Chronic Alcohol Feeding and Its Influence on c-Fos and Heat Shock Protein-70 Gene Expression in Different Brain Regions of Male and Female Rats

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Excessive ingestion of the macronutrient, alcohol, causes devastating complications in the brain leading to atrophy and impaired cognitive function with corresponding increases in morbidity and mortality and, consequently, reduced quality of life measures. The pathogenic mechanisms are unknown, but various studies have shown that the immediate early genes and heat shock (ie, stress or chaperone) proteins are increased in alcohol-exposed tissue. However, many of these studies have been performed in vitro or have failed to consider either the nutritional elements in the experimental design by appropriate use of pair-feeding or whether there are regional and/or gender differences. We hypothesized that (1) increased expression of heat shock proteins and/or oncogenes occur as a consequence of alcohol-feeding in vivo, and sensitivities are related to different (2) gender and (3) brain regions. To test this, we fed male and female rats nutritionally complete diets containing ethanol as 35% of total calories (treated) or isocaloric amounts of the same diet in which ethanol was replaced by isocaloric glucose (controls). At the end of 6 weeks, rats were killed and c-Fos and heat shock protein-70 (HSP70) mRNA analyzed in midbrain, cortex, brainstem, and cerebellum by reverse transcription-polymerase chain reaction (RT-PCR) with an endogenous internal standard, β-actin. The results showed that there were distinct regional differences (*P* at least < .05) in both c-Fos (cerebellum > cortex > midbrain and brainstem) and HSP70 (brainstem and cerebellum > cortex and midbrain). However, the only significant effect of alcohol feeding occurred in the HSP70 mRNA in midbrain of male rats, which was reduced by approximately 50% (*P* < .01). In contrast, no corresponding effect of alcohol feeding was observed in c-Fos mRNA levels in either midbrain or other regions of female rats. These data show that chronic ethanol feeding has no demonstrable effect on c-Fos mRNA expression in the brain when using nutritionally complete liquid diet regimens with concomitant pair-feeding. HSP70 mRNA, in contrast, is reduced by alcohol feeding and appears to be regional and gender dependent.

EXCESSIVE INGESTION of the macronutrient, alcohol, causes devastating complications in the brain leading to atrophy and impaired cognitive function with corresponding increases in morbidity and mortality and, consequently, reduced quality of life measures.[1,2] Changes within the ethanol-exposed brain appear to be site-specific.[3] Many factors have been proposed to account for these pathogenic effects on the brain, including increased apoptosis,[4] free radical injury,[5] disturbances in protein synthesis,[6] DNA damage,[7,8] and protein-adduct formation.[9] More recently, however, attention has focussed on the possibility that transcriptional factors, such as the immediate early genes and heat shock or stress proteins play a key role in the pathogenesis of alcohol-induced brain dysfunction.[10,11] Although the intervening steps between activation of these genes and the development of the presenting features have not been fully elucidated, a variety of candidate genes have been proposed, including c-Fos[11] and heat shock protein-70 (HSP70).[12]

However, in some of the aforementioned studies, alcohol was administered in drinking water[11,13] or studies were performed in vitro in isolated cell systems.[14] Administration of ethanol in drinking water imposes some constraints on the levels of alcohol achieved, and the nutritional state of these animals may be suboptimal (reviewed previously[15]). Furthermore, it is not certain that events occurring in response to alcohol in vitro also occur in the in vivo situation. Such methodologic limitations may explain the inconsistencies in the literature regarding the reported effects of alcohol on the brain. For example, studies have reported no effect of acute ethanol dosage on brain c-Fos expression,[16] whereas c-Fos has been shown to decline in response to acute ethanol dosage.[17] In contrast, c-Fos increases during ethanol withdrawal.[18] HSP70 in the brain either increases[12,14] or remains unaffected[19] after alcohol exposure. Additional factors, which may also possibly contribute to different conclusions in alcohol-dosing studies, relate to the region analyzed and the gender of the species studied.[20,21]

The reason for addressing issues pertaining to gender and alcohol-induced brain damage relates to clinical observations showing enhanced susceptibility of brain damage in women or gender differences in electrophysiologic and behavioral indices. For example, a comprehensive prospective study of alcoholics showed that a shorter exposure to alcohol produced the same degree of brain shrinkage in females compared with male alcoholics.[22] However, the pathogenic basis of gender sensitivity in alcoholism is unknown.

To resolve some of the issues mentioned above, we measured c-Fos and HSP70 in the brain of rats subjected to a chronic alcohol feeding regimen. Different regions of the brain were examined, and male and female rats were compared. We

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Submitted February 15, 2002; accepted June 16, 2002.

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0026-0495/02/5112-0036$35.00/0
doi:10.1053/meta.2002.35595

used what is arguably the most appropriate method for investigating the long-term effects of ethanol, namely the Lieber-DeCarli regimen. This not only induces high circulating ethanol concentrations, but also induces brain atrophy[6,23] and ensures control and ethanol-fed rats are given identical micro- and macronutrients.[15]

MATERIALS AND METHODS

Animal Treatments

A total of 20 male and 16 female Wistar rats were obtained from accredited commercial suppliers at about 60 g body weight. They were maintained in a temperature- and humidity-controlled animal house for approximately 1 week until they weighed approximately 0.1 kg. They were then ranked and divided into 2 groups of equal mean body weight and subjected to a Lieber-DeCarli alcohol-feeding regimen in which treated rats were fed a nutritionally complete liquid diet containing 35% of total calories as ethanol ad libitum.[15] There was no restriction on the amount of alcohol consumed by these rats. Controls were pair-fed the same diet in which ethanol was replaced by isocaloric glucose. After 6 to 7 weeks, animals were killed by decapitation and brains dissected on ice into the various regions. These were frozen immediately in liquid nitrogen and stored at -70°C.

Liquid Diets

Fresh liquid diets used for the chronic ethanol feeding experiment were prepared on a daily basis according to the recipe described in Table 1. A food blender was used to thoroughly mix the ingredients. To prevent the possibility of ethanol precipitating the protein in the alcohol diet, absolute ethanol was the last ingredient to be added carefully, and contents were then thoroughly stirred during the addition. The diets were freshly prepared each day and presented to the animals between 9 AM and noon. The composition of the diet is given in Table 1. Control and alcohol-containing diets were isolipidic, isonitrogenous, and isoenergetic.

Table 1. Composition of Control and Alcohol-Containing Liquid Diets

	Content (g)	Total kcal	Total kJ
Alcohol diet			
Water	366	0.0	0.0
Fresubin (mL)	600	586	2,448
Glucose	0.0	0.0	0.0
Casein	15.0	60	251
Orovite (sucrose)	2.46	9	39
Alcohol (61.8 mL)	49.08	348	1,457
Total	1,063	1,003	4,194
Control diet			
Water	366	0.0	0.0
Fresubin (mL)	600	586	2,448
Glucose	93	349	1,458
Casein	15.0	60	251
Orovite (sucrose)	2.46	9	39
Alcohol	0	0	0
Total	1,106	1,004	4,196

Composition of "Fresubin" and "Orovite 7"

	Fresubin/100 mL	Orovite 7/5 g
Protein	3.8 g	None
Fat	3.4 g	None
Carbohydrate	13.8 g	3.8 g
Energy	420 kJ	59.6 kJ
Vitamin A	60.0 μg	62.5 μg
Thiamine (vitamin B_1)	0.11 mg	1.4 mg
Riboflavin (vitamin B_2)	0.13 mg	1.7 mg
Vitamin B_6	0.12 mg	2.0 mg
Vitamin B_{12}	0.3 μg	None
Phylloquinone (vitamin K1)	10 μg	None
Vitamin E	1.0 μg	None
Nicotinamide	0.9 mg	18.0 mg
Vitamin D	0.5 μg	300.0 μg
Folic acid	20 μg	None
Vitamin C	5 mg	60.0 mg
Biotin	12 mg	None
Pantothenic acid	0.69 mg	None
Calcium	75.0 mg	None
Iodine	7.5 μg	None
Iron	1.0 mg	None

NOTE. Calorific values were calculated as follows: ethanol = 7.1 kcal/g; carbohydrate = 3.8 kcal/g; protein = 4.0 kcal/g; fat = 9.0 kcal/g; and 4.18 kJ was assumed to be = 1.0 kcal. Composition of 'Fresubin' and 'Orovite 7' was from manufacturer's specifications.

Method for c-Fos and HSP70

Total RNA was prepared from the brain tissue by standard methods described previously.[24] The levels of c-Fos and HSP70 mRNAs in the discrete brain regions were quantified by reverse transcription-polymerase chain reaction (RT-PCR) with an endogenous internal standard, β-actin, as previously described.[25] RT was performed on 1 μg total RNA for 90 minutes at 42°C in a 5-μL reaction mixture containing 25 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 5 mmol/L $MgCl_2$, 2 mmol/L dithiothreitol, 1 mmol/L each deoxynucleotide, 10 U AMV reverse transcriptase (Roche Molecular Biochemicals, Mannheim, Germany), 10 U ribonuclease inhibitor (Roche Molecular Biochemicals), and 0.8 μg oligo $(dT)_{15}$ primer (Roche Molecular Biochemicals). The RT was terminated by heating the sample at 95°C for 2 minutes.[25]

The multiplexed PCR was performed in a 20-μL reaction mixture containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 2% (vol/vol) dimethyl sulfoxide, 0.2 mmol/L each deoxynucleotide, 0.1 μmol/L each of 5′ and 3′ β-actin-specific primers, 1 μmol/L each of 5′ and 3′ c-Fos- or HSP70-specific primers, 25 ng of reverse-transcribed total RNA, and 0.5 U Taq DNA polymerase (Roche Molecular Biochemicals). The PCR amplification was performed for 26 (c-Fos) or 28 (HSP70) cycles, consisting of denaturation (94°C, 45 seconds), annealing (60°C (HSP70), or 63°C (c-Fos), 45 seconds; Table 2), and extension (72°C, 75 seconds). After 6 cycles (c-Fos) or 8 (HSP70) cycles, 0.1 μmol/L each of β-actin primer pair was added to the reaction mixture, and PCR cycles were further continued (Table 2).[25] The primer sequences used for amplification of the coding regions of c-Fos, HSP70, and β-actin were as follows: HSP70, 5′-GAGTCCTACGCCTTCAATATGAAG-3′ (forward); 5′-CATCAAGAGTCTGTCTCTAGCCAA-3′ (reverse); target sequence = 347 bp; c-Fos, 5′-AGTGGTGAAGACCATGTCAGG-3′ (forward); 5′-CATTGGGGATCTTGCAGG-3′ (reverse); target sequence = 296 bp; (reverse); β-actin, 5′-TCATGCCATCCTGCGTCTGGACCT-3′ (forward); 5′-CCGGACTCATCGTACTCCTGCTTG-3′ (reverse); target sequence = 582 bp; Table 2).[25]

The PCR products were analyzed on a 10% (wt/vol) polyacrylamide gel electrophoresis. Gels were stained with ethidium bromide, visualized with ultraviolet (UV) trans-illumination, photographed, and submitted to image analysis (see Fig 1). Quantitative image analysis of the PCR fragments was performed using the NIH image program (Scion Corp, Frederick, MD). The levels of c-Fos and HSP70 mRNAs were calculated as the ratios of optical density of the PCR products to that of the β-actin PCR product.

Table 2. Oligonucleotide Primers, Product Length, Annealing Temperatures, and Number of PCR Cycles

Gene	Primer direction	Sequence
β-Actin	Forward primer	5′-TCATGCCATCCTGCGTCTGGACCT-3′
	Reverse primer	5′-CCGGACTCATCGTACTCCTGCTTG-3′
c-Fos	Forward primer	5′-AGTGGTGAAGACCATGTCAGG-3′
	Reverse primer	5′-CATTGGGGATCTTGCAGG-3′
HSP70	Forward primer	5′-GAGTCCTACGCCTTCAATATGAAG-3′
	Reverse primer	5′-CATCAAGAGTCTGTCTCTAGCCAA-3′

	PCR Product (bp)	Annealing Temperature (°C)	Cycles	Accession No.
β-Actin	582	60, 63	20, 21	V01217
c-Fos	296	63	26	X06769
HSP70	347	60	29	L16764

Statistical Analysis

Data are presented as mean ± SEM of either 8 (female) or 10 (male) observations unless specified otherwise. Data were initially analyzed at each brain site area using a between-subjects 2 (group; control, alcohol) × 2 (gender; male, female) analysis of variance (ANOVA). Posteriori statistical analysis was conducted to unpack any statistically significant main effects or higher order interaction observed using the method of simple effects analysis. Simple effects analysis was conducted on data pairs only after descriptive scrutiny of pairs of observations using histograms suggested that a relatively large difference between pairs warranted post hoc analysis. This approach to data analysis was taken to reduce the number of comparisons made to an absolute minimum to reduce the possibility of a type 2 error occurring as much as possible. For regional differences in control rats, data were analyzed using a 1-between-subjects, 1-within-subjects mixed-group, 2 (gender; male, female) × 4 (brain region; midbrain, cortex, brain-stem, cerebellum) ANOVA with repeated measures on the second factor.[26,27] Statistically significant effects were unpacked posteriori using the method of linear contrasts following descriptive and graphical examination of the data to determine the most appropriate post hoc comparisons and to reduce the total number of multiple comparisons made. A Mauchly sphericity test was conducted on the data sets before analysis to determine if the data set centrality and spread characteristics were satisfactory for the conduct of a parametric statistical test.[26,27] A Greenhouse-Geisser epsilon correction was applied to the data analysis to modify degrees of freedom and recalculate a corrected value of *P* in the event of a statistically significant observation from the Mauchly sphericity test.[26,27]

There are a variety of approaches for dealing with the possibility of type 2 errors occuring as a result of multiple comparison procedures; for example, specifying a more conservative *P* level of significance.[26,27] We have taken the compromise approach of attempting to keep the number of multiple comparisons to a minimum by using graphic representation of the data to guide the choice of multiple comparisons, in this way keeping *P* at .05.

RESULTS

In the following results, we first describe regional and gender differences in c-Fos and HSP70 mRNA expression in control rats followed by the effects of ethanol. Mean ± SEM of all groups are displayed in Table 3.

Comparison of Brain Regions as a Function of Gender in the Control Group

c-Fos. There was no evidence of a main effect of gender (*P* = not significant [NS]); neither was there any evidence of a statistically significant higher-order interaction between gender and brain region (*P* = NS). However, a highly statistically significant main effect of brain region was observed ($P < .01$). Cortex c-Fos levels were significantly higher ($P < .05$) than midbrain c-Fos levels. Cortex c-Fos levels were significantly higher ($P < .05$) than brain-stem c-Fos levels. Cerebellum c-Fos levels were higher than midbrain ($P < .01$), cortex ($P < .01$), and brainstem ($P < .001$).

HSP70. There was no evidence of a main effect of gender (P = NS); neither was there any evidence of a statistically significant higher-order interaction between gender and brain region (*P* = NS). A borderline statistically significant main effect of brain region was observed (*P* = .06). Cortex HSP70 levels were significantly lower ($P < .05$) than brain-stem HSP70 levels. Cortex HSP70 levels were also observed to be significantly lower ($P < .01$) than cerebellum HSP70 levels. Cerebellum HSP70 levels were found to be significantly higher than midbrain ($P < .05$) HSP70 levels. Brainstem HSP70 levels were found to be significantly higher than midbrain ($P < .05$) HSP70 levels.

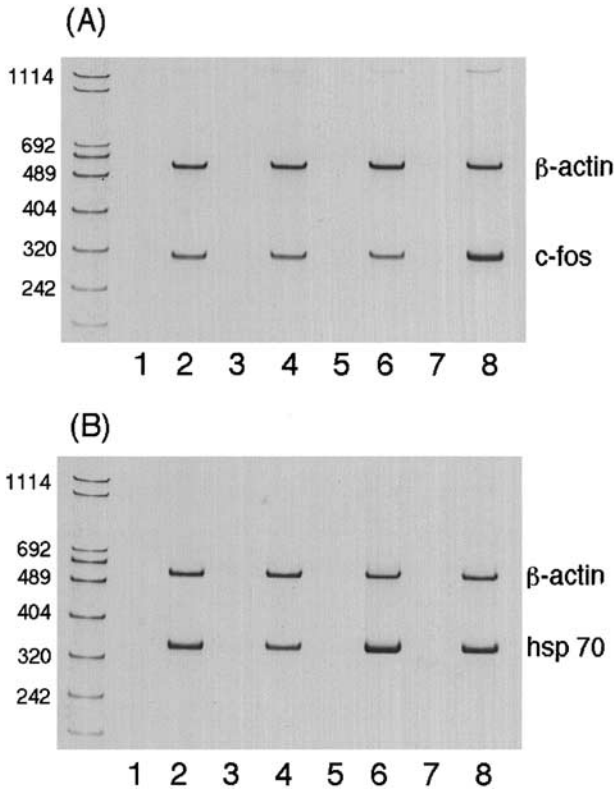

Fig 1. Ethidium bromide–stained polyacrylamide gel showing PCR products amplified from rat brain RNA. Total RNA extracted from the midbrain (lanes 1 and 2), cerebral cortex (lanes 3 and 4), brain-stem (lanes 5 and 6), and cerebellum (lanes 7 and 8) were incubated in the absence (lanes 1, 3, 5, and 7) or in the presence (lanes 2, 4, 6, and 8) of reverse transcriptase. The reverse transcription products were coamplified with (A) c-Fos and β-actin primers or (B) HSP70 and β-actin primers. A DNA standard lane is shown at the left of the gel with bands labeled in base pair lengths.

Table 3. Mean c-Fos and HSP70 Levels as a Function of Group Type and Gender

Gene and Brain Region	Control		Alcoholic	
	Male	Female	Male	Female
c-Fos mRNA				
Midbrain	0.49 (0.07)	0.46 (0.08)	0.65 (0.14)	0.54 (0.10)
Cortex	0.70 (0.08)	0.57 (0.05)	0.74 (0.10)	0.72 (0.21)
Brain stem	0.53 (0.10)*	0.39 (0.02)	0.47 (0.14)	0.37 (0.05)
Cerebellum	1.09 (0.16)	0.75 (0.11)†	1.19 (0.15)*	1.06 (0.31)
HSP70 mRNA				
Midbrain	1.44 (0.19)*	0.84 (0.13)†	0.78 (0.10)	0.92 (0.24)
Cortex	1.45 (0.33)	0.86 (0.10)†	0.84 (0.07)*	0.87 (0.11)†
Brain stem	2.09 (0.53)	0.97 (0.16)	1.15 (0.17)*	1.06 (0.25)
Cerebellum	1.70 (0.33)	1.21 (0.11)‡	1.31 (0.11)§	1.52 (0.25)

NOTE. SEM is in parentheses. Male and female rats were fed nutritionally complete liquid diets containing ethanol as 35% of total caloric intake (treated) or identical amounts of the same diet in which ethanol was replaced by isocaloric glucose (controls) for 6 to 7 weeks. At the end of the study, brain regions were dissected for analysis of c-Fos and HSP70 mRNA relative to β-actin mRNA. Data are presented as mean ± SEM of either 8 (female) or 10 (male) observations unless specified otherwise.

*n = 9; †n = 7; ‡n = 6; §n = 8 males. For a comprehensive statistical analysis of the data sets, see Results section.

Effects of Alcohol and Their Interaction With Gender

c-Fos. For all 4 regions, no statistically significant main effects of group ($P > .05$; NS) or gender (NS) were observed. There was no evidence of a statistically significant higher-order interaction between group and gender for all 4 regions (NS, in all instances).

HSP70. For the midbrain, no statistically significant main effects of group (P = NS) or gender (P = NS) were observed. A statistically significant higher-order interaction between group and gender ($P < .05$) was observed with the male control group demonstrating higher levels of HSP70 compared with the female control group in contrast to the female alcoholic group demonstrating higher levels of HSP70 compared with the male alcoholic group. There was a statistically significant difference ($P < .01$) between male groups with the control male group having significantly higher levels of HSP70 compared with the alcoholic male group. There was a statistically significant difference ($P < .01$) between the male and female control groups with the male control group having significantly higher levels of HSP70 compared with the female control group.

For cortex, brainstem, and cerebellum, there were no statistically significant main effects of group or gender (NS) for HSP70. There was no evidence of a statistically significant higher-order interaction between group and gender (NS).

DISCUSSION

In the present study, we set out to test the hypothesis that c-Fos and HSP mRNA in different brain regions increase in response to chronic ethanol feeding. Additionally, we set out to test the supposition that such responses would be regionally and gender dependent. In doing so, we compared these mRNA species in both male and female rats and demonstrated that there were no overt differences between gender of control animals. In contrast, there were distinct regional variations in mRNA levels in control animals, and the patterns of this difference were dissimilar with respect to the 2 stress genes analyzed. We were unable to show any change in c-Fos mRNA in response to alcohol. However, we showed that HSP70 mRNA was altered by chronic alcohol. This change was confined to the midbrain of male rats, and contrary to expectation, alcohol caused a marked decrease in HSP70 mRNA levels.

Lieber-DeCarli Model of Pair-Feeding

The possibility that alcohol-induced brain damage may have an etiology related to malnutrition has been addressed previously.[28] In the present studies, we used the Lieber-DeCarli feeding protocol in which both control and ethanol-fed rats received identical amounts of the same diet, albeit with differences due to the proportion of dietary energy provided by either glucose (controls) or ethanol (treated).[15] This is an important aspect of our studies for the following reasons. Inclusion of alcohol in water drinking in a free choice only results in moderate elevations in blood alcohol and either impaired weight gains or loss of weight.[29] This arises because mouse and rats have a natural aversion to alcohol and alcohol ingestion induces anorexia.[15] To resolve this, the Lieber-DeCarli regimen was developed in which alcohol, water, and nutrients are provided in a single liquid-feed. In this model, treated animals consume much more alcohol than when provided in drinking water alone.[15] In the Lieber-DeCarli protocol, controls are given the same amount of an identical diet in which ethanol is replaced by isoenergetic carbohydrate (in this case, glucose) to eliminate the possibility that the ensuing results are due to malnutrition.[15] Although some studies have included alcohol in drinking water to investigate brain biochemistry, (for example, to study trace element distribution[30] or the interaction with ethanol and other drugs, such as cocaine), they are of little use in understanding alcohol as a nutritional toxin. As a consequence, the reported effects of administering alcohol in drinking water on brain c-Fos[13] or HSP70[19] may be difficult to interpret. This is further emphasized by the fact that impaired nutritional status per se can influence brain biochemistry, such as protein synthesis[31] and proteolysis.[32] Thus, overall, we are confident in the application of the present feeding model to investigate whether ethanol perturbs gene expression of stress proteins and early intermediate or oncogenes as exemplified by HSP70 and c-Fos, respectively.

Ethanol and Brain Neuropathology

The brain is a key target of alcohol toxicity affecting functional, behavioral, morphologic, and biochemical processes in a variety of ways (reviewed in Olney et al,[33] Mann et al,[34] and Sun et al[35]). These range from DNA damage and altered gene expression[36] to amnesia, dementia, and cognitive dysfunction.[28] To elucidate the mechanisms responsible for such damage, a number of models have been developed, many that entail studies on the laboratory rat.[33,37] From the results of these studies, it is possible to infer that most biochemical pathways are affected by alcohol and include, for example, neurochemical metabolism, coupling of opioid receptors to G proteins, growth factors, glucose transporters, and cellular energy gen-

eration (for example, see Braunova et al[38]). To this list, we must also add defective abundance of HSP70 mRNA, especially in the midbrain of male rats.

However, some of the signals responsible for alcohol-induced brain damage are unknown. One possible route pertains to the increased production of corticotropin (ACTH)-releasing hormone. This will result in elevations in circulating ACTH, cortisol, and catecholamines. A role for catecholamines in alcohol-induced brain damage has previously been suggested.[39-42]

Gender Differences in Alcohol Toxicity

Clinically, it seems that females are more vulnerable to the deleterious effect of alcohol with respect to a number of variables, including psychosocial and structural abnormalities.[43] Female animals have also been shown to be more susceptible to alcohol or alcohol withdrawal than males in studies on hippocampal neurotoxicity[44] or behavioral activity.[45] Similarly, greater susceptibility is also seen in females in studies on non-neuronal pathologies, such as liver damage.[46] Some of this difference has been ascribed to consumption of diets inadequate in vitamins or periods of fasting.[43] However, as reiterated in the previous paragraphs, our studies ensured that both control and treated rats consumed identical amounts of nutritionally adequate diets supplemented with vitamins (see Materials and Methods). Furthermore, contrary to the expected changes in female rats, we observed a preferential decrease in HSP70 in male rats. We are unable to explain this, but consideration needs to be given to the fact that the susceptibility of the brain in females is not always demonstrable.[43] For example, the size of the cerebellar sulci (an index of brain damage[1]) in female alcoholics is not significantly different from controls, whereas a significant difference is observed in male alcoholics.[43] Certainly, in controlled studies on animals, administration of female hormones (estradiol and progesterone) in ovariectomized rats has been shown to modulate the effects of alcohol on the brain in terms of mu-opioid receptor binding and levels of endorphins and enkephalins.[47] It is possible to argue that the gender differences in the response of HSP70 may relate to differences in alcohol metabolizing enzymes (ie, alcohol and acetaldehyde dehydrogenases) within the brain area. However, it has been shown that the activities of these enzymes are similar in male and female rats.[48]

c-Fos

The functions and metabolic significance of oncogenes and c-Fos, in particular, has been reviewed previously.[49,50] As mentioned earlier, reduced,[17] unchanged,[16] and increased[18] c-Fos expression have been reported in alcohol studies. It seems that the response of c-Fos is dependent on the model used and a similar interpretation may be applied to our data on HSP70, which is also at variance with some reported studies in the literature (for example, Calabrese et al[12]). However, we feel that our 6-week feeding regimen study with concomitant analysis of c-Fos and HSP70 mRNA is relevant to chronic alcohol misuse, because brain atrophy can be demonstrated (see Bonner et al[6,23]). Brain atrophy is also seen in chronic alcohol misusers in the clinical setting (see Muuronen et al[51]). Our data clearly show that while there are some regional differences in c-Fos mRNA, no effect of alcohol is observed in either male or female rats. In contrast, a significant decrease in HSP70 mRNA was observed in male rats.

HSP70

The functions and metabolic significance of stress proteins and HSP70, in particular, has been reviewed previously.[52,53] In very simple terms, HSPs act as molecular chaperones and are important in processing of newly synthesized proteins or preventing the aggregation of denatured proteins by adenosine triphosphate (ATP)-dependent mechanisms.[54] Their increase in response to a variety of stresses and pathophysiologic stimuli confers cytoprotective properties on the cell, such as against injury by free radicals or oxidative stress and other forms of neuronal injury.[55,56] Based on this premise, one can infer that such a reduction in HSP70 mRNA may be detrimental. For example, the preferential localization of HSP70 to stress-exposed synapses may assist the renewal of damaged proteins within the cellular domain.[54] This may be related to the function of HSP70 in protein processing and trafficking throughout the cytocellular milieu.[57] However, our data on reduced HSP70 mRNA is not unusual. Clozapine, (an antipsychotic agent) decreases HSP70 mRNA in the brain.[25]

Regional Brain Sensitivity to Alcohol

Regional brain sensitivities to a variety of metabolic perturbations have been well documented.[58-61] Thus, for example, in experimental hypothyroidism, decreases in oxidative phosphorylation occur in cortex, but not cerebellum, midbrain, or brain stem.[61] This regional sensitivity also extends to alcohol toxicity, such as with respect to trace element levels[62] and protein metabolism.[6] However, we are presently unable to offer an explanation why HSP70 should be preferentially reduced in the midbrain of male alcoholic rats. In brains of human alcoholics, there is evidence of increased immunoreactive ubiquitin staining in the midbrain.[63] In the rat, biopterin coenzymes (ie, 6R-L-erythro-5,6,7,8-tetrahydrobiopterin; important in catecholamines and serotonin synthesis) exhibit higher levels in midbrain compared with cortex and may be related to alcohol consumption models.[64] In relation to catecholamines, midbrain is also particularly sensitive to alcohol toxicity, although the connection with biopterin levels has not been made.[65] Midbrain Na-K-ATPase activities also increase as a consequence of ethanol exposure.[66]

Comparison With Other Studies

It is important to compare the present data with other studies and question whether the current study adds or detracts from what has already been reported. In doing so, we must be mindful of the fact that the present study used a chronic regimen, which may induce a degree to tolerance and, to assess regulatory control, we examined the mRNA levels encoding HSP and c-fos rather than the proteins per se. The lack of effect of ethanol on c-fos mRNA in the present study contrasts with the reported increases in brain c-fos mRNA[67] and protein (ie, immunoreactivity)[68] in response to acute ethanol exposure. Similarly, the decrease in HSP70 mRNA in the present study contrasts with reported increases in acute studies reported by

others.[12,19] A tolerance phenomenon may well explain these discrepancies and indeed may well be an adaptive or protective mechanism against brain damage.[69,70] However, the nature of the adaptive response is unknown. We also have to be mindful of the fact that our ethanol studies were conducted using a postnatal design, which contrasts with prenatal (in utero) studies demonstrating an increase in HSP70 protein.[14] Addressing these issues in a more critical manner, we suggest that it would be of immeasurable benefit to conduct further studies using a much larger study design, examining the mRNA levels of a variety of oncogenes and heat shock proteins at numerous time points during pre and postnatal stages of development in different brains regions. Currently, the cost and practicalities of such studies are prohibitive, although some progress has been made in the use of differential display techniques in alcohol-exposed tissue.[71]

Conclusions

Our data shows that chronic ethanol feeding has no demonstrable effect on c-Fos mRNA expression in the brain when using nutritionally complete liquid diet regimens with concomitant pair feeding. HSP70 mRNA, in contrast, is reduced by alcohol feeding and appears to be regional and gender dependent. Due to the functional role of HSP70 in protein synthesis, trafficking, and cellular protection, a decrease in its encoding mRNA may confer a biologic disadvantage to additional metabolic insults or ethanol feeding itself.

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