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Ethanol-Responsive Genes in Neural Cells Include the 78-Kilodalton Glucose-Regulated Protein (GRP78) and 94-Kilodalton Glucose-Regulated Protein (GRP94) Molecular Chaperones

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SUMMARY

Previously we found that ethanol increases expression of the constitutive 70-kDa heat shock protein (Hsc70) in NG108–15 neuroblastoma × glioma cells. We suggested that known ethanol actions on cellular protein trafficking may relate to Hsc70 induction because Hsc70 functions as a molecular chaperone. Here we use a subtractive hybridization protocol to isolate ethanol-responsive genes (EtRGs). Northern blot hybridization verified ethanol-induced increases in mRNA abundance for five cDNA clones isolated from ethanol-treated NG108–15 neuroblastoma × glioma cells. DNA sequence analysis identified one EtRG as 94-kDa glucose-regulated protein (GRP94), a member of the "glucose-responsive" subgroup of stress proteins. Other identified EtRGs included an insulin-induced growth-response protein gene and an intracisternal A-type particle gene. Sequence analysis of the remaining two EtRGs showed no homology in DNA sequence databases. All EtRGs showed wide tissue expression, except SL64, which was not detected in Northern blot analyses of adult mouse or rat tissues. Ethanol also increased mRNA abundance for 78-kDa glucose-regulated protein (GRP78), a molecular chaperone known to function in glycoprotein trafficking and usually coordinately regulated with GRP94. However, ethanol induced GRP94 more than GRP78, a pattern distinct from those of other inducers of these genes. All EtRGs, including GRP94 and GRP78, showed similar ethanol concentration-dependent increases in mRNA abundance. In contrast, thapsigargin and other inducers of glucose-responsive proteins increased GRP94 and GRP78 mRNA levels without altering expression of other EtRGs. Our studies demonstrate that several molecular chaperones constitute a subset of EtRGs. Ethanol appears to regulate these EtRGs by a unique mechanism, rather than one shared by classical inducers of stress proteins.

Alcoholics show a remarkable ability to adapt to the intoxicating and sedating effects of ethanol. For example, whereas naive subjects experience severe or fatal CNS depression at blood alcohol levels above 300 mg/dl (65 mM), alcoholics can remain conscious and alert with blood alcohol levels above 1500 mg/dl (325 mM) (1). Along with the development of tolerance, alcoholics exhibit physical dependence, whereby they experience symptoms of withdrawal upon cessation of drinking. It is our hypothesis that specific alterations in CNS gene expression underlie the development of tolerance and dependence.

Ethanol-induced changes in gene expression have been documented in cultured neuronal cells (2–6) and animal models (7–12). These EtRGs include enzymes, receptors, G proteins, and other signal transduction molecules. In several instances such changes in EtRG expression appear to produce an adaptation to the acute effects of ethanol. For example, increases in γ-aminobutyric acid type A receptor function have been suggested as a major factor in the acute intoxicating effects of ethanol (13–15). Adaptive decreases in γ-aminobutyric acid type A subunit mRNA abundance have been observed in some brain regions after chronic ethanol exposure (8, 12, 16).

Our previous studies have shown that chronic ethanol exposure increases transcription of the molecular chaperone Hsc70 in NG108–15 neuroblastoma × glioma cells (17). Molecular chaperones serve to stabilize certain protein configurations during protein synthesis, processing, or trafficking (18). We therefore previously proposed that Hsc70 induction could represent a molecular adaptation to an action of ethanol on protein trafficking. Indeed, ethanol has been shown to decrease receptor-mediated endocytosis and processing of glycoproteins (19–24).

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ABBREVIATIONS: CNS, central nervous system; EtRG, ethanol-responsive gene; IAP, intracisternal A-type particle; PCR, polymerase chain reaction; GRP, glucose-regulated stress protein; HRS, insulin-induced growth-response protein; PERT, phenol-enhanced reassociation technique; Hsc70, 70-kDa constitutive heat shock protein; GRP78, 78-kDa glucose-regulated protein; GRP94, 94-kDa glucose-regulated protein.

Quantitative two-dimensional gel electrophoresis studies show that chronic ethanol exposure coordinately increases or decreases the expression of a significant number of proteins in cultured neuroblastoma cells (4). The overall adaptive response of the CNS may represent the composite of such changes in gene expression. Identification of other EtRGs may reveal clinically useful cellular targets of ethanol action, particularly if common functions or mechanisms of induction can be identified for these genes. For example, we suspect that ethanol might induce molecular chaperones other than Hsc70. Here we isolated EtRGs from cultured neuroblastoma cells and identified two of these EtRGs as the glucose-responsive molecular chaperones GRP78 and GRP94. The protein products of these genes reside in the endoplasmic reticulum and function in the processing and trafficking of glycoproteins. Agents that alter glycoprotein processing or trafficking are known to induce GRP78 and GRP94 (25). We show that, although all of the EtRGs identified here respond to ethanol with similar concentration-response profiles, they are not all chaperones or regulated by typical inducers of GRP78/GRP94. Ethanol thus induces a novel molecular response that includes a subset of molecular chaperones.

Experimental Procedures

Materials and general molecular biology procedures. All radioisotopes were purchased from NEN-DuPont. Materials for construction of cDNA libraries were from Stratagene. Linker adapters and reagents for subtracted cDNA library and multi-tissue Northern blots were obtained from Clontech. All other enzymes and reagents for recombinant DNA procedures were from Boehringer Mannheim. Other chemicals were of reagent grade from Sigma Chemical Co. Plasmids for Northern or slot blot hybridization of GRP78 and 18 S rRNA were from Dr. Amy Lee (University of Southern California) and Dr. Dan Lowenstein (University of California, San Francisco), respectively. Northern blot or plaque hybridizations and general cloning techniques were performed according to standard protocols.

cDNA library construction. We cultured NG108–15 cells as described previously, in medium containing 10% NuSerum (Collaborative Research) or in a completely defined culture medium (17). RNA for preparation of cDNA libraries was from cells grown for 4 weeks in the presence or absence of 100 mM ethanol. We isolated total RNA and poly(A)$^+$ RNA as described previously (17). cDNA libraries from control or ethanol-treated RNA were prepared using a λZapII cloning kit, exactly as described by the supplier (Stratagene). After *in vitro* packaging, libraries were titered and then amplified once before storage. The complexity of both libraries exceeded 2×10^6.

Preparation of subtracted cDNA. Subtractive hybridization was performed using a modified version of the PERT described by Travis and Sutcliffe (26). "Mass excision" of the control λZap cDNA library generated driver DNA for PERT reactions. We performed *in vivo* excision as described by the supplier (Stratagene), except that the initial incubation with R418 helper phage was for 1.5 hr. Phagemids were then used to infect XL1-Blue cells, and plasmid DNA was rescued by growth of the bacteria for 5 hr at 42° in the presence of 50 μg/ml ampicillin. Plasmid DNA was digested with *Eco*RI and *Xho*I to excise recombinant inserts before subtractive hybridization was performed.

^{32}P-labeled cDNA (cDNA$_{etoh}$) was prepared from ethanol-treated poly(A)$^+$ RNA using oligo(dT) as primer. Subtractive hybridization was performed essentially as described by Travis and Sutcliffe (26), using 200 μg of control plasmid double-stranded DNA as driver. After denaturation of driver DNA, PERT hybridizations continued for 18 hr at room temperature with constant agitation. The single-stranded DNA fraction was isolated by hydroxyapatite chromatography followed by a second round of PERT hybridization. The final single-stranded DNA fraction was used as a probe for screening a cDNA$_{etoh}$ library or was used for construction of a subtracted cDNA library (see below).

Screening of 50,000 phage from the cDNA$_{etoh}$ library was done according to standard methods, using subtracted probe ($1–2 \times 10^6$ cpm). Positive plaques were picked, rescreened with fresh subtracted probe, and plaque purified. Resulting isolates were converted to phagemid vectors by *in vivo* excision, as described by the supplier (Stratagene). Phagemid DNA was used for DNA sequence analysis (see below) or Northern blot hybridization. PCR amplification of insert regions generated recombinant portions of clones. PCR primer oligonucleotides were directed against the flanking T3 and T7 polymerase recognition sites (Stratagene).

Hybridization probes were prepared by hexamer labeling, using PCR products of positive plaques as template (27). Probes were used to screen slot blots containing RNA from control and ethanol-treated NG108–15 cells. Northern blot hybridizations verified putative EtRGs (17).

Subtracted cDNA library. We generated a cDNA library from subtracted cDNA using a PCR amplification procedure (28). Hybridization reactions used photobiotinylated driver plasmid DNA (Pierce Chemicals) for generation of subtracted cDNA. This was done to ensure removal of driver DNA before PCR amplification. After the second round of hybridization and hydroxyapatite chromatography, residual driver DNA was removed by addition of strepavidin followed by phenol extraction (29). Second-strand synthesis and S1 nuclease treatment were then performed according to standard protocols. PCR amplification adapters (Clontech) were then ligated onto the blunt-ended, double-stranded DNA, and the material was subjected to 35 cycles of amplification exactly as described by the manufacturer (Clontech). After PCR, centrifugation over Chroma-spin 100 columns (Clontech) removed primers and nucleotides. The amplified DNA was digested with *Eco*RI and ligated to *Eco*RI-digested, dephosphorylated, λZapII arms (Stratagene). After *in vitro* packaging (Gigapack Gold; Stratagene) and titering, the subtracted cDNA library was amplified once before storage.

We identified EtRGs from the subtracted cDNA library by a random selection of plaques. Phage were plated at low density (100 phage/100-mm^2 dish) and individual plaques were picked. PCR-amplified insert portions of each clone were then used as described above to identify EtRGs using slot blot and Northern blot hybridizations.

DNA sequence analysis. DNA sequence analysis of confirmed EtRGs was by the chain termination method (30) using a manual procedure (Sequenase; United States Biochemicals) or by automated DNA sequencing (University of California, San Francisco, Biomolecular Resource Laboratory). Sequences were compared with the nonredundant compiled file of DNA sequence databanks using the BLASTN and BLASTX programs, through the National Center for Biotechnology Information (31). Sequence data from unique EtRGs (SL64 and SP54) were submitted to GenBank.[1]

Results

Isolation of EtRGs by subtractive hybridization. We used a subtractive hybridization screening protocol to isolate EtRGs (Fig. 1). This approach avoids prior assumptions as to EtRG function. We used subtracted probe to screen a cDNA library constructed from ethanol-treated NG108–15 neuroblastoma × glioma cells. In addition, we isolated EtRGs by random selection of a cDNA library prepared from PCR-amplified subtracted cDNA (see Experimental Procedures). In both cases, we synthesized tracer cDNA using RNA from NG108–15 cells that had been treated with 100 mM ethanol for 4 weeks. This treatment regimen should identify genes that

[1] GenBank accession numbers for SL64 and SP54 are L31926 and L31927, respectively.

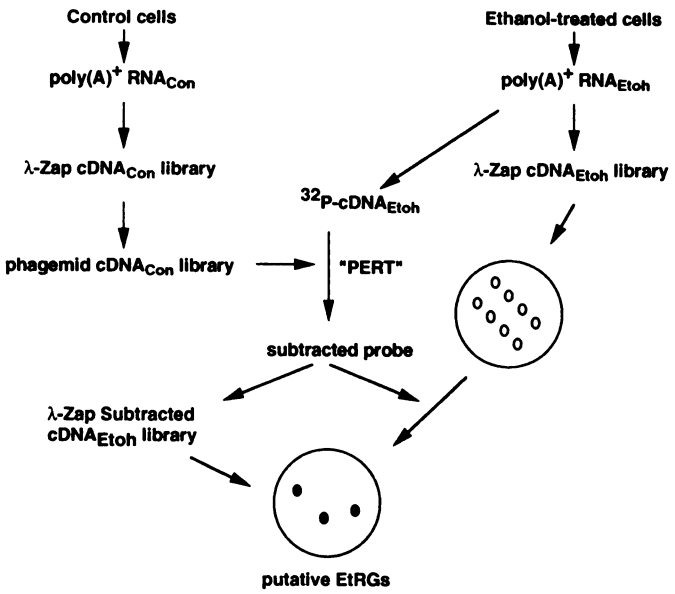

Fig. 1. Outline of subtractive hybridization protocol. Refer to Experimental Procedures for details. Parallel cultures of NG108–15 cells were grown with or without 100 mM ethanol for 4 weeks before RNA isolation. The diagram describes the use of subtractive probe for both direct screening of EtRGs and preparation of a subtracted cDNA library. Driver phagemid DNA was photobiotinylated before PERT when used for preparation of the subtracted cDNA library.

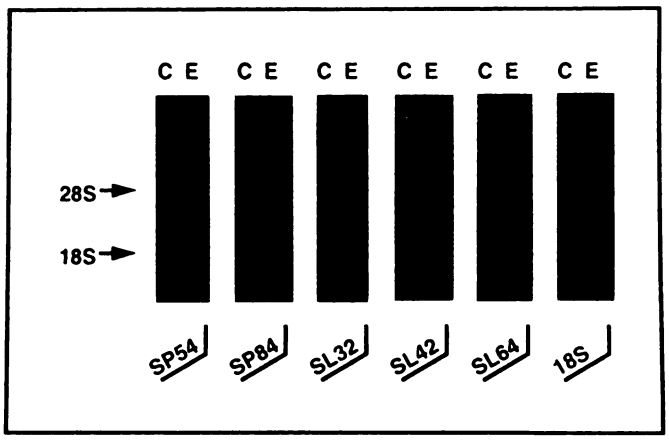

Fig. 2. Northern blot analysis of representative EtRGs. Total RNA was isolated from NG108–15 cells that had been grown in defined medium with (*E*) or without (*C*) 100 mM ethanol for 4 weeks. RNA (10 μg) was analyzed by Northern blot hybridization using probes prepared from isolated EtRGs (SP54, SP84, SL32, SL42, and SL64) or 18 S rRNA. The positions of 18 S and 28 S rRNA are indicated. Results are representative of experiments repeated twice.

undergo stable rather than transient induction with ethanol. The 4 weeks of ethanol treatment also allow detection of possible gene amplifications accompanying long term ethanol exposure. Previously we showed that ethanol induces the Hsc70 and phosducin-like protein genes to maximum levels within 24 hr of exposure and this induction is stable for up to 4 weeks as long as ethanol is present (5, 17). The concentration of ethanol (100 mM) used for these studies has previously been shown to induce EtRGs (2–5, 17) and is near the mean blood alcohol concentration seen in a large series of sober alcoholics (32).

Subtractive hybridization screening identified five cDNA clones with ethanol-induced increases in mRNA abundance on slot blot hybridization analysis (data not shown). These ethanol inductions were verified by Northern blot hybridization, as shown in Fig. 2. Table 1 gives estimated molecular weights for respective mRNA species. All clones showed mRNA inductions with ethanol. Hybridizations with 18 S rRNA (Fig. 2) confirmed equal loading of RNA for the control and ethanol-treated samples. Control hybridizations with probes for β-actin, lactate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase showed no changes with ethanol treatment (data not shown).

DNA sequence analysis and tissue distribution of EtRGs. DNA sequence analysis of the isolated EtRGs identified three genes. As shown in Table 1, clones SP84, SL32, and SL42 are GRP94, an IAP, and an HRS, respectively. The DNA sequences of SP54 and SL64 showed no significant homology to any entry in the nucleic acid databases. Identified EtRGs are hereafter referred to by their proper gene names.

We further characterized the novel EtRGs, SP54 and SL64, by studying their tissue distribution by Northern blot analysis. We included HRS in this analysis to further document the expression of this recently identified gene (33). Other investigators previously showed that GRP94 and IAP are widely expressed (25, 34). Fig. 3 shows that SP54 and HRS also have

TABLE 1

Summary of results from subtractive hybridization screening for EtRGs

EtRGs were isolated by subtractive cloning from NG108-15 cells that had been treated for 4 weeks with 100 mM ethanol. SP and SL clones were identified using subtractive probe or a subtractive library, respectively, as described in the text. Molecular sizes were calculated from Northern blot analyses, using comercially prepared molecular weight standards (BRL). DNA sequence identities were determined using the BLASTN and BLASTX programs, through the National Center for Biotechnology Information.

Clone number	mRNA size[a]	DNA sequence[b]
	kilobases	
SP54	3.6	Unique
SP84	3.0	GRP94
SL32	8.0, 3.6	IAP
SL42	3.6, 1.9	HRS
SL64	2.6	Unique

[a] Approximate size of respective mRNAs by Northern blot analysis.

[b] The sequence of >200 base pairs from SP84 was identical to that of mouse GRP94 (39). Similarly, sequencing of >200 base pairs from SL42 showed >98% identity to a cDNA for HRS that was recently isolated from rat hepatoma cells (33). The DNA sequence of 580 base pairs from SL32 was >98% homologous to an isolate of a mouse IAP gene (34). Clones listed as unique had no significant homologies identified in the Nonredundant DNA or Protein Sequence Databanks, through the National Center for Biotechnology Information.

wide tissue distributions. Our studies showed that HRS was expressed at high levels in brain and testis, in addition to other tissues also reported by Diamond *et al.* (33). Similarly, we also observed multiple forms of the HRS mRNA (see also Fig. 2), which Diamond *et al.* (33) suggested represent autoprocessed forms, because this gene product may have a function in RNA processing. SP54 was most abundant in testis and spleen.

We were unable to obtain any hybridization signal with SL64 using mouse or rat poly(A)+ or total RNA in multi-tissue Northern blot analysis (data not shown). In contrast, the same SL64 probe produced a strong hybridization signal with NG108–15 cell total RNA (Fig. 2). This suggests that the adult rat and mouse tissues surveyed either do not express SL64 or express this gene at a very low level.

Evidence that GRP78 is also an EtRG but not all EtRGs are stress proteins. The identification of GRP94 as an EtRG suggests that ethanol may act on GRPs (25). If this is the case, then ethanol should also induce GRP78, a resident

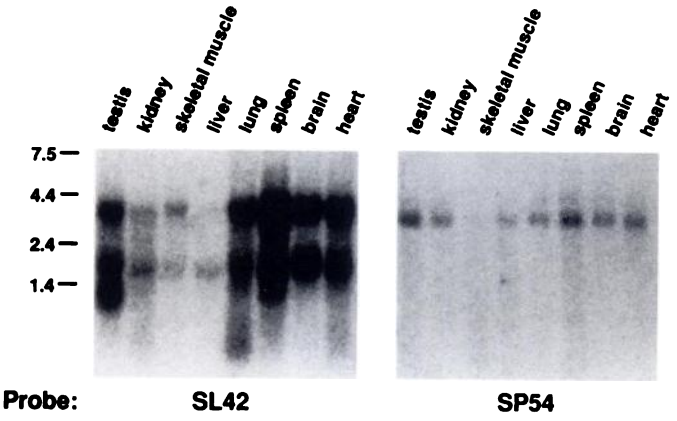

Fig. 3. Tissue expression of the EtRGs SL42 and SP54. A commercially prepared (Clontech) Northern blot of poly(A)$^+$ RNA from various mouse tissues was hybridized to probes from SL42 (HRS) and SP54. Hybridization signals were detected using a Bio-Rad GS250 PhosphorImager. *Numbers on the left*, positions and sizes (nucleotides × 10^3) of molecular weight markers.

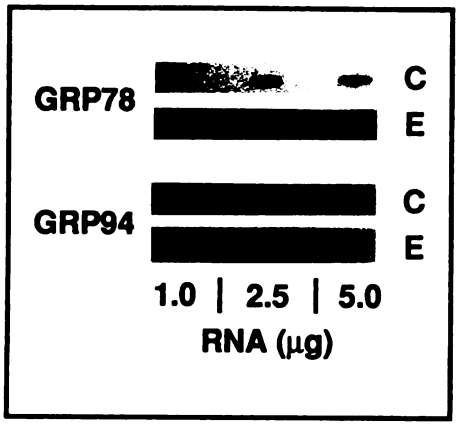

Fig. 4. Comparison of GRP78 and GRP94 responses to ethanol. Total RNA was isolated from NG108–15 cells that had been treated with (*E*) or without (*C*) 100 mM ethanol for 24 hr. RNA (1, 2.5, or 5 μg) was applied to nylon membranes using a slot blot apparatus and was then probed for either GRP78 or GRP94. Results are representative of experiments repeated 10 times.

endoplasmic reticulum protein coordinately regulated with GRP94. GRP78 functions as a molecular chaperone in glycoprotein trafficking (35). Slot blot hybridization of total RNA from NG108–15 cells treated with or without 100 mM ethanol for 24 hr (Fig. 4) showed ethanol-induced increases in mRNA abundance for both GRP78 and GRP94. Densitometry analysis of the autoradiograms determined the ethanol inductions to be approximately 2-fold for both GRP94 and GRP78 (see Fig. 6 for data from multiple experiments).

Because GRP78 and GRP94 are EtRGs, it seemed possible that some or all of the other EtRGs we have identified are also GRPs. We therefore determined whether these EtRGs responded to thapsigargin, a known inducer of GRP expression (36). Thapsigargin selectively depletes endoplasmic reticulum stores of calcium through inhibition of an ATPase required for calcium sequestration (37). Thapsigargin treatment caused striking inductions of GRP78 and GRP94 in NG108–15 cells (Fig. 5). However, no other EtRG exhibited thapsigargin-induced increases in mRNA abundance. Similarly, other inducers of GRP expression, including calcium ionophore A23187 (1 μM,

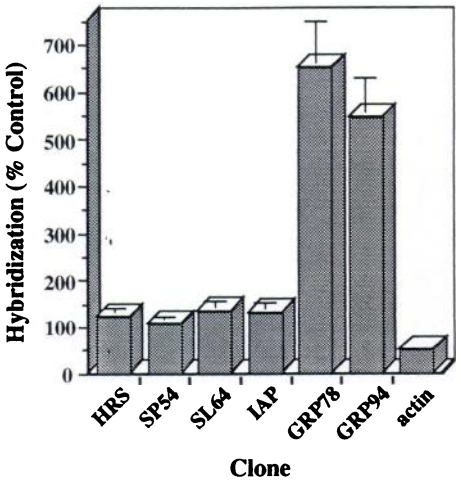

Fig. 5. Response of EtRGs to thapsigargin. NG108–15 cells were grown for 24 hr in the presence or absence of 100 nM thapsigargin. Control cultures were treated with carrier solvent (dimethylsulfoxide) alone. Total RNA was then isolated and analyzed by slot blot hybridization using the indicated probes. Hybridization was quantitated by autoradiography and computerized densitometry. Results are expressed as a percentage of hybridization obtained in control cultures and are the mean ± standard deviation from triplicate determinations. Similar results were obtained in experiments repeated three times. Similar results were seen using brefeldin A (5 μg/ml) or calcium ionophore A23187 (1 μM) to induce GRP expression (not shown).

24 hr) and brefeldin A (5 μg/ml, 24 hr), did not induce any EtRG other than GRP94 and GRP78 (data not shown).

Ethanol concentration-response curves for individual EtRGs after 24 hr of ethanol exposure. To further investigate possible differences between "stress protein-related" and other EtRGs, we determined ethanol concentration-response curves for all EtRGs identified in this study. NG108–15 cells were exposed to varying concentrations of ethanol for 24 hr and individual RNAs were quantitated by slot blot hybridization. Preliminary time course experiments showed that all EtRGs reached maximum induction levels by 24 hr (data not shown). This incubation period also corresponds to the time needed for production of signs of tolerance in animal models of chronic ethanol exposure (38). The concentrations of ethanol used similarly include a range found in actively drinking alcoholics (32) and shown to induce other EtRGs (2–5, 17). We included Hsc70 in these experiments to provide concurrent data on another ethanol-responsive stress protein.

Fig. 6 shows that the concentration-response curves for all EtRGs were similar in their overall profiles. There were no statistically significant differences between induction curves for GRP94 or GRP78 and other EtRGs (by analysis of variance), although there was a tendency for induction of GRP94, GRP78, and SL64 to plateau at lower ethanol concentrations (50 mM). The induction of GRP94 by 100 mM ethanol exceeded that of GRP78 in each experiment performed (seven experiments). The Hsc70 response in these experiments was virtually identical to our previous results on ethanol induction of this gene (17).

Discussion

Using subtractive hybridization cloning, we have isolated a number of new EtRGs and found that these include genes coding for several proteins that function as molecular chaper-

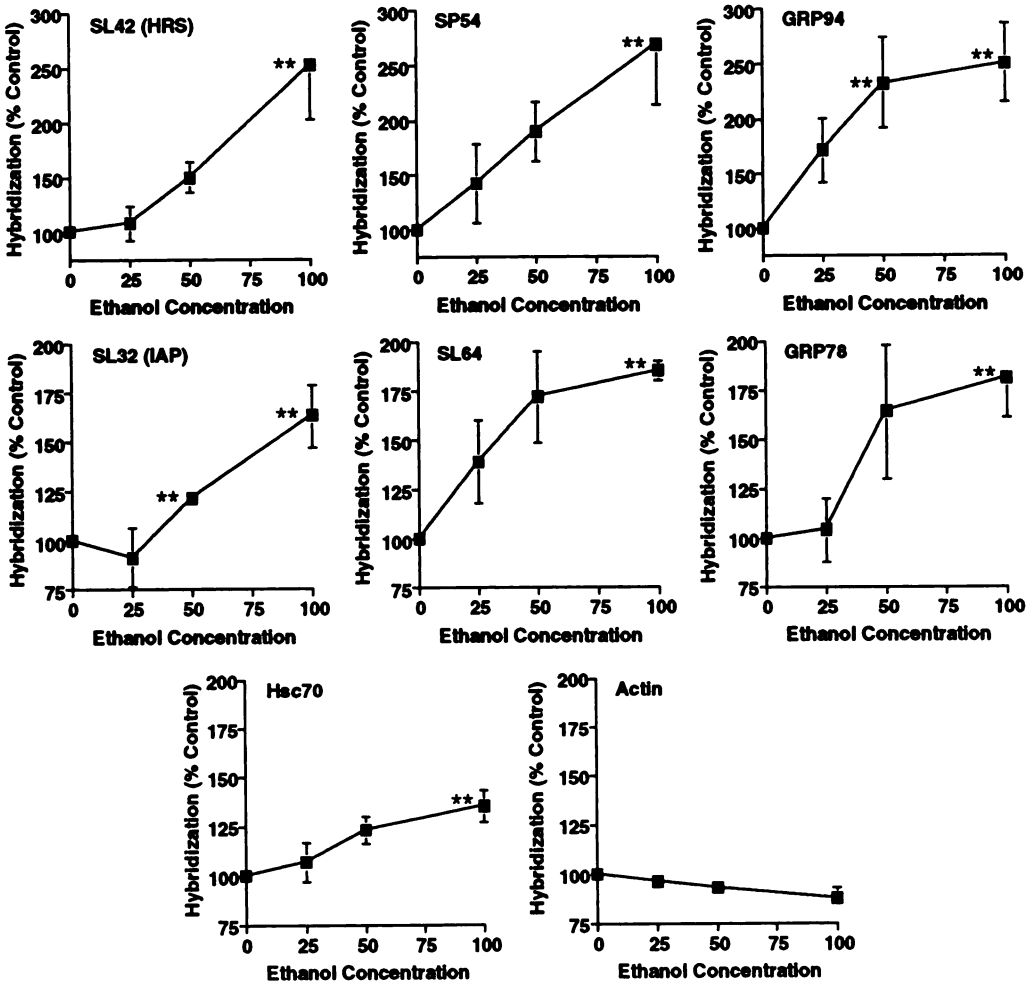

Fig. 6. Ethanol dose-response curves for EtRG mRNAs. NG108–15 cells were grown for 24 hr with the indicated concentrations of ethanol. Mock-treated control cultures were treated identically but without ethanol. Total RNA was then analyzed by slot blot hybridization using ^{32}P-labeled probes prepared from the indicated EtRGs or β-actin. Autoradiograms were quantitated by densitometry. Control blots analyzed for total poly(A)$^+$ RNA using a ^{35}S-labeled oligo(dT) probe showed no change throughout this ethanol concentration range. Results are the average ± standard error of duplicate determinations from either three (25 and 50 mM) or five (100 mM) experiments for each data point. **, Significantly different ($p < 0.05$) from control by single-group t test analysis, with Bonferroni correction for multiple groups.

ones. These studies support a growing body of evidence suggesting that changes in gene expression contribute to adaptation of the CNS to chronic ethanol exposure.

Of the EtRGs identified, one is GRP94, a transmembrane endoplasmic reticulum protein (39). GRP94 is generally coordinately regulated with GRP78, a resident endoplasmic reticulum protein (40, 41) known to function in the trafficking of glycoproteins. We found that ethanol also increases GRP78 mRNA abundance (Fig. 4). Treatments that interfere with protein glycosylation, protein trafficking, or endoplasmic reticulum storage of calcium induce both GRP78 and GRP94 (40, 41). GRP78 is consistently more responsive than GRP94 to "classical" GRP-inducing agents (40, 42–44). However, we found that ethanol induces GRP94 as much or more than GRP78 (Fig. 6). For example, the ratio for induction of GRP78 versus GRP94 was 2.84 ± 0.85 (four experiments) for 0.5 μM calcium ionophore A23187, compared with 0.70 ± 0.15 (seven experiments) for 100 mM ethanol ($p < 0.01$, unpaired t test). This suggests that ethanol differs from classical agents in the mechanism(s) of GRP induction.

Additional evidence for a unique action of ethanol comes from our finding that several other EtRGs do not appear to be molecular chaperones. These include an endogenous defective retrovirion (IAP) and HRS (Table 1). Although the HRS and IAP genes have poorly understood functions, they do not have structural similarities to other chaperones. Furthermore, GRP- or stress protein-inducing agents do not induce HRS, IAP, SP54, or SL64 (see Fig. 5 and associated discussion).

The alterations in EtRG mRNA abundance produced by ethanol are all of similar magnitude as previously identified increases or decreases in gene expression caused by ethanol (4). Subtractive hybridization cloning usually isolates genes with larger changes in expression than those noted for the EtRGs identified here. However, our subtraction procedure utilized double-stranded DNA as a driver for the hybridization reaction. This limits the total depletion of noninduced or weakly induced transcripts, particularly low abundance sequences, due to competition with reannealing of the driver DNA. The kinetics of our hybridization conditions thus favor enrichment of low abundance transcripts, particularly those with increased expression in ethanol-treated cells. Results reported here, coupled with previous reports on EtRGs (4), suggest that ethanol

produces small changes (2–3-fold) in the expression of a significant number of genes. Such a response might be expected for an agent that interacts with cellular membranes or hydrophobic portions of proteins, rather than acting on a distinct receptor.

Identification of a subset of EtRGs as the molecular chaperones Hsc70, GRP78, and GRP94 suggests that ethanol alters protein trafficking. Other investigators have indeed documented that chronic ethanol treatment decreases receptor-mediated endocytosis and processing of glycoproteins (19–24). Ethanol-induced changes in chaperone abundance could be responsible for subsequent alterations in protein trafficking. For example, increased GRP78 expression causes selective decreases in glycoprotein trafficking (45). Similarly, changes in Hsc70 expression cause compensatory alterations in protein insertion across membranes in yeast (46).

On the other hand, increases in chaperone abundance could result from primary ethanol-induced changes in protein trafficking or signaling pathways that relate to protein trafficking. For example, ethanol could decrease protein glycosylation or reduce endoplasmic reticulum calcium stores, as seen with other agents that induce GRP expression. However, because all EtRGs are not regulated as GRPs (Fig. 5) and ethanol induces a novel pattern of GRP94 ≥ GRP78 expression (Fig. 6), we suspect that ethanol acts at a site unique from that of classical inducers of GRP expression.

Fig. 6 shows that the ethanol concentration-response curves for all EtRGs, including GRP78, GRP94, and Hsc70, are very similar. Ethanol concentration-response relationships shown in Fig. 6 are also similar to results from our previous two-dimensional gel electrophoresis studies, showing ethanol-induced increases and decreases in mRNA abundance (4). Thus, although ethanol induces a subset of molecular chaperones as well as nonchaperone genes, the mechanisms for these gene inductions may be the same. For example, a common ethanol-responsive promoter element could mediate the coordinate induction of EtRGs, including a subset of chaperones. These molecular responses to chronic ethanol exposure may be secondary to acute ethanol actions such as alterations in second messenger cascades or ligand-gated ion channel function.

The role of these EtRG inductions in CNS adaptation to ethanol remains to be determined. Although all of the EtRGs isolated here showed inductions at clinically relevant ethanol concentrations (32), studies in animal models will be needed to further substantiate the potential importance of these gene inductions. Previous studies showed alterations in the expression of ligand-gated ion channels, which clearly would be suspected to have important physiological implications for CNS function. However, identification of a subset of molecular chaperones as EtRGs adds further support to suggestions that ethanol may alter protein trafficking. Alterations in protein trafficking could provide an additional mechanism for adaptive changes in the abundance of specific membrane proteins.

We suspect that CNS tolerance and dependence likely represent the cumulative effect of alterations in expression of multiple EtRGs. Our studies here, showing that the expression of three molecular chaperones is altered by ethanol, may aid in the identification of mechanisms underlying EtRG expression, because regulation of these chaperones has been studied in great detail. Identification of a subset of molecular chaperones as EtRGs also suggests that further study of ethanol actions on protein trafficking is warranted.

Acknowledgments

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