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SHORT COMMUNICATIONS

Levels of *S*-adenosylmethionine and *S*-adenosylethionine in four different tissues of male weanling rats during subchronic feeding of DL-ethionine

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Chronic feeding of ethionine to rats has been shown to produce tumors of the liver, but not of other organs [1]. The carcinogenicity of ethionine is generally attributed to its role as an antimetabolite of methionine, since the simultaneous feeding of excess methionine inhibits the carcinogenic activity of ethionine [2]. Ethionine not only inhibits enzymatic reactions requiring methionine but can also replace methionine in such reactions [3, 4]. Chronic feeding of ethionine results in the synthesis and accumulation of *S*-adenosylethionine (AdoEt), the sulfur activation product of ethionine [5], and in the decreased formation of *S*-adenosylmethionine (AdoMet) [6]. AdoMet is the chief physiological methyl donor and is utilized in numerous transmethylating reactions including those altering the structure or biological activity of macromolecules such as nucleic acids, proteins and phospholipids [7–10]. Although the hepatic metabolism of ethionine has been studied extensively [1–5], few attempts have been made to compare such metabolism with that observed in extrahepatic tissues in which tumors have not been produced. Such comparisons could be helpful in delineating the mechanism whereby ethionine exerts its carcinogenic activity. Thus, we have determined the extent to which ethionine was activated to AdoEt in liver and in three tissues resistant to the carcinogenic action of ethionine in rats fed this compound both with and without excess dietary methionine.

Materials and methods

Fischer male rats (F344/NCr), 50–60 g, were produced at the Frederick Cancer Research Facility and housed in large plastic shoe box cases. For 5 days following their arrival at this laboratory the rats were fed *ad lib.* a natural ingredient ground chow diet (Wayne Lab Blox, Allied Mills Inc., Chicago, IL). The animals were then presented with one of four different diets: chow (control group); chow + 0.8% DL-methionine (Teklad Test Diets, Madison, WI); chow + 0.3% DL-ethionine (Aldrich Chemical Co., Milwaukee, WI), and chow + 0.3% DL-ethionine + 0.8% DL-methionine. All animals were weighed and killed by decapitation between 9:00 and 11:00 a.m. at the end of 3 weeks of feeding. Liver, kidney, pancreas, and testes were removed immediately, weighed, and quickly placed on ice. Each tissue was homogenized in 2 vol. of ice-cold 0.1 M sodium acetate buffer, pH 6.0, and the protein was precipitated with 1.5 vol. of 40% trichloroacetic acid (TCA). Protein-free supernatant fractions were washed with anhydrous ether and filtered as described [11]. Samples were heated in a boiling bath for 30 min, to convert AdoMet and AdoEt to methylthioadenosine (MTA) and ethylthioadenosine (ETA) respectively [12]. After cooling to room temperature, the samples were analyzed on high performance liquid chromatography (HPLC) using an Altex Ultrasphere-ODS C_{18} column (4.6 mm × 25 cm) equilibrated with 0.05 M monobasic potassium phosphate, 35% methanol at a flow rate of 1 ml/min. Absorbance was monitored at 254 nm, and the concentrations of MTA and ETA present were determined using MTA and ETA standards prepared from AdoMet (Boehringer Mannheim, Mannheim, West Germany) and AdoEt (Sigma Chemical Co., St. Louis, MO) respectively. Endogenous MTA was not detectable in testes, pancreas and kidney. MTA levels in liver were barely detectable and were negligible relative to AdoMet levels (3% AdoMet levels). Hepatic MTA was thus not further quantitated. A second study was performed identical to the first, except that 1.5% DL-methionine was used instead of 0.8% DL-methionine and AdoMet and AdoEt were measured in liver and kidney only.

Results and discussion

The administration of 0.3% ethionine in the diet to male weanling rats for 3 weeks led to a 77% decrease in the hepatic contents of AdoMet (Fig. 1A). Ethionine feeding produced no change in the AdoMet levels in kidney but did cause a significant rise in AdoMet levels in the pancreas and testes (Fig. 1A). High levels of AdoEt were observed in the livers of rats treated with ethionine; in ethionine-fed rats the hepatic AdoEt levels were 75 times the corresponding AdoMet levels. The levels of AdoEt in kidney, testes, and pancreas were very much lower than those of liver but were still approximately 10, 21, and 10 times, respectively, the corresponding AdoMet levels. No AdoEt was observed in the liver or any of the other organs of rats not receiving ethionine (Fig. 1B). The simultaneous administration of 0.8% methionine in the ethionine-containing diet completely prevented the rise in AdoMet levels produced in testes and partially prevented the decrease in AdoMet in liver (Fig. 1A). The simultaneous administration of methione also led to a drop in AdoEt levels in all the organs studied (Fig. 1B). The methione-induced drop in renal and hepatic AdoEt levels and the alleviation by methionine of the ethionine-induced suppression of AdoMet levels were significantly greater in rats fed 1.5% methionine than in those fed 0.8% methionine. The hepatic levels of AdoMet and AdoEt observed in the latter study were as follows (expressed as: group, nmoles AdoMet/g ± S.E., nmoles AdoEt/g ± S.E. respectively): chow, 78.7 ± 3.0, 0 ± 0; chow + methionine, 141.8 ± 3.2, 0 ± 0; chow + ethionine, 29.1 ± 2.1, 1113.5 ± 39.0; chow + ethionine + methionine, 57.8 ± 2.9, 415.2 ± 6.0. The corresponding renal values of AdoMet and AdoEt were the following: chow, 23.6 ± 1.0, 0 ± 0; chow + methionine, 34.1 ± 1.0, 0 ± 0; chow + ethionine, 22.5 ± 2.0, 279.1 ± 26.0; chow + ethionine + methionine, 31.5 ± 1.0; 126.8 ± 3.0. The effects of dietary methionine on tissue AdoMet and AdoEt levels are more clear when the latter are expressed as the ratios of AdoEt:AdoMet (Table 1). The ratio of AdoEt:AdoMet was markedly higher in liver than in any other tissue under investigation (Table 1). This ratio was decreased in all tissues in response to excess methionine. With 1.5% methionine, AdoEt:AdoMet values for respective ethionine and ethionine + methionine groups for liver and kidney, respectively, were as follows: liver, 38.26 ± 3.07, 7.18 ± 0.75; kidney, 12.40 ± 1.59, 4.03 ± 0.159. Consistent with previous observations [12], the administration of 0.3% ethionine to rats for 3 weeks led to a 47% suppression in their weight gain. The liver weights of ethionine-treated rats, relative to their body weights, were 26% greater than

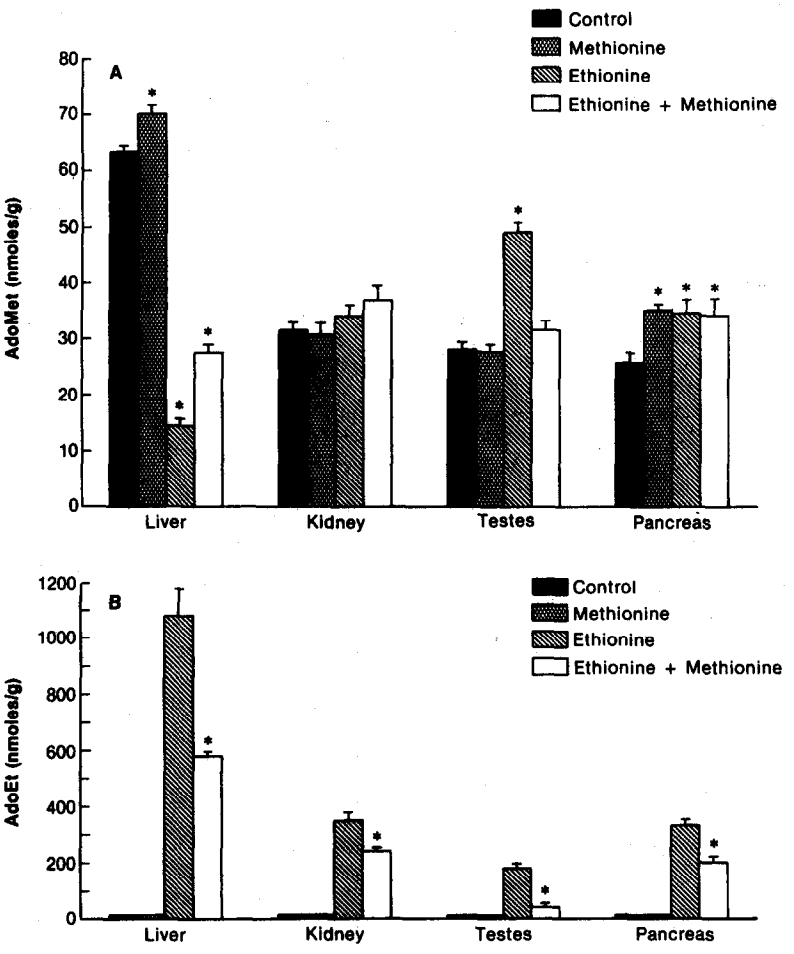

Fig. 1. AdoMet and AdoEt levels measured in four different tissues from rats that had been fed for 3 weeks with 0.3% DL-ethionine with or without 0.8% DL-methionine in the diet. Each bar represents the mean ± S.E. of five rats. An asterisk (*) indicates significantly different ($P < 0.05$) than corresponding control values for (A), while significantly different ($P < 0.05$) from corresponding ethionine-treated group for (B).

those of the controls; feeding 0.8% methionine lowered this increase to but 14%. Ethionine treatment lowered the weights of other organs relative to body weight as follows: testes 27%, pancreas 10%, and kidney 0%. Methionine supplementation completely alleviated the ethionine-induced suppression in relative weights of testes and pancreas. Ethionine feeding similarly suppressed the absolute organ weights of testes, pancreas and kidney, but exhibited no significant effect on absolute liver weights.

The present data appear to constitute the first report showing the effects of chronic ethionine feeding on AdoMet and AdoEt levels in extrahepatic tissues. Three major observations were made: (1) ethionine treatment suppressed AdoMet levels only in liver; (2) the levels of AdoEt were highest in liver but were still significant to kidney, pancreas and testes; and (3) the addition of methionine to the ethionine-containing diet completely prevented the rise in AdoMet levels in testes and partially prevented the drop in such levels in liver. These observations show that, of the four tissues studied, liver was easily the most sensitive to the establishment of a high ratio of AdoEt: AdoMet during ethionine feeding. It is thus reasonable to speculate that a high ratio of AdoEt: AdoMet may be a contributing factor to the carcinogenic activity ethionine. This proposal is supported by previous observations that AdoEt itself transforms hepatocytes in culture [13] and that a high ratio of AdoEt: AdoMet inhibits DNA methylation *in vitro* [14]. The administration to rats of acute doses of ethionine following partial hepatectomy has been shown to result in the hypomethylation of DNA [14]. Studies in this laboratory indicate that the DNA synthesized during the regeneration and cell proliferation which accompany ethionine treatment is undermethylated [15]. Although many carcinogens have been found to induce the hypomethylation of DNA [14–19], the significance of this effect in carcinogenesis is not known. There is much evidence correlating DNA hypomethylation with gene expression [20, 21]. Recent evidence indicates that, in certain specific cases at least, the expression of an oncogene is accompanied by its hypomethylation [22, 23].

The reversal by dietary methionine of the biochemical effects of ethionine are consistent with previous observations showing methionine prevention of ethionine-induced pathological lesions in testes, pancreas, and liver [2]. It is interesting that, while even 1.5% dietary methionine does not prevent completely the ethionine-induced suppression of hepatic AdoMet levels, 0.8% methionine completely prevented the hepatocarcinogenic activity of ethionine [2]. It is possible that an AdoEt: AdoMet ratio below a critical level may not adversely affect the normal

Table 1. Ratio of AdoEt to AdoMet in four different rat tissues following ethionine feeding with and without dietary supplementation with methionine*

Dietary methionine supplementation (%, w/w)	AdoEt/AdoMet			
	Liver	Kidney	Testes	Pancreas
0	75.18 ± 8.82	10.0 ± 1.01	3.64 ± 0.32	9.79 ± 0.89
0.8	22.10 ± 0.77	6.27 ± 0.46	1.38 ± 0.11	5.95 ± 0.75

* Rats (50–60 g) were fed 0.3% DL-ethionine in a chow diet with or without supplementary 0.8% DL-methionine for 3 weeks. Results are expressed as the mean ± S.E. for five to eight animals.

levels and patterns of DNA methylation. Experiments are currently underway to determine whether the altered tissue levels of AdoMet and AdoEt produced by chronic ethionine administration are accompanied by changes in DNA methylation.

In summary, the data showed that, of four rat organs studied, liver was the organ most susceptible to the effect of ethionine on the suppression of AdoMet and the accumulation of AdoEt. The effects of ethionine could generally be reversed by high dietary levels of methionine in all tissues studied. The high degree of organ specificity displayed by ethionine as a hepatocarcinogen in rats correlated well with its effects on hepatic AdoMet and AdoEt levels.

Nutrition and Metabolism Section
Laboratory of Comparative Carcinogenesis
National Cancer Institute, FRCF
Frederick, MD 21701, U.S.A.

NARAYAN SHIVAPURKAR*
LIONEL A. POIRIER

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* Address for reprint requests: Narayan Shivapurkar, Nutrition and Metabolism Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute, FCRF, Frederick, MD 21701, U.S.A.