ETHANOL-INDUCED CHANGES IN METHIONINE METABOLISM IN RAT LIVER

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SUMMARY. The administration of alcohol to rats fed a protein-restricted diet results in significant changes in the hepatic content of four enzymes of methionine metabolism. The levels of s-adenosylmethionine synthetase, cystathionine synthase, and betaine-homocysteine methyltransferase increase while the level of methyltetrahydrofolate-homocysteine methyltransferase decreases. These changes represent a reversal of the normal adaptive response to protein-restriction. The resultant impairment in methionine conservation could explain the alcohol-induced increase in the dietary lipotrope requirement.

Dietary supplements of methionine or choline can partially reverse hepatic steatosis induced by chronic ethanol administration to rats (1-3). One possible interpretation of this observation is that alcohol ingestion increases the dietary lipotrope requirement (4). In an earlier study, we examined the effect of alcohol on the content of four enzymes of methionine metabolism in livers of rats fed a standard laboratory ration (5). The activity of s-adenosylmethionine synthetase (EC 2.5.1.6, ATP: L-methionine-s-adenosyltransferase) increased significantly in alcohol-treated animals. There were no changes in the levels of either cystathionine β-synthase (EC 4.2.1.22, L-serine hydro-lyase [adding homocysteine]) or betaine-homocysteine methyltransferase (EC 2.1.1.5, betaine: L-homocysteine s-methyltransferase). We were, at that time, unable to explain the "methionine-wasting" effect of alcohol.

In the present report we have re-examined this effect by extending our observations to several diet groups and by measuring the activities of additional enzymes -- now known to be significant in the regulation of methionine metabolism. Within three days of the initiation of treatment

with alcohol, we observed significant changes in the content of enzymes in livers of rats fed a low-protein diet. The net result of the alcohol-induced changes would be a limitation in the ability to methylate homocysteine -- a reaction essential to the conservation of methionine.

METHODS. We maintained male Sprague-Dawley rats on diets containing 3.5% casein, 26% casein or 55% casein (General Biochemicals). During the second week of the dietary program we began daily isocaloric feedings either of ethanol (4.4 g/kg body weight as a 50% solution) or of glucose (as a 50% solution) by gastric intubation. The duration of alcohol treatment varied from three to ten days. We sacrificed the animals 24 hours after the last dose of alcohol.

We have described our methods for the preparation of the tissue extracts (6) and for the assays of the following enzymes: s-adenosylmethionine synthetase (7), betaine-homocysteine methyltransferase (8), cystathionine synthase (7), 5-methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13) (6), and s-adenosylhomocysteine synthase (EC 3.3.1.1, s-adenosyl-L-homocysteine hydrolase) (9). Protein was measured by the Lowry method (10).

By definition, 1 unit of enzyme activity catalyzes the synthesis of 1 nmole of product in 60 minutes. We routinely examined our data expressed as units/g liver, units/liver, units/liver/gm body weight and as specific activity (units/mg soluble protein). In this report, we have chosen to present the results in the alcohol-treated rats as a percentage of the corresponding value in the control (glucose-treated) animals. The "t" test for unpaired samples was used to evaluate statistical significance.

RESULTS. As shown in Table 1, administration of ethanol has a minimal effect on the enzyme activities in the livers of rats fed diets containing either 26% casein or 55% casein. The only significant alteration was an increase in s-adenosylmethionine synthetase in animals fed 26% casein, agreeing with earlier findings (5). When we administered alcohol to animals fed

TABLE 1

EFFECT OF ALCOHOL ON ENZYMES OF METHIONINE METABOLISM IN LIVERS OF RATS FED DIETS WITH VARYING PROTEIN CONTENT

Diet	Treatment	s-Ado Met Synthetase	CH_3-THF Enzyme	Cystathionine Synthase	Betaine Enzyme
3.5% Casein	Glucose	100	100	100	100
3.5% Casein	Ethanol	156*	72**	238*	301*
26% Casein	Glucose	44	24	217	151
26% Casein	Ethanol	68**	28	204	157
55% Casein	Glucose	198	28	141	130
55% Casein	Ethanol	166	43	133	125

Each group contained at least five rats. The rats were fed the designated diet for one week prior to the initiation of the treatment and during the treatment period of three to ten days. Results for each enzyme are expressed as the percentage of the specific activity of that enzyme in livers of glucose-treated rats fed the 3.5% casein diet. Abbreviations employed: CH_3-THF Enzyme = 5-methyltetrahydrofolate-homocysteine methyltransferase and betaine enzyme = betaine-homocysteine methyltransferase.

Statistical significance between glucose and ethanol-treated animals within each diet group:

* $P < .001$; ** $P < .05$

TABLE 2

EFFECT OF ETHANOL ON ENZYMES OF METHIONINE METABOLISM IN LIVERS OF RATS FED A PROTEIN-RESTRICTED DIET

Enzyme	Per Cent Control Value			
	U/g Liver	U/ Liver	U/g B.W.	U/mg Prot
s-Adenosylmethionine synthetase	187*	153†	148*	150†
s-Adenosylhomocysteine synthase	111	92	92	102
5-CH_3-THF methyltransferase	76‡	63‡	57†	67†
Betaine methyltransferase	272*	223*	214*	255*
Cystathionine synthase	173†	145‡	142‡	141‡
Cystathionase	126	103	101	121

We treated nine rats with ethanol and ten with glucose for four days prior to sacrifice. All of the animals received a diet containing 3.5% casein for three weeks including the treatment period. The results for the alcohol-treated group are expressed as the percentage of the value in the control (glucose-treated) rats.

Statistical significance between glucose and ethanol treated animals:

* $P < .001$; † $P < .01$; ‡ $P < .05$

the low-protein diet however, we observed significant changes in the levels of four of the enzymes. The activity of 5-methyltetrahydrofolate-homocysteine methyltransferase decreased while the activities of the other enzymes increased. A more detailed analysis of a typical study is presented in Table 2. The significance of the alcohol-induced changes in enzyme activity is not affected by the mode chosen for expression of the results. Thus, differences in specific activity reflect differences in total hepatic content of the enzyme. In addition, Table 2 highlights the specificity of the effect of alcohol treatment. The activities of s-adenosylmethionine

synthetase, cystathionine synthase and betaine-homocysteine methyltransferase increased, while the content of 5-methyltetrahydrofolate-homocysteine methyltransferase decreased, and the levels of both s-adenosylhomocysteine synthase and cystathionase remained unchanged.

In other studies, we varied the duration of alcohol administration to rats fed the 3.5% casein diet. Twenty-four hours after a single dose of alcohol, there was only one significant change -- an increase in s-adenosylmethionine synthetase. After ten days of alcohol treatment, the pattern of enzymes was no different from the pattern observed after three days (Table 2). Since the alcohol-treated animals gained weight at the same rate as the control rats, a difference in food intake is an unlikely explanation for the differences in enzyme activities.

The enzyme changes were specific for liver. Independent of diet or duration of treatment, alcohol had no effect on enzyme activities in brain or kidney.

DISCUSSION. The present studies define a mechanism for the increased dietary requirement for methionine (and choline) in animals fed alcohol together with a protein-restricted diet. Methionine metabolism in mammalian liver can be represented as a cycle with a single outlet - the cystathionine synthase reaction. Regulation of the pathway is achieved, in part, by the distribution of homocysteine among several competing reactions (8,11,12). Remethylation of homocysteine conserves the S-atom and the aliphatic portion of the original methionine molecule. In this regard 5-methyltetrahydrofolate-homocysteine methyltransferase is more significant than betaine-homocysteine methyltransferase (6,12). Utilization of the homocysteine in the cystathionine synthase reaction commits the molecule to the transsulfuration sequence -- the major route for methionine catabolism (13). In response to protein (or methionine) deprivation there is an adaptive response in the hepatic content of the relevant enzymes. Perhaps the most significant changes are a reduction in cystathionine synthase and an increase in 5-methyltetrahydrofolate-

homocysteine methyltransferase (6,8,14). By facilitating remethylation at the expense of transsulfuration, this coordinate change could maintain normal concentrations of methionine in the tissues. The data in this report indicate that alcohol administration, that results in an increase in cystathionine synthase and a decrease in 5-methyltetrahydrofolate-homocysteine methyltransferase, may impair this adaptive response.

The metabolic consequences of alcohol-induced increases in s-adenosylmethionine synthetase and betaine-homocysteine methyltransferase must be defined. It seems reasonable to speculate that an increase in the synthesis of s-adenosylmethionine might limit further the availability of methionine for protein synthesis. The increased activity of betaine-homocysteine methyltransferase may reflect an ethanol-induced increase in choline degradation (15) which would deplete further the tissue content of lipotropes. The betaine reaction is an unlikely mechanism for methionine conservation since, in the absence of dietary choline, synthesis of betaine from ethanolamine utilizes three molecules of methionine (as s-adenosylmethionine) while the betaine-homocysteine methyltransferase reaction regenerates only one molecule of methionine.

In the intact animal, the ability to conserve methionine may be compounded by other effects of alcohol. Israel et al found that alcohol inhibited intestinal absorption of methionine (16,17). Ethanol may also limit the absorption and metabolism of folic acid (18,19). Similar considerations may be relevant to the pathogenesis of human alcoholic liver disease. In that setting there may be the additional factor of deficient dietary intake of folic acid.

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