reasons, we investigated the effects of feeding on the expression of chaperone gene expression in control and ER mice. We found that feeding rapidly induced many chaperone mRNA. However, where investigated, the integrated levels of these mRNA in the 24 h after feeding were significantly lower in ER mice. Because of the novel nature of these results, we investigated the mechanism for this regulation. Studies using protein synthesis inhibitors distinguished the feeding response from the endoplasmic reticulum stress response, hypoxia and growth factor responses. Studies using dibutyryl-cAMP- and glucagon-supplemented, normal and streptozotocin (STZ)-diabetic mice suggested that postprandial changes in insulin and glucagon may be responsible for the feeding response and thereby link chaperone gene expression to feeding in several tissues, including liver.

E-mail: spindler@ucrac1.ucr.edu

1 To whom correspondence should be addressed.

2 Abbreviations used: ER, energy restriction; ERp57 and ERp72, endoplasmic reticulum protein 57 and 72; GRP75, GRP78, GRP94 and GRP170, glucose-regulated protein 75, 78, 94 and 170; HSC70, heat shock protein cognate 70; PEPCK, phosphoenolpyruvate carboxykinase; STZ, streptozotocin.
MATERIALS AND METHODS

Animals. Female mice of the long-lived F1 hybrid strain C3B10RF were used by us previously for studies of ER (9). These 24-mo-old mice were used for the feeding time course. All other mice, including adrenalectomized and sham-operated mice, were 9-mo-old male Swiss Webster retired breeders (Jackson Laboratories, Bar Harbor, ME). Mice were maintained at 20–24°C and 50–60% humidity with lights on from 0600 to 1800 h. Mice had free access to water. Sentinel mice were kept in the same room as the experimental mice, and serum samples were screened every 6 mo for titers against 11 common pathogens. No positive titers were found during these studies. Animal use protocols were approved by the institutional animal use committee of the University of California, Riverside.

Diets and treatments. Mice used for the feeding time course experiment in Figure 1 were weaned at 28 d, housed individually and fed the control (405 kJ/wk) or ER diet (225 kJ/wk) until the age of 24 mo when they were used for the experiment. These diets are defined and formulated so that dietary groups receive equal amounts of protein, corn oil, minerals and vitamins per gram of body. Dietary energy was limited by reducing the amount of carbohydrates consumed. Both groups of mice were fed on Monday, Wednesday and Friday. On Friday the mice received 1.5 times the amount fed at all other feedings. Feeding was between 0900 and 1100 h. All food was routinely consumed before the next feeding. Beginning 30 d before these studies, the control mice were fed 4.1 g (54.4 kJ) of the control diet daily at 0900 h. The 50% ER mice were fed 2.3 g (32 kJ) of the ER diet daily at 0900 h. All food was routinely consumed within 30 min. On the day of the experiment, mice were weighed after 24 h of food deprivation (before feeding). Weights were 22.96 ± 1.49 g for ER and 37.12 ± 1.19 g for control mice. For the 0 time point, five mice from each group were chosen at random and killed just before the usual feeding. For the other time points, five mice from each dietary group were randomly chosen and killed 1.5, 5, and 12 h after feeding.

The food deprivation and feeding study is presented in Figure 2. To determine glucose-regulated protein 78 (GRP78) levels during food deprivation, mice were given 5.5 g (73.2 kJ) of control diet. Most food was consumed within 5 h, after which any uneaten food was removed. Mice were killed 5 h after the start of feeding (time 0), and after 2, 4, and 6 d (n = 3). To measure the effect of feeding on GRP78 levels, mice were deprived of food for 4 d and fed 5.5 g of the control diet per day thereafter. Mice were killed and their livers were removed immediately before feeding (time 0) and 2 and 4 d after feeding (n = 3). GRP78 protein levels were quantified as described (7).

For the studies presented in Figures 3-6, mice were deprived of food for 48 h (body weight = 36.6 ± 4.2 g), fed 5.5 g of control diet at 0900 h, and killed 90 min later. Diabetes was induced by three weekly intraperitoneal injections of STZ (10 mg/100 g of body) in 50 mmol/L sodium citrate, pH 4.5. Mice were diabetic 1 wk after the last injection. Mice with blood glucose level higher than 3 g/L (16.67 mmol/L) were used. Mice injected with equivalent volumes of sodium citrate served as controls for the STZ-diabetic mice. Dibutyryl cAMP (Sigma, St. Louis, MO; 18 mg/100 g of body), theophylline (Sigma; 3 mg/100 g of body), glucagon (Sigma; 300 µg of body), dexamethasone (Sigma; 125 µg/100 g of body), cycloheximide (Sigma; 4 mg/100 g of body), and puromycin (Sigma; 10 mg/100 g of body), were administered intraperitoneally to mice as specified in the figure legends. Mice received two doses of each drug or drug combination.

Effects of feeding on hepatic GRP78 and ERp72 mRNA. Energy-restricted (ER) and control mice, fed daily for 30 d, were deprived of food for 24 h and killed (n = 5, 0 time point) or fed and killed at 0, 1.5, 5, and 12 h after feeding (n = 5 at each time point). Values are means ± SD. Their weights after 24 h of food deprivation were 22.96 ± 1.49 g for ER and 37.12 ± 1.19 g for control mice. GRP78 mRNA (A) and ERp72 mRNA (B) from control (●) and ER (□) mice were quantified using dot-blot analysis. The plus sign represents the difference between ER and control mice at each time point (Fisher’s test, P < 0.01). The asterisk represents the difference from the 0 time point within each dietary group (Fisher’s test, P < 0.01). The 0- and 24-h time points are the same dataset.

RNA isolation and quantification. Mice were killed by cervical dislocation, and livers, kidneys and muscles were removed. Muscle from the hind legs and back was removed and pooled for each mouse. Tissues were flash frozen in liquid nitrogen. Approximately 0.2 g of frozen tissue was homogenized for 40 s in 4 mL of TRI Reagent (Molecular Research Center, Cincinnati, OH) using a Tekmar Tissuemizer (Tekmar, Cincinnati, OH) at a setting of 55. RNA was isolated as described by the TRI Reagent supplier. RNA was resuspended in FORMAzol (Molecular Research Center) and Northern and dot blots were performed using 20 and 10 µg of RNA, respectively. The RNA was analyzed using Northern blots to verify its integrity. Dot blots were used to quantify mRNA levels (7,14). Specific mRNA levels were normalized to the level of total RNA and/or mRNA present in each sample using hybridization with radiolabeled complementary DNA to 18S rRNA and/or transcription factor S-II, as indicated in the figure legends (7,16). The murine endoplasmic reticulum protein 72 (ERp72) 2.5 kb cDNA was excised with BamHI from pcD72–1 (17). The 1235-bp murine GRP75 coding fragment was excised with HindIII from pG7z-PBP1.8 (18). A 1.5-kb coding fragment of GRP78 cDNA was produced by digestion of p3C5 with EcoRI and PstI (19). A 1.4-kb hamster GRP94 coding fragment was produced by EcoRI and Sall digestion of pA43 (19). A 664-bp coding fragment of rat calreticulin was produced by polymerase chain reaction from GT10.U1 (20). The entire 2.4-kb cDNA of murine protein disulfide isomerase was excised from pGEM59.4 with SacI and BamHI (17). A 1-kb coding fragment of GRP78 cDNA was produced by digestion of p3C5 with EcoRI and PstI (19). A 1.4-kb hamster GRP94 coding fragment was produced by EcoRI and Sall digestion of pA43 (19). A 664-bp coding fragment of rat calreticulin was produced by polymerase chain reaction from GT10.U1 (20). The entire 2.4-kb cDNA of murine protein disulfide isomerase was excised from pGEM59.4 with SacI and BamHI (17). A 1-kb coding fragment of hamster GRP170 cDNA was excised with EcoRI and Xhol from pCstrmII (21). The 1.9-kb cDNA of murine ERp57 was excised with HindIII and SstI from pErP61 (22). The 1-kb cDNA of murine heat shock protein cognate 70 (Hsc70) was excised with EcoRI and Xhol from pCstrmII (21). The 1.9-kb cDNA of murine ERp57 was excised with HindIII and SstI from pErP61 (22). The 1-kb cDNA of murine heat shock protein cognate 70 (Hsc70) was excised with EcoRI and Xhol from pCstrmII (21).
FIGURE 2 Effects of food deprivation and feeding on GRP78 protein levels in mice determined by Western blot analysis. (A) Represents GRP78 levels 5 h after feeding (time 0), and after 2, 4 and 6 d of food deprivation. (B) Represents GRP78 levels at the indicated times during feeding after a 4-d period of food deprivation. The values are means ± SD (n = 5).

TN). The fragments were isolated by agarose gel electrophoresis and radioactively labeled using a [32P]-labeled antisense RNA probes using a MAXIScript kit as described by the supplier (Ambion, Austin, TX). These constructs were used as template for the synthesis of a 200-base fragment including all of exon 7 and the first 56 bases of intron 7 also was produced. The T7 RNA polymerase promoter was ligated to these polymerase chain reaction fragments. The hybridization of the 257-base RNA probe with GRP78 premRNA protected the 143 bases complementary to exon 7. A 277-bp cDNA fragment of S-II cDNA was synthesized and subcloned into pT7/T3 (16). [32P]-labeled RNA probes for the sense and antisense transcripts were synthesized in vitro and RNase protection assays performed. Hybridization with S-II mRNA protected the entire 277-base region of the probes. Protection of only the sense strand probes was detected. Fragmentations were analyzed using a 5% acrylamide/8 mol/L urea gel. Quantitation of the hybridized fragments was performed with ImageQuanNT (Molecular Dynamics, Sunnyvale, CA).

RNase protection assays. Two probes of the junction region of intron 7 and exon 7 of the GRP78 gene were produced by polymerase chain reaction using mouse genomic DNA as template. A 257-base fragment including all of exon 7 and the first 113 bases of intron 7 was produced. A 200-base fragment including all of exon 7 and the first 56 bases of intron 7 also was produced. The T7 RNA polymerase promoter was ligated to these polymerase chain reaction fragments using a Lig`nScribe kit as described by the supplier (Ambion, Austin, TX). These constructs were used as template for the synthesis of [32P]-labeled antisense RNA probes using a MAXIScript kit as described by the supplier (Ambion). RNase protection assays were performed with an RPA II kit as described by the supplier (Ambion). Hybridization of the 257-base RNA probe with GRP78 premRNA protected all 257 bases corresponding to exon 7 and the first 113 bases of intron 7. Hybridization of the 200-base RNA probe to premRNA protected 200 bases corresponding to all of exon 7 and the first 56 bases of intron 7. Hybridization of either probe to GRP78 mRNA protected the 143 bases complementary to exon 7. A 277-hp cDNA fragment of S-II cDNA was synthesized and subcloned into pT7/T3 (16). [32P]-labeled RNA probes for the sense and antisense transcripts were synthesized in vitro and RNase protection assays performed. Hybridization with S-II mRNA protected the entire 277-base region of the probes. Protection of only the sense strand probes was detected. Fragmentations were analyzed using a 5% acrylamide/8 mol/L urea gel. Quantitation of the hybridized fragments was performed with ImageQuanNT (Molecular Dynamics).

Plasma glucose and insulin. Plasma glucose, insulin and glucagon concentrations were determined using glucose (HK) 10 (Sigma), rat insulin radioimmunoassay and glucagon radioimmunoassay kits (Linco Research, St. Charles, MO).

Statistical analysis. The data shown in Figure 1 are expressed as means ± SD for five mice at each time point and analyzed using a one-way ANOVA followed by Fisher’s test. The analysis determined whether individual time point means differed from time 0 means within each dietary group. It also determined the differences between the means of the control and ER groups at each time point. Differences of P < 0.001 were considered significant. Data in Figure 3–6 are expressed as means ± SD. Significance of difference was determined with either Student’s unpaired t test (P < 0.05) or a one-way ANOVA followed by Fisher’s test (P < 0.001). All statistical analyses were performed with Minitab Statistical Software (Minitab, State College, PA).

RESULTS

Effects of ER and feeding on hepatic chaperone mRNA. Feeding of food-deprived mice rapidly induced the abundance of GRP78 and Erp72 mRNA (Fig. 1, A and B). These mRNA were induced several-fold 1.5 to 5 h after feeding (P < 0.01). Consistent with our previous reports, GRP78 and Erp72 mRNAs were lower in the ER mice after 24 h of food deprivation (0 time, P < 0.001) (8). The integrated levels of chaperone mRNA over the entire period were lower in the ER mice. Similar results were obtained for Hsc70, Erp57 and calreticulin mRNA (data not shown). These results suggest that the history of consumed energy affected the integrated levels of chaperone mRNA after feeding.

Energy consumption altered chaperone protein abundance. We examined the response of chaperone protein levels to energy intake. GRP78 protein began to decline after 2 d of food deprivation, assuming a lower steady-state level after a total of 4 d (Fig. 2A). Thus, in the absence of feeding-induced spikes of GRP78 mRNA, chaperone levels fell by ~30%. When mice that had been deprived of food for 4 d were fed daily, GRP78 protein returned to control levels in 4 d (Fig. 2B). These results are consistent with the greater stability of most chaperones with respect to their mRNA.

Feeding induced many chaperone mRNA in a number of tissues and species. The levels of nine chaperones were determined in mice 1.5 h after feeding. Hepatic GRP78 mRNA was induced approximately threefold (P < 0.001), and the mRNA for Erp57 (P < 0.001), Erp72 (P < 0.001), Grp94 (P < 0.001), Grp170 (P < 0.01), protein disulfide isomerase (P < 0.01), calreticulin (P < 0.01), and Hsc70 (P < 0.001) were induced to varying degrees by feeding (Fig. 3A). Hsc70 mRNA, which codes for the most abundant cytoplasmic chaperone, was induced nearly threefold. No change was found in the mitochondrial chaperone Grp75 (P > 0.05).

Feeding-related chaperone mRNA induction found in the liver (P < 0.05), extended to at least the kidney (P < 0.01) and muscle (P < 0.01; Fig. 3B). Hsc70 mRNA was also induced in these tissues (data not shown). In other studies, we found that a similar induction of hepatic chaperone mRNAs occurs in rats, indicating that this response is not species-specific (data not shown).

FIGURE 3 Effects of food deprivation in mice on gene and tissue specificity of the chaperone feeding response. (A) Quantitation of hepatic chaperone mRNA abundance in mice deprived of food for 48 h (n = 6, open bars) or from mice deprived of food for 48 h, fed and killed 1.5 h later (n = 6, closed bars). (B) Liver, kidney and muscle GRP78 mRNA from mice deprived of food for 24 h (n = 4) and from mice deprived of food for 24 h, fed, and killed 1.5 h later (n = 5). The data in B were from different mice than used in A. The mRNA were quantified by dot-blotting and Northern blotting, and similar results were obtained with each RNA sample using either of these techniques. The dot-blotting results are shown. Asterisks indicate significantly different from food-deprived mice (t test, ***P < 0.05; **P < 0.01; ***P < 0.001).
Feeding induced the hepatic GRP78 primary transcript. The level of gene expression regulated by feeding was investigated using RNase protection studies. Liver RNA purified 1.5 h after feeding protected more of a 257-base probe fragment representing the primary transcript (compare Fig. 4A, lanes 10–12 with lanes 7–9). Similar results were obtained with a second probe for the primary transcript, which produced a 200-base fragment (compare Fig. 4A, lanes 16–18 with lanes 13–15). In each case, RNA from fed mice also protected more of a 143-base probe fragment representing tRNA.

Quantification of these data indicated that feeding induced GRP78 mRNA (Fig. 4B; P < 0.001) and its precursor (Fig. 4C; P < 0.001) to the same extent. Similar results were obtained using a probe spanning the third intron and fourth exon (data not shown). In addition, when these probes were used to examine the primary and mature GRP78 transcript levels in ER mice, the results indicated that ER reduced the level of expression of both transcripts to the same degree (data not shown). These data suggest that feeding and ER have nuclear effects on chaperone expression, feeding positively and ER negatively.

Inhibitors of protein synthesis. To investigate the basis for the feeding response, we used inhibitors of protein synthesis. Food-deprived mice were treated with levels of cycloheximide or puromycin sufficient to inhibit >95% of protein synthesis in the liver (24). Cycloheximide strongly and rapidly induced GRP78 mRNA in both food-deprived and fed mice (Fig. 5A; P < 0.001). Puromycin modestly induced GRP78 mRNA in food-deprived mice (P > 0.05). Feeding induced the chaperone mRNA in puromycin-treated mice (Fig. 5A; P < 0.001). Thus, the feeding induction apparently does not require de novo protein synthesis. These results suggest that a rapidly turning-over factor may repress chaperone mRNA levels in food-deprived mice.

As a positive control, the known effects of the protein synthesis inhibitors on PEPCK mRNA were determined. Food deprivation induced and feeding repressed PEPCK mRNA (Fig. 5B; P < 0.001) (25). Cycloheximide increased PEPCK mRNA in both food-deprived and fed mice (25). Puromycin strongly induced PEPCK mRNA in both food-deprived and fed mice (P < 0.001). These effects indicate that the inhibitors were efficacious in our studies.

Pancreatic and adrenal hormones and serum glucose. The physiological hallmarks of the food deprivation/feeding transition are increased circulating insulin and decreased circulating glucagon. In the studies shown in Figure 6A, food-deprived and fed sham-injected mice had serum glucose concentrations of 4.67 ± 0.28 and 6.72 ± 0.44 mmol/L (0.84 ± 0.05 and 1.21 ± 0.08 g/L), serum insulin concentrations of 0.5 ± 0.2 and 1.3 ± 0.2 nmol/L, and serum glucagon concentrations of 0.14 ± 0.02 and 0.08 ± 0.01 μg/L, respectively.

To investigate whether these hormones were involved in the feeding responsiveness of chaperone mRNA, we examined the effects of cAMP, glucagon and STZ-induced diabetes on GRP78 mRNA. Administration of either dibutyryl-cAMP or glucagon significantly reduced the response of GRP78 mRNA to feeding (Fig. 6A; P < 0.001). STZ-induced diabetes also blunted the response to feeding (P < 0.001) but did not modify the mRNA levels after food deprivation (P > 0.05). The combination of STZ-diabetes and cAMP obliterated the postprandial induction of GRP78 mRNA (P < 0.001). Together, these results suggest that glucagon, acting to increase intracellular cAMP levels, suppresses chaperone gene expression. Furthermore, insulin was required for the full feeding response.

The possible role of adrenal hormones in the postprandial induction of GRP78 mRNA was examined using adrenalectomized mice (Fig. 6B). Adrenalectomy had no effect on GRP78 mRNA levels after food deprivation (P > 0.05). Adrenalectomy increased the magnitude of the postprandial induction by approximately twofold (P < 0.001). The induction of Erp72, GRP94 and GRP170 was also enhanced in the adrenalectomized mice (data not shown). Dexamethasone did not affect GRP78 mRNA expression in food-deprived or fed adrenalectomized mice (Fig. 6B; P > 0.05). These results suggest that glucocorticoids are not responsible for the chaperone response to feeding.

**FIGURE 4** Effects of feeding on hepatic GRP78 mRNA and premRNA abundance in mice. (A) Representative RNase protection studies for hepatic GRP78 premRNA and mRNA in mice deprived of food for 48 h or 1.5 h after feeding. Hepatic RNA was hybridized either to probe 1, which produced a 257-base protected fragment (labeled GRP78 premRNA in lanes 7–12), or to probe 2, which produced a 200-nucleotide protected fragment (labeled GRP78 premRNA in lanes 13–18). With both probes, GRP78 mRNA protected a 143-nucleotide fragment labeled GRP78 mRNA. An internal control S-II probe produced a protected fragment 277 nucleotides long, labeled S-II mRNA. Lane 1, RNA markers. Lanes 2–6, hybridization of the indicated probes with yeast tRNA. Lanes 7–12, hybridization of probe 1 and the S-II probe with RNA from food-deprived (lanes 7–9) and fed (lanes 10–12) mice. Lanes 13–18, hybridization of probe 2 and the S-II probe with RNA from food-deprived (lanes 13–15) and fed (lanes 16–18) mice. Quantification of the abundance of the protected fragments representing the GRP78 mRNA (B) and premRNA (C). The intensity of the protected fragments was quantified and normalized (n = 6). Open bars are food-deprived and filled bars are fed RNA levels. Significant differences are indicated as in Figure 3.

**DISCUSSION**

Here we show that feeding rapidly increased the level of mRNA for the endoplasmic reticulum and major cytoplasmic chaperone genes. Feeding induced the level of the GRP78 primary transcript, suggesting transcriptional regulation of its expression, because other means of nuclear regulation of gene
expression are much less common. GRP78 protein levels were also regulated by feeding. The GRP78 mRNA response occurred in liver, kidney and muscle of mice, and in the liver of rats. Feeding seemed to release GRP78 expression from the effects of an unstable inhibitor and seemed to require high insulin and low glucagon and cAMP levels. Glucocorticoids did not seem to affect the response.

We previously reported data that support an association between energy consumption and chaperone mRNA levels. GRP78 mRNA and protein levels are lower in the livers of mice subjected to ER (7,8). Chaperone mRNA levels also are directly related to the average amount of energy consumed by mice (14). Moreover, when ER mice are fed a control diet, chaperone mRNA increases over a period of 2 wk to the levels found in control mice (8). However, only in the studies reported here has a rapid cause and effect relationship between food consumption and chaperone mRNA expression been reported. Because this feeding-related pathway functions in vivo, in unstressed mice, it is very likely to be of equal or greater importance than the other pathways described for the physiological regulation of the gene.

The data presented here make it likely that the feeding response does not involve a previously reported pathway for chaperone regulation. Endoplasmic reticulum-related stress induces endoplasmic reticulum chaperone gene transcription via the endoplasmic reticulum stress response element, which contains an unfolded protein response element (26). This is the most studied pathway for controlling the expression of endoplasmic reticulum chaperones. Two other pathways involving the cellular response to hypoxia and to growth factors also have been described (27,28). However, the regulatory pathway described here seems to be completely novel. GRP78 mRNA was induced by the protein synthesis inhibitor cycloheximide (Chx) or puromycin (Pmy), and killed 1 h after the second injection. Food-deprived Chx and food-deprived Pmy mice received two injections of vehicle 1 h apart. Fed + Chx and fed + Pmy mice were injected with vehicle 6 h before and 30 min after feeding and killed 1 h later. Food-deprived + Sham mice were sham-operated mice injected with vehicle 6 h before and 30 min after feeding and killed 1 h after the last injection. Food-deprived Adx + Sham were adrenalectomized mice injected with vehicle 7.5 h and 1.5 h before killing. Fed Adx + Sham were adrenalectomized mice injected with vehicle 6 h before and 30 min after feeding and killed 1 h later. Food-deprived Adx + Dex were adrenalectomized mice injected with Dex 7.5 h and 1.5 h before killing. Fed Adx + Dex were adrenalectomized mice injected with Dex 6 h before and 30 min after feeding and killed 1 h later. Values are means ± SD, n = 6. Bars without common letters differ (Fisher’s test, P < 0.001).

Because many chaperones are relatively stable proteins,
their protein levels change more slowly than their mRNA levels. GRP78 protein has a half-life of over 24 h in cultured cells (31). However, its mRNA in mouse liver must be much less stable, because it changes rapidly in response to feeding (Fig. 1). The slow changes in chaperone protein levels effectively integrate the feeding-related peaks in chaperone mRNA into longer-term adjustments of chaperone protein levels to energy intake. Interestingly, long-term 40% ER decreases GRP78 protein levels by 65% (9), approximately twice the reduction produced by 4 d without food. ER also reduces ERP72 and calreticulin by 60%, ERP57 by 35%, and GRP170 by 40% (9). These results suggest that longer-term reductions in dietary energy intake produce greater reductions in chaperone protein than acute food deprivation does. We have found that reduction of endoplasmic reticulum chaperones by ER results in secretion of twice as much albumin, 63% more 1-antitrypsin, and 250% more of a 31.5-kDa protein 2 h after their synthesis (9). These increased rates of plasma protein secretion may reduce serum protein dwell time and be a major contributor to the reduced glycoxidation damage found in ER animals.

The direct relationship between energy consumption and chaperone levels may have other health consequences. Elevated chaperone levels prevent apoptosis, and decreased chaperone levels enhance apoptosis (32), a function implicated in tumorigenesis (33). Approximately 21% of mice die of hepato-toma, mostly late in life, and 78% of mice of this strain die of some form of neoplasia, and this death rate accelerates dramatically with age (34). ER, which lowers chaperone levels, decreases or delays cancer incidence (34). Furthermore, two 5-d cycles of acute food deprivation increase apoptosis in preneoplastic lesions and reduce rates of chemical carcinogenesis (35,36). Thus, the effects of ER and acute food deprivation on chaperone levels may be the source of at least a part of their anti-neoplastic effects. The effects of ER on the expression of other apoptosis-related genes and on genes for xenobiotic detoxification may also contribute to the anti-neoplastic effects of ER (15).

Our studies suggest that insulin and glucagon may act together to regulate hepatic chaperone mRNAs in vivo (Fig. 6A). Feeding is known to decrease glucagon and increase insulin levels. Both glucagon and dibutyryl-cAMP blunted the feeding induction of GRP78 mRNA. Thus, glucagon seems to be a negative regulator of GRP78 expression in vivo. The feeding induction of GRP78 mRNA was significantly reduced in STZ-diabetic mice. This result and the absence of a feeding response in STZ-diabetic dibutyryl-cAMP-treated mice suggest that the action of both hormones is required for the full response. However, the results could be due to the effects of other factors that are either insulin and/or cAMP responsive or that change levels after feeding. Additional studies will be required to distinguish these possibilities.

A number of previous studies have shown that cAMP and insulin are capable of regulating GRP78 gene expression. A binding site for the cAMP-response element binding protein has been identified in the 5′-region of the gene (37). It differs from a cAMP response element by only one nucleotide. Transcription of the GRP78 promoter is stimulated by cAMP in GH3 pituitary cells and FRTL thyroid cells but not in several other cell types (19,38,39). Likewise, there is a perfect AP-1 site at position −210 flanking the mouse GRP78 gene (40). AP-1 sites mediate the insulin responsiveness of several hepatic genes (41). Insulin increases GRP78 protein synthesis in L929 cells that had been previously incubated in serum-free medium for at least 24 h (42). These data, coupled with our results, suggest that in some cells and tissues, insulin and glucagon signaling pathways are involved in chaperone gene regulation. Thus, chaperone synthesis can be modulated by physiological effectors distinct from those modulated by injurious stress.

Because food deprivation increases and feeding decreases circulating glucocorticoid levels (43), we investigated the effects of glucocorticoids on the feeding response. The results were not consistent with a major role for glucocorticoids in the feeding induction of GRP78 mRNA (Fig. 6B). Feeding induced the gene in adrenalectomized mice. Furthermore, the feeding response was not affected by dexamethasone administration. Therefore, glucocorticoids do not seem to participate in the feeding response. In fact, the feeding induction of the GRP78 gene was approximately doubled in adrenalectomized mice. Thus, some adrenal hormones, perhaps catecholamines, may blunt the induction of this chaperone by feeding.

In summary, we have shown that feeding rapidly and strongly induced the mRNA for the major cytoplasmic chaperone, HSC70, and most endoplasmic reticulum chaperones examined. Feeding also induced endoplasmic reticulum chaperone mRNA in at least three different tissues. Chaperone mRNA abundance may be regulated by feeding and ER. Feeding seemed to release chaperone gene expression from the effects of an unstable inhibitor. Insulin seemed to enhance, and glucagon and cAMP seemed to mediate the feeding response. These results suggest that postprandial changes in glucagon levels may be the primary mediator of the response. Gastrointestinal and adrenal hormones but not glucocorticoids also may have a role in the feeding response. This response seems to link energy consumption to chaperone expression in at least three tissues of mice.

LITERATURE CITED


FEEDING-REGULATED CHAPERONE GENE EXPRESSION


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