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Ethanol Effects on Methionine Metabolism in Rat Liver (33353)

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Dietary supplements of methionine and choline can partially reverse the hepatic steatosis caused by chronic ethanol administration of rats (1). This finding can be interpreted as an increase in the dietary lipotrope requirement secondary to alcohol feeding (2). In order to define the enzymatic basis for this phenomenon, we have studied the effect of ethanol feeding on four enzymes of methionine metabolism in rat liver—methionine-activating enzyme (ATP:L-methionine-*S*-adenosyltransferase, E.C.2.5.1.6); cystathionine synthase; cystathionase (L-homoserine hydro-lyase (deaminating), E.C.4.2.1.15); and betaine–homocysteine methyltransferase (E.C.2.1.1.5). The four enzymes catalyze reactions (1) through (4), respectively:

Methionine + ATP ⟶ *S*-adenosylmethionine + PP_i + P_i, (1)

Serine + homocysteine ⟶ cystathionine, (2)

Cystathionine ⟶ α-ketobutyrate + cysteine + NH_3, (3)

Betaine + homocysteine ⟶ methionine + dimethylglycine. (4)

Experimental Procedures. Male, Sprague-Dawley rats (weight 150–200 g) maintained on Wayne Lab Blox were used routinely. At the time of sacrifice, the animals were stunned and then exsanguinated by carotid transection. The tissues were removed, chilled immediately, and tested either individually or as pooled specimens. Homogenization was performed in ice-cold 0.03 *M* potassium phosphate buffer, pH 6.9, in a Sorvall Omni-Mixer at half speed for 2 min. The extracts were centrifuged at 8000*g* for 15 min at 4°. The supernatant was used for all assays which were performed on the day of sacrifice.

We used our usual assay methods (3, 4). Each is a specific and direct measurement of product formation. Since each of the assay systems is unsaturated with respect to one of the substrates, direct comparisons between the different enzyme activities cannot be made. The results are expressed as mμmoles of product/time/mg of protein. No significant differences in interpretation occurred when we employed units/g of wet weight of liver; units/animal; or units/g of body weight.

All chemicals were of reagent grade and were obtained from commercial sources. Merck, Sharp and Dohme generously supplied the actinomycin D.

Results. Chronic administration of ethanol. The specific activities of methionine-activating enzyme and cystathionase in rat liver were increased significantly in rats given ethanol (4.4 g/kg daily for 14 days) as a supplement to a normal laboratory ration (Table I). The alcohol feeding did not affect the levels of cystathionine synthase and betaine–homocysteine methyltransferase. Ethanol-treated animals gained weight at the same rate as the glucose-supplemented control rats and the alcohol caused no significant change in liver weight, liver fat, or extractable protein per gram of liver. The increase in specific activities therefore reflects a net increase in methionine-activating enzyme and cystathionase. The increase in methionine-activating enzyme occurred within 5 days following the beginning of the ethanol feeding and was unchanged thereafter.

The enzyme levels also were measured in

TABLE I. Effect of Chronic Ethanol Administration on Enzymes in Rat Liver.[a]

Treatment	MAE	Cyst. synth.	C-ase	BH Enz
Control	61	365	24	17
Treated	96[b]	391	36[b]	18

[a] The data represent the mean values for the specific activities of the enzymes in liver extracts prepared from each of the six rats in the two groups. The animals weighed 175 g initially and 250 g at the time of sacrifice on the fifteenth day. Each treated animal received 14 daily doses of ethanol (4.4 g/kg as a 50% solution) by gastric intubation. Control animals were given an equal volume of isocaloric glucose solution. Abbreviations and units: MAE = methionine-activating enzyme (mμmoles/mg of protein/60 min). Cyst. synth. = cystathionine synthase (mμmoles/mg of protein/135 min). C-ase = cystathionase (mμmoles/mg of protein/30 min). BH Enz = betaine homocysteine methyltransferase (mμmoles/mg of protein/15 min).

[b] $p < .01$

extracts prepared from the brains of these animals. Ethanol administration did not alter the content of methionine-activating enzyme or of cystathionine synthase. We did not detect betaine–homocysteine methyltransferase in rat brain and because of the low level of cystathionase in this organ we could not interpret small changes.

By administering daily, parenteral supplements of L-methionine (0.33 mmoles) and choline (0.2 mmoles) concurrently with the alcohol, we were able to measure the effect of these lipotropes on the enzyme changes induced by ethanol (Table II). Neither the methionine nor the choline prevented the increase in methionine-activating enzyme or cystathionase. Indeed, when given to the control rats, both agents had the same effects as ethanol and caused significant increases in the two enzymes. Methionine-activating enzyme levels, already increased by ethanol treatment, were further augmented by choline or methionine administration. We did not observe this phenomenon with the other enzymes.

Single dose of ethanol. The effect of a single, large dose of ethanol on the specific activities of the enzymes in rat liver is shown in Table III. There was a marked increase in the specific activity of methionine-activating enzyme and lesser increases in cystathionine synthase and betaine–homocysteine methyltransferase. The magnitudes of the increments in these last two enzymes were approximately equal in all experiments and the ratio of activities remained unchanged. The single dose, unlike the chronic feeding of alcohol, did not affect the levels of cystathionase.

No increase in methionine-activating enzyme was found within 8 hr following the administration of alcohol, and the maximum response was reached 18–24 hr after treatment. The enzyme activity did not change in response to single doses of ethanol of less than 6.0 g/kg.

TABLE II. Effects of Choline and Methionine on Liver Enzymes in Rats Fed Ethanol.[a]

Treatment	MAE	Cyst. synth.	C-ase	BH Enz
Control	56	324	22	17
+ methionine	87	391	40	22
+ choline	88	351	50	24
Treated	88	341	40	20
+ methionine	95	355	38	20
+ choline	114	364	35	23

[a] The data represent the mean values for duplicate determinations of the specific activities of each enzyme in pooled extracts from each group of four rats. The mean weight of the animals was 180 g initially and 227 g at the time of sacrifice. The control animals received glucose and the treated animals received ethanol as described in Table I. The L-methionine (50 mg) and choline chloride (25 mg) were administered by daily intraperitoneal injections. Abbreviations and units: as defined in Table I.

TABLE III. Effect of a Single Dose of Ethanol on Enzymes in Rat Liver.[a]

Treatment	MAE	Cyst. synth.	C-ase	BH Enz
Control	64	237	38	14
Treated	146[b]	360[c]	39	23[b]

[a] The data represent the mean values for the specific activities of the enzymes in liver extracts prepared from each of the five rats in the two groups. The animals (mean wt. 190 g) were given either ethanol (6.5 g/kg as a 50% solution) or an equal volume of isocaloric glucose by gastric intubation. The rats were fasted for the 18 hr between intubation and sacrifice. This period of fasting may explain the difference in the control levels of cystathionine synthase as compared to Tables I and II (5). Abbreviations and units: as defined in Table I.

[b] $p < .001$.

[c] $p < .02$.

The acute effect of ethanol was unaffected by the sex of the animals, the antecedent diet, or the period of fasting which followed the feeding of alcohol. Female and male rats showed the same pattern of responses even though the level of methionine-activating enzyme in the control females was 1.8 times that of the males. Cystathionine synthase and betaine–homocysteine methyltransferase also responded to alcohol; the control and posttreatment values showed no sex differences. Cystathionase, which is 1.5 times greater in the untreated female than in the untreated male, was unaltered by the single feeding of ethanol. Pretreatment of male rats with a vitamin-supplemented, low protein diet (General Biochemicals) for 14 days did not alter the pattern of responses to ethanol. This diet does however affect both the basal enzyme levels and their response to fasting (5). The pattern of enzyme changes was also unaffected when the animals were allowed to eat following the administration of the ethanol.

The administration of ATP, α-tocopherol, or asparagine may prevent the histopathological changes which follow the acute administration of large doses of ethanol (6–8). We found that ATP (35 mg/100 g × 3 doses, intraperitoneally), α-tocopherol (15 mg/100 g, intraperitoneally) and asparagine (200 mg/100 g, by gastric tube) failed to prevent an increase in the specific activity of methionine-activating enzyme.

Mechanism of action of ethanol on hepatic methionine-activiating enzyme. We could not demonstrate any direct effect of alcohol on methionine-activiating enzyme. Both the addition of ethanol to the reaction mixture *in vitro* and the preincubation of the liver extract with alcohol were ineffective. The methods for the preincubation experiments were designed to allow the production of various intermediates in ethanol metabolism including acetaldehyde, acetoin and 5-hydroxy-4-ketohexanoic acid (9). We could not demonstrate activation of control extracts coincubated with preparations from the ethanol treated animals (untreated extract, heat inactivated extract and protein free extract). Passage of the extracts derived from the ethanol treated rats through a column of Sephadex G-25 resulted in no loss of acitivity.

The increase in hepatic methionine-activating enzyme probably represents new enzyme synthesis since ethanol has no effect in animals concurrently given puromycin (6 mg/100 g every 4 hr). Actinomycin D (0.12 mg/100 g) also prevents increases in the level of methionine-activating enzyme which further suggests that the new protein formation is dependent on DNA directed RNA synthesis. The response of the control animals to actinomycin D is of interest. The drug itself caused a 50% increase in hepatic methionine-activating enzyme content as well as in specific activity. Since an increase does not occur with puromycin alone we have evidence that the template directing the synthesis of this enzyme is relatively stable.

Discussion. The pathway for methionine metabolism and the interrelationship of methionine and choline are illustrated in Fig. 1. The major functions of methionine are (a) utilization for protein synthesis; (b) conversion to *S*-adenosylmethionine, the primary biological methyl group donor, and (c) conversion by means of the transsulfuration pathway to cystathionine, cysteine, and further derivatives of cysteine.

Little is known of the mechanisms which

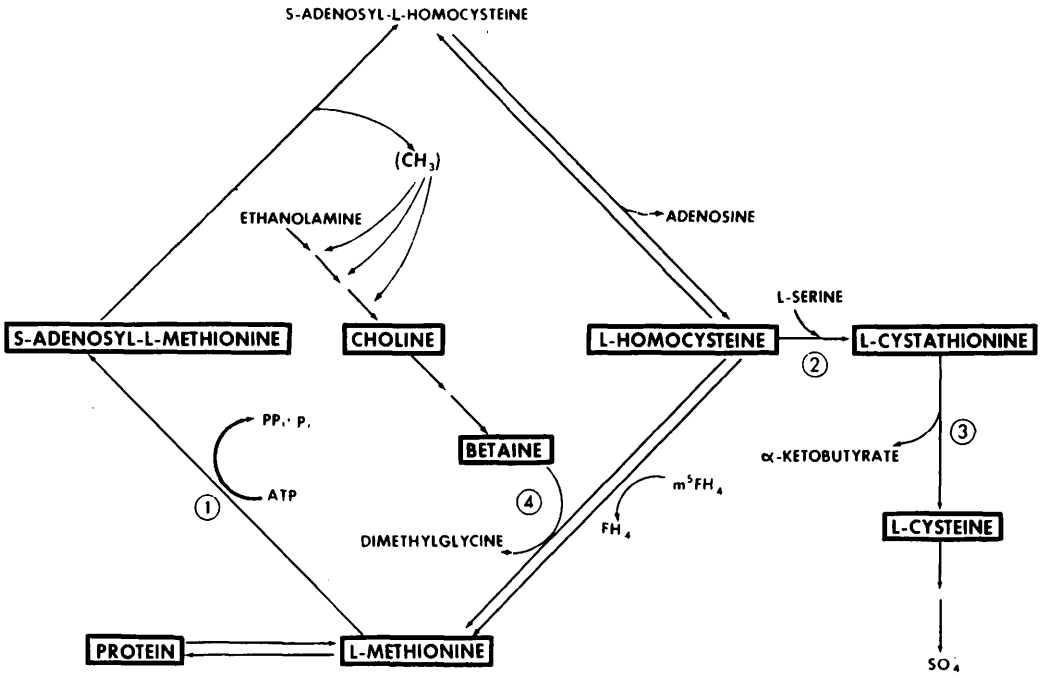

FIG. 1. Major pathways for methionine metabolism in mammalian tissues. P_i and PP_i = inorganic phosphate and pyrophosphate; FH_4 = tetrahydrofolic acid; and m^5FH_4 = N^5-methyltetrahydrofolic acid. The many transmethylation reactions, including the methylation of ethanolamine (as phosphatidylethanolamine) to choline (as phosphatidylcholine), are schematically represented by the formation of (CH_3). In this study we assayed the reactions designated: (1) methionine-activating enzyme; (2) cystathionine synthase; (3) cystathionase, and (4) betaine-homocysteine methyltransferase.

regulate methionine metabolism. From studies of patients who are deficient in cystathionine synthase and cystathionase, we can conclude that the enzymes are relatively insensitive to the tissue levels of their substrates and products (3, 10, 11). There is no evidence for the coordinate control of the enzyme activities in rat tissues. Dietary changes and hormone treatment may significantly alter one enzyme without affecting the others (5). It seems likely that methionine metabolism is regulated by a balance of competing enzyme reactions at certain key points. One regulatory site is formed by the reactions utilizing homocysteine. Conversion to cystathionine is irreversible whereas remethylation (by betaine or N^5-methyltetrahydrofolic acid) regenerates methionine. The methionine-sparing effect of cyst(e)ine can be explained by a reduction in the synthesis of cystathionine (4). Another possible regulatory site is in the reactions which utilize methionine as substrate. At this point activation of methionine to *S*-adenosylmethionine is in competition with the utilization of methionine for protein synthesis.

We have demonstrated that ethanol administration significantly increases hepatic methionine-activating enzyme. With chronic treatment we observed an increase in cystathionase. During acute ethanol feeding we observed equivalent increases in cystathionine synthase and betaine–homocysteine methyltransferase. Since this latter change is "balanced" and since cystathionase is distal to the methionine cycle, we may reasonably confine our discussion to the possible effects of the increase in methionine-activating enzyme. Changes in the level of this enzyme may lead to an increased utilization of available methionine for *S*-adenosylmethionine synthesis and a decrease in the rate of protein synthesis may result. Increased levels of *S*-adenosylmethionine would facilitate trans-

methylation reactions including those reactions which result in the formation of choline and its derivatives. Few studies are available which directly test this hypothesis. Chronic ethanol feeding increases rat liver phospholipids but this is not specific (triglycerides are more markedly increased). Increased phosphatidylethanolamine rather than augmented transmethylation could explain the increase in phospholipids (12). Some data can be obtained from studies of sex determined differences in, and estrogen effects on, methionine metabolism. The extrapolation is justified by the fact that the pattern of enzymes in the male rat chronically fed ethanol resembled that in the female rat and in the male rat treated with estradiol (5). In all three situations we noted a marked increase in cystathionase and methionine-activating enzyme. Several studies have shown that female rats or feminized male rats incorporate greater amounts of the methyl group of methionine into phospholipid choline, liver RNA, and liver proteins (13, 14).

The relationship between the enzyme changes which resulted from chronic ethanol administration and hepatic steatosis is unclear. In these studies the livers from the treated animals showed no gross fat. In addition treatment with agents known to inhibit lipid accumulation did not affect the alcohol induced changes in enzymatic activity. Therefore, it is unlikely that an increase in liver lipid caused the increase in methionine-activating enzyme. But we do not believe that the induction of methionine-activating enzyme is the prime event leading to steatosis. Instead, we suggest that the increased level of this enzyme renders the animal more susceptible to methionine deficiency despite a normal dietary intake and perhaps more susceptible to the direct hepatotoxic effect of ethanol. This interpretation is consistent with the increased sensitivity of female rats to methionine (choline) deprivation and to alcohol ingestion (15, 16). It might also explain why treatment with lipotropic agents only partially reverses the pathology of chronic ethanol feeding.

Summary. Ethanol administration, in either chronic feeding or acute experiments, increased the level of methionine-activating enzyme in rat liver. The increase in methionine-activating enzyme did not occur in animals treated with puromycin or actinomycin D. The simultaneous administration of methionine or choline failed to inhibit this effect of ethanol. Prolonged ethanol feeding also led to an increase in hepatic cystathionase while acute intoxication resulted in a balanced increment in cystathionine synthase and betaine–homocysteine methyltransferase. We discussed the possible relationship of these results to the increased lipotrope requirement which attends alcohol consumption in rats.

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