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Effect of Hypoxia on Hepatic DNA Methylation and tRNA Methyltransferase in Rat: Similarities to Effects of Methyl-Deficient Diets

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Abstract Young rats were maintained in a 10% oxygen atmosphere for 2, 6, and 10 days and administered normal rat chow and water ad libitum. Thereafter, their hepatic S-adenosyl-L-methionine (AdoMet) and activity and mRNA levels of AdoMet synthetase were assayed. AdoMet levels decreased by 45% after 10 days; hepatic AdoMet synthetase also declined by ~40%. In rats with low hepatic AdoMet, the mRNA level of AdoMet synthetase also declined by up to 80%. No significant change in AdoMet or AdoMet synthetase was noted in pair-fed normoxic rats. DNA hypomethylation was determined in terms of incorporation of [^{3}H]methyl of AdoMet incorporated at unmethylated sites in DNA in reactions mediated by methylases *Hpa*II and *Sss*I. As compared to the normal hepatic DNA, [^{3}H]methyl group incorporation in the 10-day hypoxic DNA was almost double in the *Hpa*II-mediated reaction and ~10-fold in the *Sss*I-mediated reaction. Hepatic tRNA methyltransferase activity doubled after 10 days of hypoxia. However, hypoxic rats showed no detectable mRNA transcripts for c-*myc* and c-*fos* oncogenes on Northern blot analysis. These observations show that because of subnormal activity of AdoMet synthetase, hypoxic liver is depleted of AdoMet, even when the animals are administered a complete diet. However, unlike rats on chronic lipotrope-deficient diets, hypoxic rats on a complete diet show no aberrant expression of oncogenes.

Key words: hypoxia, S-adenosylmethionine, DNA methylation, hypomethylation, t-RNA methyltransferase

Diets containing limiting amounts of lipotropes such as methionine, choline, folic acid, and vitamin B_{12} reportedly promote chemical carcinogenesis [Lombardi and Shinozuka, 1979; Newberne and Rogers, 1986; Hoffman, 1984; Yokoyama et al., 1985]. In addition, chronic administration of these diets, also called methyl-deficient diet (MDD), causes spontaneous hepatoma in laboratory rats without any exogenous carcinogen [Ghoshal and Farber, 1984; Mikol et al., 1983]. These animals have subnormal hepatic S-adenosyl-L-methionine (AdoMet), hypomethylated DNA, and increased activity of tRNA methyltransferase [Wilson et al., 1984; Dizik et al., 1991; Wainfan et al., 1984, 1986; Wainfan and Poirier, 1992]. Hepatic insufficiency of AdoMet is considered a pivotal event in the development of biochemical abnormalities and spontaneous carcinogenesis. The abnormalities are corrected when rats are transferred to a normal diet.

We previously reported that AdoMet levels were significantly subnormal in isolated hepatocytes exposed to acute hypoxia [Shan et al., 1989; Aw et al., 1991]. We also found that levels of hepatic AdoMet and AdoMet synthetase activity were significantly lower in rats exposed to physiologic hypoxia of 10% oxygen for 9 days [Chawla and Jones, 1994]. These changes occurred even though rats received regular food containing appropriate amounts of all nutrients, including lipotropes. If hypoxia-associated depletion of hepatic AdoMet also affected DNA methylation, such an effect would be of general importance because DNA methylation has a significant role in the regulation of gene expression [Newberne and Rogers, 1986; Hoffman, 1984; Razin and Cedar, 1991].

Abbreviations used: AdoMet, S-adenosyl-L-methionine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPLC, high-performance liquid chromatography; MDD, methyl-deficient diets; MOPS, 3-N-(morpholino)propanesulfonic acid; SDS, sodium dodecyl sulfate; 1 × SSC, 0.15 M sodium chloride plus 0.015 M sodium citrate buffer.

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In this paper, we examine changes in hepatic AdoMet, AdoMet synthetase, and mRNA of AdoMet synthetase in rat exposed to hypoxia for 2, 6, and 10 days. In addition, we examine whether alterations in hepatic AdoMet pool effect the methylation of DNA and activity of tRNA methyltransferase. Our findings show that the AdoMet deficiency is significant only after 10 days of hypoxia; furthermore, this deficiency results in hypomethylated DNA and increased activity of tRNA methyltransferase in rat liver. Thus, moderate in vivo hypoxia results in decreased methylation similar to that observed for MDD, even when the rats are administered standard laboratory food containing all nutrients.

MATERIALS AND METHODS

All reagents were of highest chemical purity and were obtained from Sigma Chemical Co. (St. Louis, MO), except as indicated. Radiolabeled methionine was from Amersham (Arlington Heights, IL), and radiolabeled AdoMet was from New England Nuclear (Boston, MA). Reagents for Northern blot were of molecular biology grade and were obtained from Gibco-BRL or Sigma Chemical Co. RNAzol was from Biotecx (Houston, TX). The cDNA probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was a 1.1-kb fragment of the human gene obtained from Clontech (Palo Alto, CA). The probe cross hybridizes strongly with the rat mRNA. The random prime labeling kit was from Stratagene (La Jolla, CA). HPLC analysis was performed on Waters 600 E system with Waters 486 tunable detector, Waters WISP, and Waters 746 data module. Methylases for DNA methylation were from New England BioLabs (Beverley, MA). A molecular-weight RNA ladder, 0.24–9.5 kb, was from Gibco-BRL (Gaithersburg, MD).

Animals

All animal studies were approved by the Emory University Animal Care and Use Committee. Male Sprague Dawley rats, age 5–6 weeks and weighing 120–140 g, were used and were provided a standard laboratory rat diet and water ad libitum. They were housed in temperature and humidity controlled environments on a 12-h light–dark cycle.

In a typical experiment, six rats were made hypoxic using a previously published protocol [Aw et al., 1991; Chawla and Jones, 1994] and were housed in a chamber with regulated inflow of air mixed with nitrogen to lower the amount of oxygen to 10% under normobaric conditions for 2, 6, and 10 days. Also included in each experiment were a group of six control rats on ad libitum standard rat diet and water and six pair-fed rats that received a decreased amount of the diet to gain the same weight as the hypoxic rats. Their food intake and body weight were monitored and the bedding was changed on alternate days. For this purpose, animals under hypoxia were briefly exposed to normoxia (≤ 15 min) during bedding changes.

Methods

Assay of S-adenosylmethionine and S-adenosylhomocystene in hepatic tissue. The assay was performed as described previously observing the precautions in handling, storage, and processing of the hepatic tissue [Chawla and Jones, 1994; Eloranta, 1977; Gordon et al., 1987]. Frozen liver tissue was homogenized at 4°C using a Polytron tissue homogenizer with 5% sulfosalicylic acid (4 ml/g tissue) and centrifuged. The supernatant was filtered and chromatographed on a Waters Radial Pak 8-mm × 100-mm cation-exchange column. Standard solutions of AdoMet and S-adenosylhomocysteine were prepared in 5% sulfosalicylic acid; S-adenosylethionine was used as an internal standard for tissue extracts and standard solutions. A binary solvent system consisting of solvent A (1 mM ammonium formate, pH 4.0) and solvent B (1.0 M ammonium formate, pH 4.0, containing 0.8 M ammonium sulfate) was delivered according to the following gradient. Up to 10 min, 0% B; 10–30 min, 3% B; 30–40 min, 20% B; 40–45 min, 80% B; 45–52 min, 0% B. The flow rate was 1 ml/min, and A_{254} of the eluate was monitored. Analyses were performed by manually injecting the samples with WISP, which ensured that the injection was both uniform and reproducible. Elution times were determined in relation to the S-adenosylethionine peak.

Assay of hepatic S-adenosylmethionine-synthetase. The assay was performed according to the method of Suma et al. [1986], as described by us [Chawla and Jones, 1994]. Hepatic cytosol was the source of the enzyme and was isolated and aliquoted and stored at −80°C until used for assays. Protein contents of cytosol were determined according to Lowry using bovine serum albumin as a standard [Lowry et al., 1951]. The assay involved monitoring the conversion of (methyl-^{3}H)methionine to AdoMet in a

pH 9.0 Tris buffer containing $MgCl_2$, KCl, dithiothreitol, methionine, and ATP. After 10-min incubation at 37°C, the reaction was stopped by adding 20 μl 2M $HClO_4$ containing 5 mM methionine and centrifuged at 13,000*g* for 2 min. The supernatant fluid was applied to a cellulose phosphate paper; unreacted labeled methionine was washed off the paper with a phosphate buffer (pH 7.0). ^{3}H-AdoMet was eluted from the paper with 1.5 M NH_4OH, and its radioactivity was measured. Hepatic AdoMet synthetase activity was expressed as pmol of methionine converted to AdoMet per mg cytosol protein/10 min.

Northern blot analysis of hepatic RNA for AdoMet synthetase. The assay was as described before [Chawla and Jones, 1994]. All procedures were carried out using sterile reagents, solvents, and equipment using standard precautions to avoid any contamination [Chawla and Jones, 1994]. A 25-mer oligonucleotide probe complementary to the region 1178–1154 of the cDNA sequence of rat liver AdoMet synthetase [Horikawa et al., 1989] was synthesized by the Microchemical Facility of the Emory University Cancer Center on ABI 380 B Oligonucleotide DNA synthesizer following the manufacturer's instructions. The sequence of the probe was as below: 5′–TTGGGGACCTC-CCAGGGAAACT- CGC–3′. The homogeneity of the synthesized oligonucleotide was established on the basis of its analysis by C18 reverse-phase HPLC analysis and by gel electrophoresis. The oligo probe was labeled by the addition of ^{32}P to its 5′ end using T4 Kinase and a specific activity of 3–5 × 10^9 cpm/μg was routinely obtained.

RNA was isolated from freshly frozen liver (~500 mg) by homogenizing it with RNAzol B (2 ml/100 mg tissue) as described previously [Chawla and Jones, 1994]. Its purity was estimated from its A_{260}:A_{280} ratio, and its concentration was estimated from its A_{260}. The isolated RNA was aliquoted, stored at −20°C, and used within 2 weeks. Aliquots of RNA from different rats (20 μg each) were electrophoresed in duplicate on a 1.2% agarose gel containing 6.6% formaldehyde in 1× MOPS at 5 V/cm. The gel was washed and blotted to a nitrocellulose membrane by capillary blotting for 18 h in 20 × SSC; the membrane was heated at 80°C for 2 h; one set of duplicate lanes was cut and stained.

The membrane was prehybridized overnight at 67°C by shaking it in a sealed bag with 10 ml of the hybridization solution, consisting of 0.125 M phosphate buffer containing 7% SDS, 10 mg/ml of bovine serum albumin (BSA), 10% dextran sulfate, 1.25 mM EDTA, and 250 mM NaCl. An aliquot of the labeled probe, mixed with boiled sheared salmon sperm DNA, was then added to the bag, and the membrane was shaken for another 20 h at 67°C. The membrane was washed twice with 1× SSC at 25°C for 30 min, twice in 0.25 × SSC for 30 min at 25°C, once with 0.1 × SSC at 25°C, and once with 0.1 × SSC at 43°C. All SSC solutions contained 0.5% SDS. The membrane was next autoradiographed between two intensifying screens at −70°C for 48–72 h.

In order to probe it with GAPDH, the membrane was boiled in 0.1% SDS for 10 min and then prehybridized overnight at 42°C in 5 × SSC also containing 1% SDS, 5 × Denhardt's solution, 100 μg salmon sperm DNA, and 50% formamide. The cDNA probe was labeled by random prime labeling (Stratagene) technique. The hybridization was carried out overnight at 42°C in a sealed bag containing 7 ml of the prehybridization solution and 10% dextran sulfate and 10^7 cpm of the labeled probe. The membrane was washed twice with 2 × SSC containing 1% SDS for 30 min at 55°C, dried, and autoradiographed between two intensifying screens at −70°C for 72 h.

Assay of Hepatic DNA Methylation

Hepatic DNA was isolated according to published procedures [Ausubel et al., 1987]. Frozen liver (1–2 g) was ground in liquid nitrogen in a precooled mortar; the powder was mixed in a buffer (pH 8.0) containing 10 mM Tris, 100 mM NaCl, and 25 mM EDTA containing 0.5% SDS and 100 μg/ml proteinase K. After incubation at 50°C for 16 h, the mixture was extracted with a mixture of phenol/chloroform/isoamyl alcohol (25:24:1). DNA in the aqueous layer was precipitated by adding 2 vol of absolute ethanol and one-half vol of 7.5 M ammonium acetate. The precipitate was redissolved in 10 mM Tris pH 7.6 containing 1 mM EDTA. The mixture was treated with 10 μg/ml of RNase and re-extracted with chloroform/isoamyl alcohol/phenol. DNA was resuspended in 10 mM Tris, pH 7.5–0.1 mM EDTA buffer; its concentration was estimated by A_{260}. The DNA suspension was aliquoted and stored at −20°C. Methylation of DNA was carried out using *Hpa*II (methylates internal C of . . CCGG . .) and *Sss*I (methylates C of CpG) with modifications of the vendor's

instructions [D. Bednarik, personal communication].

Purified DNA (3 μg) was incubated with 3 units of the enzyme and 2 μCi of [^{3}H]AdoMet (~2.8 nM) in 50 μl of the buffer supplied by the vendor. For *Sss*I, the buffer was 50 mM NaCl, 10 mM Tris, 10 mM $MgCl_2$, and 1 mM of dithiothreitol, pH 7.0. For *Hpa*II, the buffer was 50 mM Tris, 10 mM EDTA, and 5 mM mercaptoethanol. The mixture was incubated at 4°C overnight; unlabeled AdoMet was then added. Total AdoMet concentration was 160 μM for *Sss*I and 80 μM for *Hpa*II. Additional enzyme was added at this stage—4 units per tube of *Sss*I or 3 units per tube for *Hpa*II. After 3-h incubation at 37°C, 5 vol of ice-cold trichloroacetic acid (TCA) was added to the mixture, bringing its final concentration to 10%. The mixture was centrifuged and the pellet was washed with TCA and centrifuged at 10,000*g* for 30 min. The pellet was dissolved in 50 μl of 0.1 N NaOH and counted for its radioactivity.

Assay of hepatic tRNA methyltransferase. The procedure of Wainfan et al. [1984, 1986] was used. Briefly, rat liver cytosol was dialyzed against a large volume of a buffer containing 10 mM Tris, 5 mM $MgCl_2$, and 5 mM mercaptoethanol, and its protein content was determined. The dialyzed cytosol was used as a source of the tRNA methyltransferase activity in a heterologous system using *Escherichia coli* $tRNA^{Nfmet}$ as a substrate. After 90-min incubation, the reaction was terminated by the addition of 2 ml of 1.5 M hydroxylamine, pH 7.5 followed by incubation at 35°C for 10 min. Thereafter, the labeled tRNA was precipitated and assayed for radioactivity.

RESULTS

Conditions employed in this study for in vivo hypoxia were not life-threatening and were similar to those we previously employed to investigate drug metabolism in hypoxic liver [Aw et al., 1991]. After the first 24 h of acclimation, rats appeared normal and healthy. However, the average daily food intake of the hypoxic group was noted to be about 30% lower than that of the control group and gain in body weight was significantly reduced (62.5 ± 4.6 g vs. 90.3 ± 12.0 g; $P < 0.05$). A pair-fed group of rats was therefore included in each experiment to provide a normoxic control group with approximately the same weight gain as the hypoxic rats.

To determine the time course of the effects of hypoxia on AdoMet, we measured hepatic AdoMet and AdoMet synthetase activity after in vivo exposure to hypoxia for 2, 6, and 10 days. For this purpose, rats were anesthetized with ketamine and their livers were promptly frozen in liquid nitrogen. The control liver concentration of AdoMet was 69.6 ± 8.7 nmol/g liver, a value similar to that reported previously [Aw et al., 1991; Chawla and Jones, 1994; Gordon et al., 1987]. AdoMet levels did not change significantly after 2 or 6 days of hypoxia but dropped to 40.5 ± 10.2 nmol/g liver after 10 days ($P < 0.05$ compared to control) (see Figure 1A). AdoMet levels did not change significantly in the pair-fed group (57.6 ± 6.3 nmol/g liver). For these analyses, the authenticity of the AdoMet peak in the liver extract was confirmed by (1) monitoring the increase in the peak height after adding a known amount of the metabolite to the extract, and (2) monitoring the disappearance of the peak after treating the extract with an equal volume of 1.0 M NaOH. Hepatic levels of S-adenosylhomocysteine in hypoxic rats were not significantly altered, as compared to those in the control group (38.9 ± 12.9 vs. 46.3 ± 17.4 nmol/g tissue; $P > 0.05$). The authenticity of S-adenosylhomocysteine peak was confirmed by adding a known amount of the metabolite to the extract.

AdoMet synthetase activity was measured to determine whether it could explain subnormal hepatic AdoMet. This activity declined from 0.97 ± 0.2 nmol AdoMet formed/mg protein/10 min in the control group to 0.60 ± 0.07 in the 10-day hypoxia group ($P < 0.05$) (Fig. 1B). The enzymatic activity in the control group is similar to that reported by others [Suma et al., 1986]. Interestingly, in the pair-fed group, AdoMet synthetase activity (0.81 ± 0.19 units) did not differ significantly from those of the control group. Therefore, a limited supply of oxygen, and not lower food intake, is responsible for subnormal hepatic AdoMet and AdoMet synthetase.

The concentration of mRNA of AdoMet synthetase was measured to determine whether the lower enzymatic activity could be related to its decreased mRNA expression. The results are shown in Figure 2A. The upper panel shows a single band of mRNA of AdoMet synthetase at 3.6 kb, identical to its reported size [Horikawa et al., 1989]. The molecular size of the band was estimated from the RNA ladder as shown in Figure 2A. In lanes 2 and 3, the intensity of

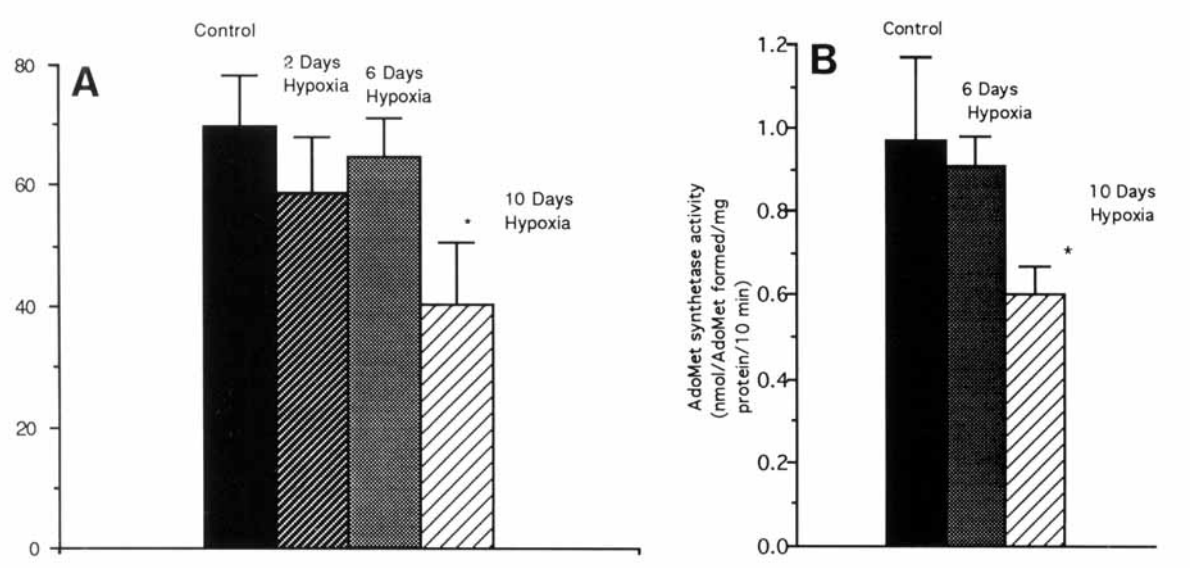

Fig. 1. **A:** Bar graph showing AdoMet levels (nmol/g liver) in control group of rats and in rats after hypoxic treatment for 2, 6, and 10 days. See text for details of the procedure. Each experiment was performed three times. The results shown are from one single experiment. The values are ave ±SD. *, the value differs significantly from that of the control group. **B:** Bar graph showing AdoMet synthetase activity (nmol of AdoMet formed/mg cytosol protein/min) in control group of rats and in rats after 10-day hypoxic treatment. The values are ave ±SD. *, the value differs significantly from that of the control group.

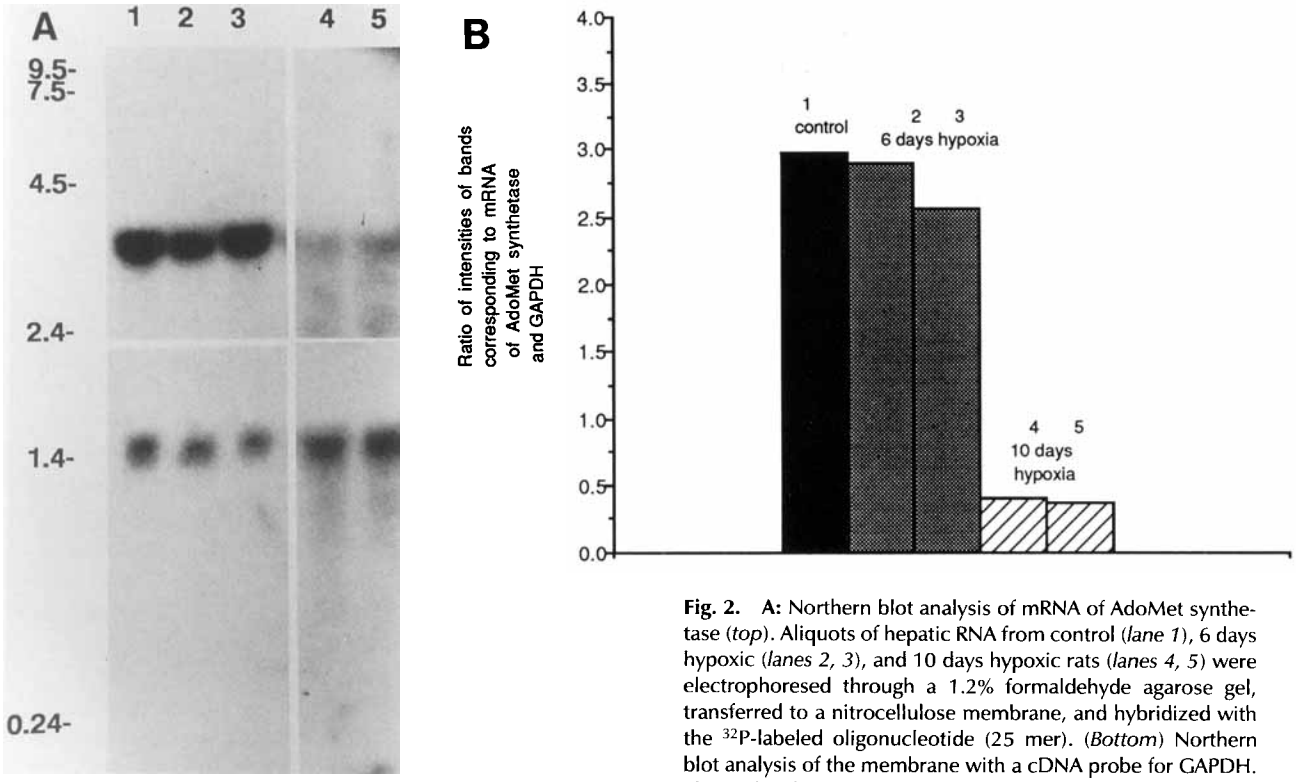

Fig. 2. **A:** Northern blot analysis of mRNA of AdoMet synthetase (*top*). Aliquots of hepatic RNA from control (*lane 1*), 6 days hypoxic (*lanes 2, 3*), and 10 days hypoxic rats (*lanes 4, 5*) were electrophoresed through a 1.2% formaldehyde agarose gel, transferred to a nitrocellulose membrane, and hybridized with the ^{32}P-labeled oligonucleotide (25 mer). (*Bottom*) Northern blot analysis of the membrane with a cDNA probe for GAPDH. The molecular size of the bands was estimated from the RNA ladder, as shown. See text for details. **B:** Bar graph showing ratios of intensities of bands corresponding to mRNA of AdoMet synthetase and GAPDH from **A.**

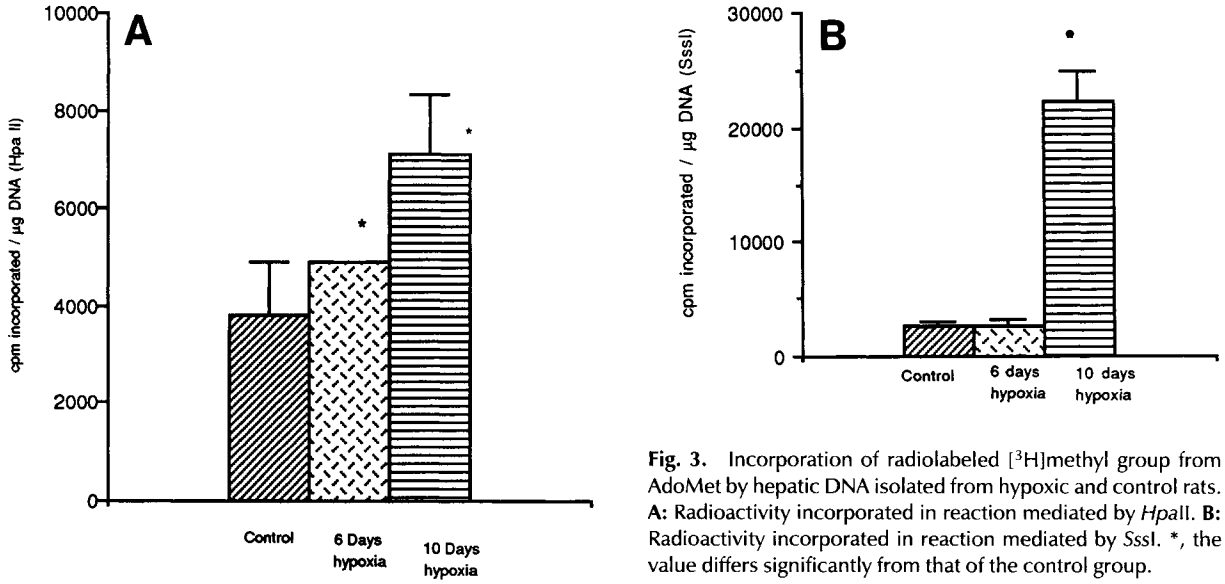

Fig. 3. Incorporation of radiolabeled [^{3}H]methyl group from AdoMet by hepatic DNA isolated from hypoxic and control rats. **A:** Radioactivity incorporated in reaction mediated by *Hpa*II. **B:** Radioactivity incorporated in reaction mediated by *Sss*I. *, the value differs significantly from that of the control group.

mRNA after 6 days of hypoxia was essentially unchanged, as compared to that of the control rats (lane 1). After 10 days of hypoxia (lanes 4 and 5), intensity of the mRNA decreased significantly to ~20% of the control level. The ratios of intensities of mRNA of AdoMet synthetase and GAPDH were measured by a laser densitometric scan and are shown in Figure 2B; the data confirm the conclusion that the loss of mRNA of AdoMet synthetase became significant after 10 days. The change in mRNA of AdoMet synthetase was observed in only three rats with AdoMet levels of < 35 nmol/g tissue.

The effect of hepatic AdoMet insufficiency on DNA methylation was examined because of reported relationship between AdoMet deficiency and DNA hypomethylation in MDD-fed rats [Wainfan and Poirier, 1992]. In this study, DNA hypomethylation was investigated in terms of the radioactivity incorporated from labeled AdoMet. An increased incorporation of the labeled methyl group indicates an increase in unmethylated sites in the hypoxic DNA molecule. The *Hpa*II-mediated incorporation of radioactivity (CCGG specificity) in 10-day hypoxic rat liver DNA was nearly twice as much as in the control liver (7.105 ± 1.208 vs. 3.814 ± 1.120; $P < 0.01$) (Fig. 3A). The *Sss*I-mediated incorporation of radioactivity (CpG specificity) in hypoxic DNA was about ninefold more than in the control liver DNA (22,414 ± 2,504 vs. 2,567 ± 366; $P < 0.005$) (Fig. 3B). Thus, a limited availability of hepatic AdoMet in hypoxic liver probably increased the unmethylated sites of DNA, as measured by [^{3}H]methyl incorporation, using the above enzymes.

Figure 4 shows changes in the tRNA methyltransferase activity as a result of hypoxia. This activity in hypoxic rat liver was twofold greater than that in the control group. The assay conditions and the substrate selected reportedly favor the methylation of N^2 of guanine and measure the activity of N^2-guanine tRNA methyltransferase II [Dizik et al., 1991; Wainfan et al., 1984, 1986]. In subsequent assays, a precipitate obtained by 60% saturated ammonium sulfate treatment of cytosol was also used as a source of the methyltransferase activity for the assay as described in [Wainfan et al., 1984, 1986; Colonna and Kerr, 1980], and the results were similar to those reported in Figure 4.

DISCUSSION

A significant decline in hepatic AdoMet and AdoMet synthetase activity was noted only after 10 days of hypoxia, when AdoMet decreased by about 45%. This decline was probably due to decreased AdoMet synthetase activity which also decreased by about the same extent. A limited availability of the substrates—methionine and ATP—was probably not responsible for the subnormal AdoMet. This conclusion is based on the observations that the pair-fed rats in this study did not have subnormal AdoMet or AdoMet synthetase, and our previous study showed that chronically hypoxic cells adapt with reduced functional capacity that allows preservation of ATP by reducing activities of ATP using en-

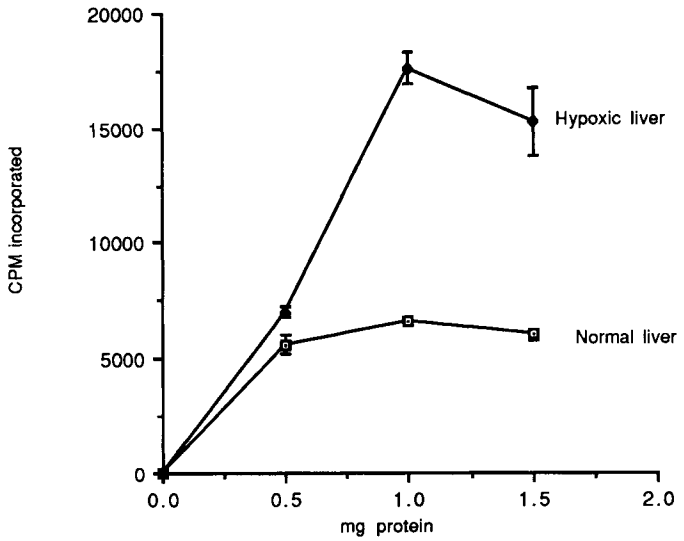

Fig. 4. Methylation of *Escherichia coli* $tRNA^{Nfmet}$ as catalyzed by hypoxic and control rat liver enzyme. The horizontal axis shows the amount of purified cytosol protein and the vertical axis shows the amount of radioactivity as [^{3}H]methyl group from AdoMet incorporated by tRNA substrate.

zymes [Aw et al., 1991]. Furthermore, the decline in AdoMet synthetase is probably due to the low concentration of its mRNA. Hypoxia could affect the rate of transcription of the mRNA or its half-life, or both. The mechanism by which hypoxia exerts its regulatory effect is not understood and further studies are planned to investigate this issue. It is, however, of interest to note that hypoxia can alter several metabolic processes at the transcriptional level. For example, erythropoietin gene expression is modulated by hypoxia [Schuster et al., 1989].

In addition to being an intermediate in the conversion of methionine to cysteine, AdoMet methylates a variety of molecules including DNA, RNA, histones, and biologic amines [Newberne and Rogers, 1986; Hoffman, 1984]. Limited availability of this substrate may be expected to affect the methylation of these molecules. This point has been well established in rats chronically administered MDD. These rats had hypomethylated DNA and increased tRNA methyltransferase activity and an aberrant expression of certain oncogenes [Wainfan and Poirier, 1992]. Hypomethylation of DNA was proposed as the biochemical basis for the spontaneous hepatocarcinogenesis in rats on MDD. The significance of increased tRNA methyltransferase in this group of rats is not fully understood, although it may have a role in the interaction of tRNA with amino-acyl synthetases or with ribosomal binding [Colonna and Kerr, 1980]. In the present study, in vivo chronic physiologic hypoxia resulted in hepatic depletion of AdoMet, even though the rats consumed regular rat chow. This depletion also resulted in hypomethylated DNA and increased tRNA methyltransferase activity, similar to results reported in MDD-treated rats. Methylation of DNA may be affected by a limited availability of AdoMet or an increase in S-adenosylhomocysteine. The ratio of AdoMet/S-adenosylhomocysteine is often a predictor for methylation. The hypoxic animals had no significant change in their hepatic S-adenosylhomocysteine concentrations; it is therefore safe to conclude that subnormal AdoMet alone affected the DNA methylation profile. Furthermore, in this study, we could not detect any aberrant expression of oncogenes reported in rats on MDD (data not shown). This may be due to a short duration of hypoxia and/or due to the fact that dietary deprivation of lipotropic factors (e.g., choline) may be required for the aberrant gene expression. Further work is planned to investigate this issue.

Subnormal hepatic AdoMet and AdoMet synthetase have also been reported in the liver of patients with alcoholic cirrhosis and the decline is of the same magnitude as that observed in hypoxic rat. For example, our previous studies identified an acquired block in the hepatic conversion of methionine to AdoMet in cirrhotic subjects which impaired their capacity to de novo synthesize cysteine or choline [Chawla et

al., 1984, 1989]. Gaull et al. [1981] previously observed that human cirrhotic liver was deficient in AdoMet. Cabrero et al. [1988] reported subnormal AdoMet synthetase activity—but no change in AdoMet levels—in cirrhotic liver. Because of the reported correlation between alcohol consumption and hepatic hypoxia [Fukui et al., 1990; Hijioka et al., 1991] and between alcoholic cirrhosis and hepatic hypoxia [Moreau et al., 1988, 1989], it may be postulated that abnormalities in AdoMet metabolism in alcoholic cirrhosis may be due in part to an imbalance between the hepatic requirements and the supply of oxygen. Also of interest in this regard is the observation that a large proportion of cirrhotic patients is at an increased risk to develop hepatocellular carcinoma [Misslbeck and Campbell, 1985; Kew and Popper, 1984]. This correlation has been explained as due to hepatitis B virus infection, ingestion of hepatotoxins, and/or malnutrition [Hoffman, 1984; Misslbeck and Campbell, 1985]. If human cirrhotic liver is indeed chronically deficient in AdoMet and malnourished, these factors may be other contributory factors in development of hepatoma among the cirrhotic population.

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