Acute Exposure to the Nutritional Toxin Alcohol Reduces Brain Protein Synthesis In Vivo

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Few studies have measured brain protein synthesis in vivo using reliable methods that consider the precursor pool, and there is a paucity of data on the regional sensitivity of this organ to nutritional or toxic substances. We hypothesized that different areas of the brain will exhibit variations in protein synthesis rates, which might also be expected to show different sensitivities to the nutritional toxin, ethanol. To test this, we dosed male Wistar rats with ethanol (75 mmol/kg body weight) and measured rates of protein synthesis (ie, the fractional rate of protein synthesis, defined as the percentage of the protein pool renewed each day; k_s, %/d) in different brain regions 2.5 hours later with the flooding dose method using L-[4-^{3}H] phenylalanine. In the event that some regions were refractory to the deleterious effects of ethanol, we also predosed rats with cyanamide, an aldehyde dehydrogenase inhibitor (ie, cyanamide + ethanol), to increase endogenous acetaldehyde, a potent neurotoxic agent. The results indicated the mean fractional rates of protein synthesis in the cortex was 21.1%/d, which was significantly lower than either brain stem (30.2%/d, $P < .025$), cerebellum (30.1%/d, $P < .01$), or midbrain (29.8%, $P < .025$). Ethanol significantly decreased protein synthesis in the cortex (21%, $P < 0.01$), cerebellum (19%, $P < .025$), brain stem (44%, $P < .025$), but not in the midbrain (not significant [NS]). However, significant reductions in protein synthesis in the midbrain occurred in cyanamide + ethanol-dosed rats (60%, $P < .0001$). Cyanamide + ethanol treatment also reduced k_s in the brain stem (66%, $P < .001$), cortex (59%, $P < .001$), and cerebellum (55%, $P < .001$). In conclusion, the applicability of the flooding dose technique to measure protein synthesis in the brain in vivo is demonstrated by its ability to measure regional difference. Impaired protein synthesis rates may contribute to or reflect the pathogenesis of alcohol-induced brain damage.

ATROPHY OF THE BRAIN is one of the most devastating complications and destructive outcomes of alcohol abuse.[1,2] Studies on the mechanism of these phenomena have focused on the possibility that defects in protein synthesis contribute to this pathology (for example, see Preedy et al[3]). These studies have shown that alcohol affects brain protein synthesis in a number of ways, but most of the data is conflicting, and many do not address the fundamental question of whether alcohol perturbs brain protein synthesis in vivo. Thus, in vitro, ethanol reduces mRNA translational efficiencies of astrocytoma cells,[4] concomitant with decreases in labelling of [^{35}S]-methionine of 40S ternary and 80S initiation complexes, possibly via modifications of eukaryotic initiation factor-2, ie, eIF-2.[4] On the other hand, in vitro ethanol exposure has been shown to be ineffective in altering protein synthesis in cell-free extracts of brain, whereas acute in vivo dosing is reported to increase protein synthesis measured in extracts of cytoplasmic ribosomes from the cerebellum and cortex.[5]

Chick embryos exposed to ethanol also show reduced protein synthesis rates measured by either in vivo or vitro methods.[6,7] In chronic studies (> 1 week), postnatal chronic ethanol ingestion has been suggested to increase brain protein synthesis in rats,[8,9] which is similar to the conclusions drawn from indirect measures of protein synthesis (by assay of RNA-rich intranucleolar bodies using histologic analysis) in hamster cerebellar Purkinje cells and motor neurons.[10] In contrast, in chronic studies in vivo using the Lieber-DeCarli regimen, protein synthesis in vitro has been reported to decrease.[11] However, there is a paucity of data on the acute effects of ethanol, although one study has shown that ethanol administration for 3 days decreases protein synthesis in vivo.[12]

A plausible explanation for this variability in the literature pertains to the fact that only one or a few selective regions were analyzed without considering the possibility that different regions exhibit distinct responses (for example, see Peters and Steele[12]). Furthermore, to obtain a precise assessment of protein synthesis using labelled amino acids, studies should ideally be performed in vivo using methods that take into account the specific radioactivity's of the aminoacyl tRNA (ie, the labelling of the amino acid at the site of protein synthesis, S_{tRNA}) and the product (ie, amount of label in protein).[13,14] While the measurement of the product presents few practical difficulties, assessing S_{tRNA} is complicated because of its low abundance and its extreme lability. To overcome this, the flooding dose method has been developed.[13,14] In simple terms, the flooding dose as developed by McNurlan et al[15] assumes that S_{tRNA} values can be assessed via the measurement of the specific radioactivities of the free amino acid in acid soluble pools of tissue extracts (ie, $S_{i;}$[13,14]). Recent studies have suggested that values for S_i agree well with S_{tRNA}, and the former is more amenable for high-throughput analysis.[13,14] The applicability of the flooding dose method in assessing the relative rate of protein synthesis in the different regions of the brain in vivo have not been widely explored apart from one study in which a flooding dose of [^{35}S] methionine was used with quantitative histoautoradiography to measure synthesis rates in different regions.[16] In another study, synthesis rates in the whole brain were determined by directly measuring S_{tRNA}.[17] Nevertheless, the flooding dose method, as adapted for phenylalanine, has numerous practical advantages, particularly when sampling

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minute amounts of tissue, thus making it amenable for analyzing different brain regions of small animals.[18]

The aim of this study was to address some of the issues raised above and investigate the relative effects of ethanol on brain protein synthesis in intact animals. Protein synthesis rates were established for cerebellum, brain stem, midbrain, and cortex using the flooding dose method with L-[4-^{3}H]phenylalanine as a radiolabel. In the event that ethanol did not cause any significant effect on brain protein synthesis, we also pretreated rats with cyanamide, an aldehyde dehydrogenase inhibitor. This has the effect of raising the levels of extremely toxic metabolite acetaldehyde, which has previously been used to determine whether any tissue or region is refractory to adverse pathophysiologic stimuli.[19]

MATERIALS AND METHODS

Male Wistar rats, supplied by Bantin and Kingman (Alderborough, Hull, UK), were used for all experiments. L-[4-^{3}H]phenylalanine was obtained from Amersham International (Amersham, Bucks, UK). Cyanamide was from The Sigma Chemical Company (Poole, Dorset, UK). Other chemicals and reagent were obtained from various commercial suppliers and were of optimum purity. Ethanol (2%, vol/vol) was removed from L-[4-^{3}H]phenylalanine via rotary evaporation after addition of a known quantity of unlabeled phenylalanine as a carrier. A solution of 150 mmol/L phenylalanine in distilled water was added to the radiolabeled stock solution to a final concentration of approx. 25 μCi of L-[4-^{3}H] phenylalanine/mL. The solution was filtered through a Millipore (Watford, England) filter, 0.22 μm pore size, for injection at a dosage of 1 mL/100 g body weight. The mixture was stored at −20°C between use.

Experimental Teatment

All rats were allowed access to a standard laboratory diet and water ad libitum up to and including experimentation. The rats were maintained in a home office-approved animal house at a temperature of 20°C to 25°C, with a 12-hour light/12-hour dark cycle and humidity control. The rats initially weighed 50 to 70 g and on reaching a weight of approximately 150 g, they were ranked in order of body weight and assigned to 1 of 4 groups of equal mean body weight. We divided the rats into groups of equal body weight rather than randomize them. This is because it has been shown that there is a direct correlation between growth rates of rats (which would be expected to be expressed by body weights) and rates of tissue (albeit muscle) protein turnover.[20,21] Even in apparently homogenous groups of rats with similar ages and weights, protein synthesis rates correlate with growth and, by inference, body weights.[22] Thus, it is inappropriate to randomize rats for studies on protein metabolism. This has also been reiterated by studies showing significant differences in body weight in a homogenous group of rats subjected to a randomization process (Preedy VR, unpublished).

The experimental protocol was divided into 2 parts, namely: a "pretreatment" stage for 30 minutes and a "treatment" stage of 2 and $\frac{1}{2}$ hours. Thus, the groups were (pretreatment + treatment): A, saline + saline; B, saline + ethanol; C, cyanamide + saline; and D, cyanamide + ethanol. Body weights of the groups A, B, C, and D were 155 ± 2, 154 ± 3, 157 ± 3, 158 ± 2 g, respectively. In the pretreatment stage, rats were injected intraperitoneally with (0.5 mL/100 g body weight) of either saline or cyanamide. At 30 minutes, the rats were injected in the treatment stage with an intraperitoneal injection (1 mL/100 g body weight) of either saline or ethanol. After a further 2 hours and 20 minutes, the rats were injected with a flooding dose of L-[4-^{3}H] phenylalanine to measure protein synthesis. The following doses were used: saline, 0.15 mol NaCl/l; ethanol, 75 mmol/kg body weight; cyanamide, 0.50 mmol/kg body weight.

At 10 minutes after injection of isotope, rats were killed by decapitation so that the brains were immediately plunged into an ice water slurry.

Analytical Methods

The fractional rate of protein, ie, the percentage of tissue protein renewed each day (ie, k_s, %/d) was measured using the method originally described for leucine[23] and subsequently modified for phenylalanine.[18] A "flooding dose" of L-[4-^{3}H]phenylalanine was injected into rats via a lateral tail vein (150 mmol/L; 1 mL/100 body weight) while the animal was restrained in a "J-cloth" disposable paper towel.[18] At 10 minutes after isotope injection, the rats were decapitated, and the head quickly submerged in a beaker containing an ice water slurry to terminate protein synthesis. The brains were then removed on ice and the regions dissected. The exact time between isotope injection and immersion of the head in the ice water slurry was recorded and constituted "t" minutes.[18] Effectively, this was only a few seconds after killing the rats. All tissues were frozen in liquid nitrogen and finally stored at −70°C until they were processed for the determination of the specific radioactivity of phenylalanine in free and protein bound amino acid pools.[18]

Tissue Processing for Phenylalanine-Specific Radioactivities

Mixed protein homogenates were precipitated in 0.2 mol/L perchloric acid (PCA), centrifuged, and acid supernatants containing free amino acids decanted and neutralized with saturated tripotassium citrate to precipitate the $KClO_4$.[18] The precipitate was removed by centrifugation (2,000 × g, 10 minutes, 4°C) and supernatants stored at 0°C to 4°C until they were used to measure the specific radioactivity of free phenylalanine (S_i).[18] Pellets from mixed homogenates were washed twice in 12 to 14 mL 0.2 mol/L PCA, suspended in 10 mL 0.3 mol/L NaOH and incubated at 37°C for 1 hour during which period samples were vortexed, thus ensuring protein lumps were thoroughly broken up. The solubilized protein was reprecipitated with 2 mL ice-cold 2 mol/L PCA. The mixture was then gently mixed and centrifuged, and the supernatants were carefully decanted to waste and protein pellets repeatedly washed 6 to 8 times in 12 to 14 mL 0.2 mol/L PCA to remove free radioactivity.[18] Finally, 3 mL 6 mol/l HCl was added to the protein pellets and samples acid hydrolyzed for 36 hours at 105°C in a Grant dry block heater.[18] Dry residues of the hydrolysates were then suspended in 4 mL sodium citrate buffer and stored briefly at 0°C to 4°C until conversion of phenylalanine to β-phenylethylamine (β-PEA).[18]

The determination of phenylalanine specific radioactivity's in acid supernatants (S_i) and protein hydrolysates (S_B) have been described in detail elsewhere.[18] The concentrations of β-PEA in samples were obtained using a fluorimetric assay involving incubation of samples with leucylalanine and ninhydrin.[18] Fractional rates of protein synthesis were calculated from the formula: $k_s = (S_B \times 100)/(S_i \times t)$, %/d. Where S_B is the specific radioactivity of phenylalanine covalently bound in protein; S_i is the specific radioactivity of the free amino acid (ie, phenylalanine) in the intracellular pool at the end of the labeling period; t is the isotope incorporation time in days.[18] The rats were killed 10 minutes after injection, and the heads were placed into an ice-water slurry immediately (usually within a second of decapitation). Thus, the mean value of t was 10.00 minutes. Potential differences in the rate of protein synthesis in the 4 regions were not due to the rate at which different regions were cooled. This statement is supported by studies showing that the tissue hypoxia/anoxia occurring after killing has an apparent inhibitory effect of protein synthesis (Koll M, Preedy VR, Garlick PJ, unpublished data).

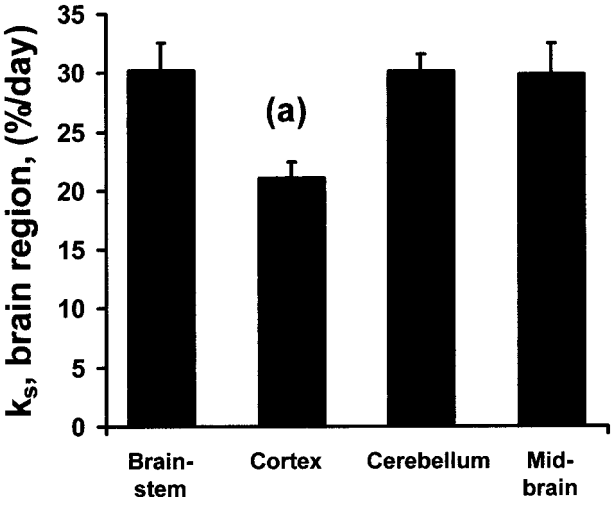

Fig 1. Protein synthesis in different regions of the rat brain. Protein synthesis, k_s (percentage of the protein pool renewed each day; %/d), in brain stem (n = 6), midbrain (n = 7), cerebellum (n = 7), and cortex (n = 5) of control rats (ie, saline-injected animals in group A rats. Data are mean ± SEM. Statistical differences between means were determined by LSD using the pooled estimate of variance. Significance indicated when *P* < .05. (a) Cortex *v* either cerebellum, *P* < .01; midbrain, *P* < .05 or brain stem, *P* = .01.

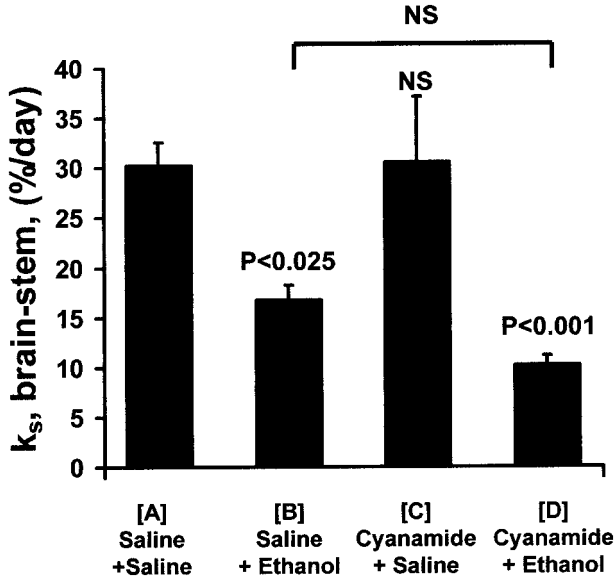

Fig 2. Protein synthesis in rat brain stem in response to ethanol and cyanamide. Young male Wistar rats were divided into 4 groups and subjected to a 30-minute "pretreatment" phase of saline or cyanamide dosage followed by a 150-minute "treatment" phase of ether saline or ethanol dosage. At 10 minutes before the end of the treatment stage, protein synthesis was measured in the brain stem of (A) saline + saline controls (n = 6); (B) saline + ethanol (n = 5); (C) cyanamide + saline (n = 5); and (D) cyanamide + ethanol (n = 6)-treated groups. For other details, see Materials and Methods. Data are mean ± SEM. Statistical differences between means were determined by LSD using the pooled estimate of variance. Significance indicated when *P* < .05.

Analysis of Data and Statistical Methods

All results are expressed as means ± SEM (n = 4 to 7). Data was tested for significant differences between means with the LSD method using the pooled estimate of variance for comparisons between different groups. Significance was indicated at $P < .05$.

RESULTS

Rates of Protein Synthesis in Different Brain Regions

The data in Fig 1 show the regional differences in k_s within the brain. Rates of protein synthesis in the cortex were significantly lower than either brain stem, midbrain, or cerebellum ($P < .05$ for all comparisons). Conceivably, lower rates of protein synthesis in cortex could be ascribed to higher S_i values. However, the only significant difference obtained when comparing all regional S_i values in control rats of group A, pertained to cortex versus midbrain ($P < .05$); all other comparisons between S_i values in the 4 regions of brain of saline + saline-injected rats were not statistically significant (Table 1).

Effects of Ethanol

In response to ethanol alone (groups A *v* B), rates of protein synthesis declined significantly in the brain stem (44%; $P < .025$; Fig 2), cortex (21%, $P < .01$; Fig 3), cerebellum (19%; $P < .05$; Fig 4). However, there was no significant change in the midbrain (Fig 5).

Table 1. Specific Radioactivities of Free Phenylalanine in Different Regions of the Brain After a 10-minute Labeling

Region	Specific Radioactivity of S_i in the Brain (dpm/nmol)			
	Group A	Group B	Group C	Group D
Brain stem	45.4 ± 5.8 (7)	45.6 ± 4.6 (6)	43.0 ± 8.2 (5)	49.2 ± 3.3 (6)
Cortex	56.1 ± 2.6 (6)*	55.2 ± 1.7 (7)	55.9 ± 11.7 (4)	57.0 ± 2.9 (6)
Cerebellum	44.5 ± 1.5 (7)	44.7 ± 1.5 (7)	47.5 ± 4.7 (5)	48.0 ± 4.2 (6)
Midbrain	40.3 ± 4.2 (7)*	33.5 ± 2.6 (7)	34.7 ± 2.3 (5)	35.4 ± 2.7 (6)

NOTE. Free phenylalanine specific radioactivities in homogenates of rat brain. (A) Saline + saline controls; (B) saline + ethanol; (C) cyanamide + saline, and (D) cyanamide + ethanol-treated groups. Data are mean ± SEM (n). Differences between means for a particular region were assessed using the pooled estimate of variance.

*The only significant difference obtained when comparing all S_i values in control rats of group A pertained to cortex *v* midbrain; all other comparisons between saline-injected rats were not significant. In any one region, there was no statistically significant differences between any of the 4 treatment groups (ie, A *v* B, C, or D or B *v* D; $P > .05$ (not significant) in all instances).

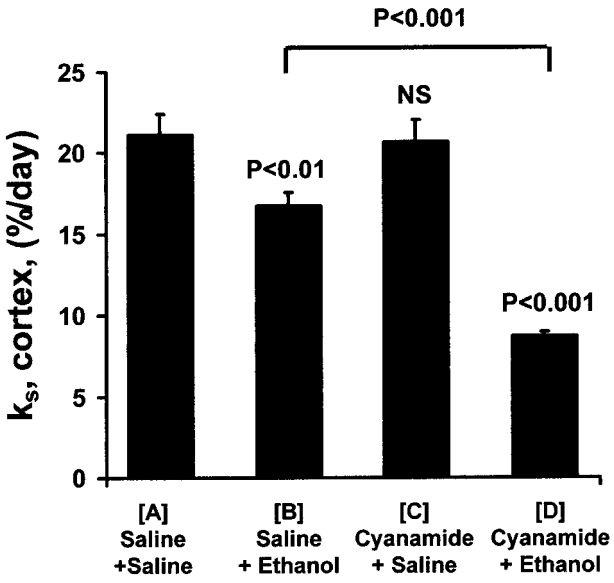

Fig 3. Protein synthesis in rat cortex in response to ethanol and cyanamide. Fractional protein synthesis rates (k_s, %/d) in cortex of (A) saline + saline controls (n = 6); (B) saline + ethanol (n = 7); (C) cyanamide + saline (n = 4); and (D) cyanamide + ethanol (n = 6)-treated groups. For other details, see Materials and Methods. Data are mean ± SEM. Statistical differences between means were determined by LSD using the pooled estimate of variance. Significance indicated when $P < .05$.

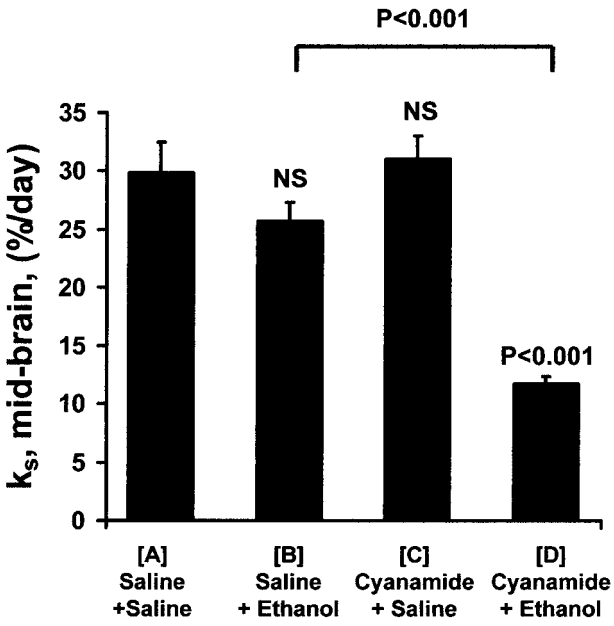

Fig 5. Protein synthesis in rat midbrain in response to ethanol and cyanamide. Fractional protein synthesis rates (k_s, %/d) in midbrain of (A) saline + saline controls (n = 5); (B) saline + ethanol (n = 7); (C) cyanamide + saline (n = 4); and (D) cyanamide + ethanol (n = 6)-treated groups. For other details, see Materials and Methods. Data are mean ± SEM. Statistical differences between means were determined by LSD using the pooled estimate of variance. Significance indicated when $P < .05$.

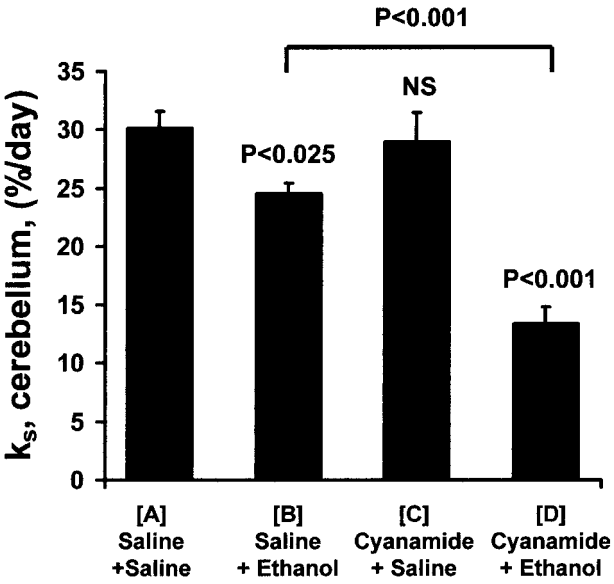

Fig 4. Protein synthesis in rat cerebellum in response to ethanol and cyanamide. Fractional protein synthesis rates (k_s, %/d) in cerebellum of (A) saline + saline controls (n = 7); (B) saline + ethanol (n = 7); (C) cyanamide + saline (n = 5); and (D) cyanamide + ethanol (n = 6)-treated groups. For other details, see Materials and Methods. Data are mean ± SEM. Statistical differences between means were determined by LSD using the pooled estimate of variance. Significance indicated when $P < .05$.

Effects of Increasing Acetaldehyde

Increasing endogenous alcohol-derived acetaldehyde (groups A *v* D) depressed protein synthesis in the brain stem (−66%, $P < .001$; Fig 2), cortex (−59%, $P < .001$; Fig 3), cerebellum (−55%, $P < .001$; Fig 4) and midbrain (−60%, $P < .001$; Fig 5). However, when we compared the response of the ethanol-treated rats (group B) with those of the cyanamide + ethanol-treated rats (group D), there was no apparent potentiation by cyanamide in the brain stem, but there were in the other 3 brain regions (changes between 45% and 54%, $P < .001$ in all 3 regions; Figs 3 through 5).

None of the changes due to ethanol or cyanamide could be ascribed to alterations in the enrichment of the precursor S_i (Table 1). In any one region, there was no statistically significant difference between any of the 4 treatment groups (ie, A *v* B, A *v* C, A *v* D, B *v* D, or other permutations; $P > .05$ in all instances).

DISCUSSION

In this study, we have demonstrated that acute ethanol administration reduces the rate of brain protein synthesis in vivo (Table 2). This decrease in k_s was observed in most regions studied except in the midbrain. However, with an increased acetaldehyde level, the midbrain showed a marked suppression of protein synthesis rates. The reason for analyzing the different regions of the brain was based on the observation that this organ exhibits regional sensitivities in response to perturbations in metabolism (such as malnutrition[24] or alterations in thyroid

Table 2. Summary of Change in Protein Synthesis in Response to Ethanol and Cyanamide

Region	% Change Saline *v* Ethanol (A *v* B)	% Change Saline *v* Cyanamide + Ethanol (A *v* D)	% Change Ethanol *v* Cyanamide + Ethanol (B *v* D)
Brain stem	−44*	−66†	−39 (NS)
Cortex	−21‡	−59†	−48†
Cerebellum	−19*	−55†	−45†
Midbrain	−14 (NS)	−60†	−54†

Abbreviation: NS, not significant.
*$P < .025$.
†$P < .001$.
‡$P < .01$.

hormone status,[25] for example). This differential regional sensitivity also extends to alcohol.[26-30] For example, corticotropin-releasing factor in brain of alcohol preferring mice show increases in frontal cortex, but a paradoxical decline in medulla-pons compared with their nonpreferring mice counterparts.[29] Similar contrasting effects are also observed in studies on the human brain.[31] In the aforementioned study on oxidative stress, the activity of the antioxidant enzyme catalase declined in the corpus-callosum, but increased in the frontal cortex[31] (for reviews on the effects on alcohol on regional brain damage, see Harper[32] and Harper and Kril[33]).

Protein Synthesis in Different Brain Regions

Compared with other mammalian tissues, such as the hepatointestinal and musculoskeletal systems, the pathophysiologic factors that regulate brain protein synthesis are poorly understood. However, there are a few studies in which measurement of protein synthesis in brain has been performed using the flooding dose technique. This has seen wide applicability in vivo in such diverse conditions, such as insulin-like growth factor (IGF)-1 treatment,[34] mu-opioid receptor stimulation,[35] starvation and surgical stress,[36] and liver damage and repair,[37] for example. However, one of the basic assumptions in some of the early studies was the notion that the brain is homogenous, and all regions respond similarly to pathologic stimuli.

In the present study, all regions had similar protein synthesis rates except the cortex, which was lower. This had no relationship on subsequent sensitivities to the modulating effects of either ethanol or cyanamide + ethanol where midbrain was the least sensitive. The brain stem showed the greatest decrease in protein synthesis, ie, 44%. This is arguably one of the greatest decreases in tissue protein synthesis in 2.5 hours using our dosing regimen with ethanol. We do not know the reasons why some regions of the brain were more significantly affected by ethanol. Certainly, regional brain sensitivities to metabolic perturbations have been well characterized, and it is possible a similar phenomenon occurs in response to ethanol.[38,39]

We need to raise the question why, in the current study, the regional differences in brain protein synthesis are so much smaller than those observed by other investigators using quantitative autoradiography after ^{35}S methionine injection in vivo.[16] In the aforementioned study, a total of 24 brain regions were investigated and synthesis rates expressed as nanomoles of 100 $g^{-1} \cdot min \cdot g^{-1}$.[16] The intertissue variability in synthesis rates was considerable; for example, rates in the stria terminalis were less than a fifth of the rate in the thalamus.[16] Such variability may arise if there were different abundance of proteins with varying methionine concentrations, although we do not have evidence to support this. However, in the present study, data were expressed as fractional rates although only 4 regions of the brain were examined. Limitations in resources and time precluded us from examining more regions.

Alcohol, Tolerance, and Increasing Levels of Acetaldehyde

In our previous studies on the role of chronic alcohol ingestion, fractional rates of protein synthesis in the brain were measured with a flooding dose of radiolabeled phenylalanine and shown to be unaltered after 3 weeks of ethanol feeding.[30] The susceptibility of the brain to acute ethanol dosage was confirmed by the observation that 2.5 hours after ethanol dosage, rates of brain protein were reduced. The contrasting differences between acute and chronic ethanol exposure may be explained by a "tolerance phenomenon" reflecting an adaptive mechanism, which contributes to limiting the damage induced by ethanol in this organ.[40] Such tolerance has been observed in terms of the motor impairment and other behavioral indices induced by alcohol.[41] However, we are unable to explain the nature of the adaptive response or its underlying molecular or cellular basis, although this is an area of considerable interest (see reviews, Crabbe[42]).

Acetaldehyde, the first metabolite of ethanol metabolism, has been postulated to mediate the numerous actions of alcohol on the brain.[43] This is a highly reactive intermediate of ethanol metabolism and permeates the blood-brain interface.[43] Acetaldehyde has also been shown to cause structural and conformational modifications of brain proteins.[44] However, due to the extreme volatility of acetaldehyde, it can only be injected into rats at very low molar concentrations, less than 5 mmol/kg body weight. Pretreatment of ethanol-dosed rats with cyanamide increases endogenous acetaldehyde, a technique used in a number of studies to assess the role of this metabolite in vivo.[45] Nevertheless, it is important to emphasize that these studies were not designed to be a comprehensive investigation into the possible role of acetaldehyde, but rather whether rates could be altered at all in an extreme pathologic situation of alcohol toxicity.

Although we did not measure acetaldehyde in the present study, we have done so in previous experiments using a different set of young rats, but using an identical dosing protocol.[19] Thus, blood acetaldehyde levels increase 6-fold in rats treated with ethanol (mean, 31 nmol/mL) compared with saline (mean, 5 nmol/mL[19]). In cyanamide + ethanol-dosed rats, blood acetaldehyde increases to 2,495 nmol/mL.[19] Tissue levels in liver and muscle increase to a similar order of magnitude with cyanamide + ethanol dosing.[19] The brain has an inherent ability to oxidize alcohol and acetaldehyde, although it is not known whether acetaldehyde generated in the brain directly was responsible for the further suppression of protein synthesis.[46] We do not have regional brain acetaldehyde concentrations, but data would be unreliable using present techniques, as it is extremely volatile. Theoretically, tissues need to be dissected within seconds,[47] whereas practically a much longer

period was required to dissect the brain (see Materials and Methods).

Overall, cyanamide pretreatment had a more dramatic decline on protein synthesis rate than ethanol alone. It has already been shown that acetaldehyde-mediated influences are important facets of alcoholic brain tissue damage.[43] Studies from our research group and others have also shown the importance of acetaldehyde in mediating pathogenic changes in other tissues, such as intestine and heart.[48,49]

Possible Mechanisms

It is important to raise in this section potential mechanisms for the regional sensitivities in brain protein synthesis to alcohol and to identify distinguishing features of the midbrain that might protect it from the alterations induced by alcohol.

Brain protein synthesis might be affected by covalent binding with tissue proteins (e.g., cell membranes and subcellular organelles) to form acetaldehyde-protein adducts.[44] Previous studies have shown that protein adducts form in several other tissues in vivo as a consequence of ethanol exposure, including muscle[50] and liver.[51] Cyanamide + ethanol treatments enhance adduct formation in the liver.[52] However, the pathogenic significance of this protein-adduct formation, in relationship to brain pathology and protein synthesis, has yet to be elucidated. Cytotoxic reactive oxygen species may also be generated in the brain during alcohol exposure.[53] Alternatively, the detailed changes in initiation factors and other subcellular regulators of protein synthesis described for ethanol-exposed muscle and liver[54] may also apply to brain.[4] Changes in blood flow do not, however, appear to be affected by acute ethanol dosage.[55]

The midbrain exhibited a smaller change in protein synthesis when compared with the other 3 regions. This observation is not unusual. For example, heat shock protein (HSP) mRNA in the midbrain is paradoxically reduced in rats fed ethanol for a chronic period compared with the brain stem, cortex, and cerebellum, which shows no significant change.[56] Although this does not fully explain the refractory response of the midbrain, it does serve to illustrate that this region has different metabolic responses to pathophysiologic stimuli.[56] In a murine study using an acute ethanol dosing regimen, choline acetyltransferase activities increased in cortex, midbrain, and cerebellum (and 4 other regions not studied in our own experiment). Additional ethanol dosing potentiated this effect only in the midbrain and not the other 6 regions.[57] Neuroprotection in the midbrain may be due to the acute stability of antioxidants, such as glutathione or superoxide dismutase compared with their decrease in other regions.[58,59] Site-specific differences in amino acid concentrations may also contribute to the different regional responses to protein synthesis in alcohol-dosed rats.[60]

Methodologic Considerations

Potentially, these studies could be criticized on the grounds that there are serious methodologic errors or limitations inherent in the experimental design, in particular, relating to the maturity of the rats and the mode of ethanol dosing and the method for measuring protein synthesis. However, we do not believe this to be the case for the following reasons. First, alcohol consumption in young people is a growing or established problem in most westernized countries, and features of brain damage are seen in young alcohol abusers.[61] Second, the blood levels of ethanol (i.e., 50 to 60 mmol/L[62]) achieved with this dosing regimen (75 mmol/kg body weight) are similar to levels occurring in pathophysiologic situations. For example, clinical studies have reported levels of 40 to 60 mmol/L,[63] 76 to 111 mmol/L,[64] and 38 to 100 mmol/L.[65] With respect to the intraperitoneal route of alcohol dosage, we are aware that other techniques of administration can be used in acute ethanol-dosing studies, such as the intravenous or intragastric routes.[66] However, intraperitoneal dosing as used in the present study, ensures greater bioavailability of alcohol. It circumvents problems encountered when alcohol is given by gavage in which its absorption or first pass-metabolism by alcohol dehydrogenase can be modulated by either pharmacologic agents or nutritional status.[67] Intravenous ethanol dosage may possibly cause red blood cell lysis.[66]

Conclusions

In conclusion, the applicability of the flooding dose technique to measure protein synthesis in the brain in vivo is demonstrated by its ability to measure regional difference. Impaired protein synthesis rates may contribute to, or reflect, the pathogenesis of alcohol-induced brain damage.

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