Vol. 90, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 12, 1979 Pages 777-782

EFFECTS OF ETHIONINE ON tRNA METHYLATION IN MALE AND FEMALE RATS

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Received August 13,1979

SUMMARY:

Administration of hepatocarcinogens to rats results in an increase in tRNA methyltransferase activity in the target tissues. Ethionine is active as a carcinogen only in female rats and only in females is this increase in enzyme activity seen. However, ethionine also causes the formation of methyl-deficient tRNA in the liver. Other hepatocarcinogens do not do this. Ethionine is equally effective in this action in males and females. Thus, the two actions of ethionine are completely separable, and the methyl-deficiency of tRNA is caused by an activity not identical with the carcinogenic one.

INTRODUCTION:

It has been reported that administration of ethionine to female rats results in an increase in tRNA methylating enzymes and the production of methyl deficient tRNA (1,2,3). Several carcinogens were shown to affect the methylation of tRNA causing an increase in the methyltransferases within a few days to two weeks. The increases are seen only in the target tissue and, in the case of those that are effective against only one of the sexes, only in the sex that would develop tumors after treatment with that agent (6).

We noted further that only the ethionine-treated rats developed methyl-deficient tRNA. So that although changes in the activity of the enzyme appeared to be a characteristic of the effects of carcinogens, the production of methyl deficiency was associated only with ethionine, an

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The following abbreviations have been used in this text:
Dimethylaminoazobenzene = DMAB; 2 acetylaminofluorene = AAF;
N formylnethionine = Nfmet and trichloroacetic acid = TCA

0006-291X/79/190777-06$01.00/0

analog of methionine. In order to ascertain the interrelationship between the two actions of ethionine, we decided to compare further the responses of male and female rats to ethionine.

METHODS

Animals: A diet of Purine chow supplemented with 0.5% D,L-ethionine, 0.06% dimethylaminoazobenzene (DMAB) or 2 acetylaminofluorene (AAF) was given to male and female CFN Wistar rats. Control animals received the same diet without carcinogens and water ad libitum.

RNA Substrates: Methyl deficient tRNA was prepared from cells of E coli B harvested in logarithmic growth phase; and livers of female rats given injections of DL-ethionine (250 mg/kg) plus adenine (120 mg/kg) for 2 days (1). N formylmethionine (Nfmet) tRNA was purchased from Sigma Chemical Co., St. Louis, Mo. Livers were minced with scissors, homogenized at 4° for 30 sec in 0.01 M Tris pH 7.0, 0.15 M NaCl. 0.006 M EDTA and 0.005 M mercaptoethanol and then stirred with an equal volume of buffer-saturated phenol for 30 min at 4° and 30 min at room temperature. Isolation of RNA from the phenol was the same as for E coli (4)

tRNA-Methylating Enzyme Assays: All operations were carried out at 4°. The livers were homogenized in 0.25 M sucrose 0.005 M $MgCl_2$, centrifuged for 20 min at low speed to remove cellular debris, and then centrifuged at 100,000 x g for 70 min (S-100 preparation). The enzyme assay measured the ability of preparations from the various tissues to catalyze the transfer of the methyl-^{14}C moiety from S-[methyl-^{14}C]adenosylmethionine to the tRNA substrate. Details of the methods have been described previously (1).

Each incubation tube contained 20 to 100 µg tRNA, 0.1 to 0.15 µCi S-[methyl-^{14}C]adenosylmethionine (specific activity, 52 mCi/ mmol; International Chemical and Nuclear Corp., Irvine, Calif), and S-100 enzyme preparation in a final buffer concentration of 0.3 M ammonium acetate, 0.01 M Tris 0.005 M $MgCl_2$ and 0.005 M mercaptoethanol (pH 8) in a volume of 0.4 to 0.5 ml. The mixture was incubated at 35° for 50 to 60 min; 0.2 ml of 1.5 M hydroxylamine (pH 7.5) was then added to each tube, and the sample were incubated at 35° for 10 min longer. Samples were chilled, and 2 mg carrier RNA followed by 4 ml of 0.5 M NaCl in 75% ethanol were added. After 2 hr at -15°, precipitates were collected by centrifugation in the cold and then extracted with 2 M NaCl. RNA was reprecipitated from the extracts by addition of 60% trichloroacetic acid (TCA) solution to give a final concentration of 10%. The precipitates were washed and assayed for radioactivity as described previously (1).

RESULTS

tRNA preparations were obtained by identical procedures from male and female rats that had received one of three carcinogens. They were then used as methyl acceptors in the standard assay. The tRNA from livers of control animals and from those that had received acetylaminofluorene (AAF) or dimethylaminoazobenzene (DMAB) for two days did not accept significant

TABLE 1

Methylation of tRNA from Liver of Carcinogen-Treated Rats

Source of Liver	Methylation
Control animals	60±10
Female-Fed Ethionine	670±10
Male-Fed Ethionine	673± 8
Male-Fed AAF	43±15
Male-Fed DMAB	70±27

Values given are cpm/50 μg tRNA/50 min (cf Methods). Enzyme source was liver of untreated rats, 1.7 mg protein was used per assay. Under these conditions an equal amount of E coli B tRNA accepted 4000±200 cpm of methyl groups. RNA was prepared as was "methyl deficient" tRNA (cf Methods).

TABLE 2

tRNA Methylase Activities of Ethionine-Treated and Control Rat Liver

Enzyme Source	Activity
Female-controls	1.1
Female-Fed Ethionine (14 days)	2.1
Male-Controls	0.56
Male-Fed Ethionine (16 days)	0.53
Male-Controls	0.51
Male-Fed Ethionine (7 days)	0.50

Values are given a p mol/min/mg protein. E coli B tRNA was used as acceptor (cf Methods).

amounts of methyl groups in this assay, in which the transfer was catalyzed by S 100 preparations from normal rat liver (Table 1). In other studies (data not shown) tRNA from rats treated with AAF or DMAB for 1,3,5, and 10 days was also found not to be a methyl acceptor with rat enyzme. However when rats fed ethionine were the source of the tRNA there was extensive transfer of methyl groups. The acceptor ability of the tRNA was about the same for male and female ethionine-treated rats (Table 1).

S-100 preparations from ethionine-treated females but not males showed an increased activity in the methylation of heterologous tRNA (Table 2). With E coli B tRNA, S-100 prepartions from females fed 14 days showed a

TABLE 3

Methylation of E coli Nfmet tRNA by Liver Extracts from Ethionine-Fed and Control Rats

Enzyme Source	10 μg tRNA	6 μg tRNA
Female Control	5000	3500
Female-Fed Ethionine (14 days)	7400	5000
Male Control	2260	1693
Male-Fed Ethionine (16 days)	1685	1418

Values are cpm/50 min in total RNA. Each assay contained 1.6 mg protein (cf Methods).

specific activity twice that of extracts from control rats, which is inherently about twice that of extracts from males. The preparations from treated and untreated males had the same specific activity, after both 7 and 16 days of feeding. Previous studies had shown that the stimulation with females was about 1.5 after 7 days (5)

In other studies in our laboratory we have found that the use of specific amino acid-acceptor tRNAs often gave more information than did the use of total tRNA mixtures. Of particular interest, has been formylmethionine tRNA. We therefore evaluated the ability of extracts of treated and untreated male and female rats to catalyze the methylation of this tRNA. With two different levels of Nfmet tRNA, extracts from ethionine-treated females showed about fifty percent more activity than those from control females. The control male rats gave preparations with about half as much activity as the females and ethionine treatment of males caused no increase (Table 3).

DISCUSSION:

It is apparent from these data that ethionine has two distinct actions as the biphasic response curve reported previously had suggested (1). One of these, the increased enzyme activity is apparently related to carcinogenicity because with ethionine it is seen only in the females, the sex that developed hepatomas, and this is parallel to the specificity of sex and organ site seen with AAF and DMAB (6,7). The other action,

that of inhibiting methylation, is presumably due to the fact that, as a homolog of methionine, ethionine can be converted to an anabolite that is able to interfere with tRNA methylation. This results in the production of methyl deficient tRNA that can accept methyl groups from S-adenosyl methionine under the influence of normal rat tRNA methyl transferases. This occurs with both males and females. Since only females develop tumors with ethionine, we can conclude that inhibition of tRNA methylation is not related to the action of ethionine as a carcinogen.

The clarification of the apparent conflict between the data obtained with ethionine and those with AAF and DMAB supports the conclusion that the increase in the activity of tRNA methylases is a common effect of heptocarcinogens, at least, if not of all carcinogens. The increased activity is exerted with homologous as well as heterologous acceptors. The relationship of the higher methylase activity to increases seen with some, but not all, non-malignant growth changes must be elucidated before the full significance of this phenomenon can be understood (8,9).

We have reported earlier the production of a dialysable inhibitor by ethionine-fed and -injected rats (1). Presumably, this is the active *in vivo* inhibitor. Purification and identification of the structure of this inhibitor in males and females will be necessary for absolute proof of this hypothesis.

ACKNOWLEDGMENTS

The authors wish to thank George F. Brown and Howard Brody for their excellent and conscientious assistance. This investigation was supported in part by USPHS Grants HL 09011 and CA 08748.

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