S-Adenosylmethionine and S-Adenosylhomocysteine Metabolism in Isolated Rat Liver

EFFECTS OF L-METHIONINE, L-HOMOCYSTEINE, AND ADENOSINE*

(Received for publication, April 14, 1980)

Dennis R. Hoffman, Donald W. Marion, William E. Cornatzer, and John A. Duerre
From the Departments of Biochemistry and Microbiology, Ireland Research Laboratory, University of North Dakota, Grand Forks, North Dakota 58202

The effects of varying concentrations of L-methionine, L-homocysteine, and adenosine on the tissue levels of S-adenosylmethionine (AdoMet) and S-adenosylhomocysteine (AdoHcy) were investigated in perfused liver. In the normal liver, the intracellular concentration of AdoMet was dependent upon the availability of methionine. In the presence of high concentrations of methionine the maximum level of AdoMet attainable was 300 nmol/g of liver. The exogenous concentration of methionine did not alter the hepatic concentration of AdoHcy (8 to 20 nmol/g) while adenosine or homocysteine blocked hydrolysis of AdoHcy resulting in elevated levels of AdoHcy (400 to 800 nmol/g) and AdoMet (300 to 600 nmol/g). The addition of both adenosine (4 mM) and homocysteine (3.4 mM) to the perfusate further increased the levels of AdoHcy (4g M/mM) and AdoMet (1.2 g M/mM). As the concentration of AdoHcy increased, significant amounts of this compound were released into the perfusate, while AdoMet was not detected. Under all conditions where AdoHcy accumulated in the cell, a concomitant increase in the AdoMet level occurred. Apparently AdoHcy acts as a positive effector of the S-adenosylmethionine synthase. The hepatocytes did not take up significant amounts of [methyl-14C]AdoMet from the perfusate nor were any [14C]methyl groups from this compound incorporated into histones, DNA, or phospholipids. In contrast, [14C]methyl groups were readily incorporated into these macromolecules from exogenous [methyl-14C]methionine. The addition of adenosine (4 mM) and homocysteine (3.4 mM) shifted the AdoMet:AdoHcy ratio from 8.2 to 0.3. Under these conditions, transmethylation was inhibited markedly.

S-Adenosylhomocysteine, one of the products of all transmethylation reactions involving S-adenosylmethionine, acts as a competitive inhibitor of most, if not all, of these reactions. The affinity of most methyltransferases for this inhibitor has been observed to be greater than the affinity for the substrate, AdoMet1 (1). In the liver, the AdoMet concentration has been reported to be 70 to 90 nmol/g (2-6) and the AdoHcy concentration was 40 to 60 nmol/g (2-4). At this ratio of substrate to inhibitor, the methylation of numerous compounds would be inhibited grossly. This has led several investigators to propose that AdoHcy acts as a bioregulatory compound (7-11). Recently we (1) reported that the hepatic concentration of AdoHcy increased markedly in tissues following death of the animal. The estimated in vivo concentration was 5 nmol/g in developing liver and increased to 13 nmol/g in the adult. The level of AdoMet in the liver (60 to 90 nmol/g) that we obtained did not differ significantly from that reported by others (2-6). The concentration of AdoMet in the rat brain was 40 to 50 nmol/g during development and decreased to 20 to 30 nmol/g of tissue in the adult (1, 12), while the concentration of AdoHcy rarely exceeded 1 nmol/g (1). Therefore, the ratio of AdoMet to AdoHcy is so large in these organs that it is doubtful that the latter compound would have any effect on most methyltransferase reactions under normal conditions.

The primary mechanism for the breakdown of AdoHcy in eucaryotic cells is via the reversible S-adenosylhomocysteine hydrolase (EC 3.3.1.1). Catabolism of AdoHcy yields homocysteine and adenosine while both products have been demonstrated to have an inhibitory action on the hydrolysis of this compound (13-16). The equilibrium of the reaction, however, favors condensation. Degradation of adenosine by adenosine deaminase and homocysteine conversion to cystathionine or methionine enables the hydrolytic reaction to proceed in normal cells (Scheme I). Adenosine deaminase is present in the liver, as well as most other organs, in relatively high concentration, particularly during development (1, 17). Mudd and colleagues (18, 19) propose that homocysteine is cycled repeatedly through methionine prior to being eliminated via cystathionine. Methylation of homocysteine to methionine occurs by two mechanisms. Betaine homocysteine methyltransferase is present primarily in the liver; however, Finkelstein suggested that 5-methyltetrahydrofolate homocysteine methyltransferase is a more significant contributor of cellular methionine (20).

The entrance of AdoHcy into the cell appears to be regulated. Perfusion studies have revealed that rat liver cells are impermeable to exogenous AdoHcy (21) while the excretion of this compound from liver is uncertain. Intravenous administration of AdoHcy to the rat primarily yields S-adenosyl-y-thio-a-ketobutyrate in the urine (22). This compound is formed through oxidative deamination of AdoHcy in the kidney (23). However, S-adenosyl-y-thio-a-ketobutyrate has not been detected in plasma or urine from normal rat (1, 22).

The objectives of this study were to determine 1) if the concentrations of AdoMet and AdoHcy in the liver would be affected by varying the level of adenosine, homocysteine, or methionine, 2) if the ratio of AdoMet to AdoHcy could be altered by varying these substrate concentrations, 3) the effect...
of altered levels of AdoMet and/or AdoHcy on the activities of several methyltransferases, and 4) if AdoMet and/or AdoHcy are excreted from hepatocytes.

**EXPERIMENTAL PROCEDURES**

**Materials**—L-[methyl-14C]Methionine (9.4 μCi/μmol) was purchased from New England Nuclear and diluted with unlabeled methionine obtained from ICN Pharmaceuticals, Inc. S-Adenosyl-L-[methyl-35S]methionine (58.4 μCi/μmol) was purchased from New England Nuclear and diluted with unlabeled AdoMet prepared by the method of Schienk and DePalma (24). L-Homocysteine thiolactone and adenosine were obtained from Calbiochem. L-Homocysteine was obtained from ICN Pharmaceuticals, Inc. S-Adenosyl-L-Hcy are excreted from hepatocytes.

**Animals**—Sprague-Dawley rats weighing 180 to 200 g, maintained on Purina rat chow ad libitum. Livers were isolated and perfused according to the procedure of Alvares and Ray (26). Animals were under ether anesthesia as the bile duct, portal vein, and thoracic inferior vena cava (in that order) were cannulated prior to removal of the liver from the body. Heparin (Grade II) and bovine serum albumin were purchased from Sigma Chemical Co. Vydac exchange resin was obtained from Rohm and Haas Co. Thiacrine hydrochloride was obtained from Parke Davis, and dexamethasone was obtained from Aldrich Chemical Co. Radiochemicals were purchased from New England Nuclear and diluted with unlabeled materials. L-Adenosylhomocysteine hydrolase was purchased from Sigma Chemical Co. Heparin (Grade II) and bovine serum albumin were purchased from Sigma Chemical Co. Vydac exchange resin was obtained from Rohm and Haas Co. Thiacrine hydrochloride was obtained from Parke Davis, and dexamethasone was obtained from Aldrich Chemical Co. Radiochemicals were purchased from New England Nuclear and diluted with unlabeled materials.

**Experimental Procedures**—Amino acids were fractionated by chromatography on Amberlite CG-120 resin (23), and the effluent was collected with a fraction collector. Aliquots of each 10-ml fraction were analyzed for amino acids with the aid of a Technicon autoanalyzer, and radioactivity was determined by Packard liquid scintillation spectrometer. On this resin, adenosine eluted just after homocysteine and was quantitated by ultraviolet absorbance at 280 nm. The perfusate (2.0 ml) was prepared for chromatography by deproteinization with trichloroacetic acid (5%) and centrifugation at 10,000 g for 10 min. One drop of 30% H2O2 was added to completely oxidize homocysteine and cysteine.

AdoMet and AdoHcy were fractionated by high pressure liquid chromatography on Vydac cation exchange resin as described previously (1). Fractions of the effluent were collected with the aid of a fraction collector for the determination of radioactivity. Prior to chromatography, aliquots of the perfusate (1.0 ml) were deproteinized by the addition of 0.5 ml of 0.3 m mercaptoethanol/15% sulfoalicylic acid, centrifugation at 10,000 g for 10 min, and filtration through 1-μm porosity glass fiber filter paper. AdoMet and AdoHcy were extracted from liver biopsy samples as follows. The frozen tissue was weighed and homogenized in 0.1 M mercaptoethanol/5% sulfoalicylic acid (1 to 2 ml/g), sedimented by centrifugation, and filtered as described above. This procedure was repeated twice and the filtrates pooled.

The extent of methylation of macromolecules in the liver was measured by quantitation of [3H]methyl incorporated. Nuclear, microsomal, and cytosolic fractions were isolated from the liver samples by differential centrifugation (1). Histones (28) and phosphatidylcholine (29) were isolated as described previously. The DNA was purified by a modification of the procedure of Kiesseck and Morris (30). The DNA was precipitated from crude liver extracts with 2 volumes of 95% ethanol and collected by centrifugation at 20,000 g for 5 min. The crude DNA was solubilized in 1.4 M NaCl by intermittent stirring for 1 h. The solution was centrifuged at 25,000 g for 10 min and the precipitate discarded. The DNA was precipitated from the supernatant fluid by addition of 1.5 volumes of 95% ethanol, sedimented by centrifugation, dissolved in 0.5 M NaOH, and heated at 60°C for 10 min. The DNA was precipitated by addition of HCl and 1.0 M perchloric acid, sedimented by centrifugation, and washed twice with 0.5 M perchloric acid at 0°C. The DNA was hydrolyzed in 1.0 M perchloric acid at 70°C for 25 min. An aliquot was removed for the determination of radioactivity, and the remainder was quantitated by the method of Burton (31).
Utilization of Methionine by Isolated Livers—The rate of utilization of L-[methyl-\(^{14}\)C]methionine when added to the perfusate as a single low dose is presented in Fig. 1A. The initial rate of uptake was 135 ± 41 nmol/min/g of liver, while methionine was exhausted from the perfusate within 30 min after the time of addition. At this point the specific radioactivity of the resultant AdoMet was about 16% of that of the exogenous methionine. Turnover of the intracellular AdoMet was evident from a decrease in the specific activity to 7% at 90 min. The radiolabeled methyl groups from the AdoMet were readily utilized for the methylation of histones and DNA (Fig. 1B). It is evident from the data in Fig. 1A that methionine uptake is reduced in the presence of exogenous homocysteine. When [methyl-\(^{14}\)C]AdoMet was added to the perfusate, only trace quantities of radioactive methyl groups were detectable in histones or DNA. Furthermore, less than 0.5% of the exogenous AdoMet was retained by the liver after 90 min (Fig. 1A). The addition of glucose to the system was without effect.

Formation of AdoMet and AdoHcy from Various Substrates by Isolated Livers—The rate of formation of AdoMet and AdoHcy from various substrates added in a single dose is presented in Figs. 2 and 3. In the absence of exogenous substrates, livers from normal rats maintained a level of 32 ± 13 nmol of AdoMet and 12 ± 7 nmol of AdoHcy/g of tissue. Introducing 50 \(\mu\)M methionine resulted in a significant increase in the level of AdoMet which reached a maximum of 120 nmol/g of liver in 20 min (Fig. 2A). At this point, the methionine in the perfusate was exhausted (Fig. 1A), and the AdoMet level decreased gradually (Fig. 2A). If the exogenous methionine concentration was elevated to 2.25 mM, the liver concentration of AdoMet increased to about 300 nmol/g in 15 min and remained at that level throughout the time course of the experiment (Fig. 2B). Analysis of the perfusate revealed that 40% of the methionine was utilized during the 90-min period. Regardless of the concentration of methionine added to the perfusate, the level of AdoHcy in the tissue did not vary significantly (Fig. 2, A and B). If 50 \(\mu\)M homocysteine was added to the perfusate, the results were comparable to those obtained with an equivalent amount of methionine (data not presented). Under these conditions, the carbon 1 pool does not appear to be limited. When the concentration of homocysteine was elevated to 2.25 mM, the tissue level of AdoHcy increased markedly (Fig. 2C). As the tissue level of AdoHcy increased, this compound was released from the cells into the

**RESULTS**

**Utilization of Methionine by Isolated Livers**—The rate of utilization of L-[methyl-\(^{14}\)C]methionine when added to the perfusate as a single low dose is presented in Fig. 1A. The initial rate of uptake was 135 ± 41 nmol/min/g of liver, while methionine was exhausted from the perfusate within 30 min after the time of addition. At this point the specific radioactivity of the resultant AdoMet was about 16% of that of the exogenous methionine. Turnover of the intracellular AdoMet was evident from a decrease in the specific activity to 7% at 90 min. The radiolabeled methyl groups from the AdoMet were readily utilized for the methylation of histones and DNA (Fig. 1B). It is evident from the data in Fig. 1A that methionine uptake is reduced in the presence of exogenous homocysteine. When [methyl-\(^{14}\)C]AdoMet was added to the perfusate, only trace quantities of radioactive methyl groups were detectable in histones or DNA. Furthermore, less than 0.5% of the exogenous AdoMet was retained by the liver after 90 min (Fig. 1A). The addition of glucose to the system was without effect.

**Formation of AdoMet and AdoHcy from Various Substrates by Isolated Livers**—The rate of formation of AdoMet and AdoHcy from various substrates added in a single dose is presented in Figs. 2 and 3. In the absence of exogenous substrates, livers from normal rats maintained a level of 32 ± 13 nmol of AdoMet and 12 ± 7 nmol of AdoHcy/g of tissue. Introducing 50 \(\mu\)M methionine resulted in a significant increase in the level of AdoMet which reached a maximum of 120 nmol/g of liver in 20 min (Fig. 2A). At this point, the methionine in the perfusate was exhausted (Fig. 1A), and the AdoMet level decreased gradually (Fig. 2A). If the exogenous methionine concentration was elevated to 2.25 mM, the liver concentration of AdoMet increased to about 300 nmol/g in 15 min and remained at that level throughout the time course of the experiment (Fig. 2B). Analysis of the perfusate revealed that 40% of the methionine was utilized during the 90-min period. Regardless of the concentration of methionine added to the perfusate, the level of AdoHcy in the tissue did not vary significantly (Fig. 2, A and B). If 50 \(\mu\)M homocysteine was added to the perfusate, the results were comparable to those obtained with an equivalent amount of methionine (data not presented). Under these conditions, the carbon 1 pool does not appear to be limited. When the concentration of homocysteine was elevated to 2.25 mM, the tissue level of AdoHcy increased markedly (Fig. 2C). As the tissue level of AdoHcy increased, this compound was released from the cells into the
per fusate. After the 90-min period, the level of AdoHcy in the perfusate was 50 μM. The accumulation of AdoHcy could result either via synthesis (S-adenosylhomocysteine hydrolase) or inhibition of hydrolysis by homocysteine. The latter would appear to be the most likely since L-homocysteine has been found to be a potent inhibitor of the hydrolysis of AdoHcy (13-16), and the intracellular pool of adenosine has been reported to be 0.3 to 0.7 nmol/g of tissue (32, 33).

The results obtained when a single large dose of adenosine was added (Fig. 3A) were comparable to those obtained with homocysteine alone. Adenosine acts as an inhibitor of the hydrolysis of AdoHcy (13-16) resulting in the accumulation of this compound. An increase in the endogenous level of AdoMet occurred. To account for the elevated levels of these compounds, substantial quantities of endogenous methionine and/or homocysteine must be available. The addition of homocysteine (0.5 mM) in the presence of adenosine further increased the tissue levels of both AdoMet and AdoHcy (Fig. 3C). If the level of homocysteine in the perfusate was increased to 3.4 mM, the rate of AdoMet synthesis increased (Fig. 3D); however, net cellular levels were not affected. In contrast, AdoHcy synthesis continued at a relatively constant rate over the 90-min time course.

Throughout these experiments, it was apparent that whenever the intracellular level of AdoHcy increased a concomitant increase in AdoMet occurred. This phenomenon is well illustrated in Fig. 3B, where a low dose of adenosine and homocysteine was added to the perfusate. Within 15 min the concentration of AdoHcy in the cell increased 6-fold; at this point, the adenosine was exhausted (Fig. 3B, inset) and subsequently, AdoHcy levels decreased. The concentration of AdoMet in the cell paralleled that of AdoHcy throughout this experiment.

When the normal liver was perfused with methionine, little or no AdoHcy or AdoMet was detected in the perfusate (Fig. 4). No AdoMet was released regardless of the intracellular concentration of this compound. In contrast, whenever AdoHcy accumulated intracellularly, it was released from the hepatocytes. In all these experiments the AdoHcy concentration in the perfusate was equivalent to about one-tenth that found in the tissue. When high concentrations of both adenosine and homocysteine were employed (Fig. 3D), the total quantity of AdoHcy in both liver and perfusate after 90 min was about 70 μmol. Most of the AdoHcy would appear to be the result of synthesis via reversal of the S-adenosylhomocysteine hydrolase since methylation is blocked (Table I) and AdoMet does not turn over. Under these conditions, a net rate of synthesis of about 120 nmol of AdoHcy/min/g of liver resulted.

Throughout all of these experiments, adenosine was utilized by the hepatocytes at a rapid rate of 1100 ± 300 nmol/min/g (see Fig. 3, insets). When homocysteine was available, approximately 20% of the adenosine was utilized for AdoHcy synthesis. The remainder of the adenosine was metabolized via adenosine deaminase to form inosine (1, 34) or adenosine kinase to form adenosine 5'-monophosphate (35).

In contrast to adenosine, the utilization of exogenous homocysteine was limited. In these experiments the uptake of homocysteine could be attributed entirely to AdoMet (methionine) and AdoHcy biosynthesis. Significant quantities of cystathionine, cysteine, and α-ketobutyric acid were detected only when serine was available. When serine (4.0 mM) was added in the presence of 4.0 mM adenosine and 3.4 mM homocysteine, the rate of removal of homocysteine was approximately 80 nmol/min/g of liver (data not presented).
AdoHcy-The extent of methylation of histones, DNA, and phosphatidylethanolamine after perfusing the liver with 50 mM L-
[35S]methionine was set at 100% (Table I). The further addition of adenosine and homocysteine (0.5 mM each) to the perfusate did not alter the AdoMet:AdoHcy ratio nor did it affect the rate of formation of AdoHcy. Inhibition of Macromolecular Methyltransferases by AdoHcy—The extent of methylation of histones, DNA, and phospholipids after perfusing the liver with 60 μM L-
[35S]methionine was set at 100% (Table I). The further addition of adenosine and homocysteine (0.5 mM each) to the perfusate did not alter the AdoMet:AdoHcy ratio nor did it affect the rate of formation of AdoHcy. Inhibition of histone, DNA, and phosphatidylethanolamine was almost completely inhibited. These results support the homocysteine-conserving cycle proposed by Mudd et al. (18, 19). In contrast to homocysteine, adenosine is metabolized rapidly. This can occur by several competing enzymes, i.e. adenosine deaminase, adenosine kinase, and S-adenosylhomocysteine hydrolase. The S-adenosylhomocysteine hydrolase from rat liver has a high affinity for adenosine; however, AdoHcy could be synthesized only if ample homocysteine were available since the Michaelis constant of the enzyme for this compound is much greater.

Inhibition of histone, DNA, and phosphatidylcholine (PtdCho) in the presence of [35S]methionine (50 μM) was arbitrarily set at 100%. Values are the means of three determinations with standard deviations.

<table>
<thead>
<tr>
<th>Addition</th>
<th>AdoMet</th>
<th>AdoHcy</th>
<th>Ratio of AdoMet to AdoHcy</th>
<th>% Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>45 ± 13</td>
<td>8 ± 6</td>
<td>5.6 ± 1.8</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>Hcy (0.5 mM)</td>
<td>156 ± 14°</td>
<td>19 ± 9</td>
<td>8.2 ± 2.7</td>
<td>107 ± 12</td>
</tr>
<tr>
<td>Ado (0.5 mM)</td>
<td>1250 ± 190°</td>
<td>4000 ± 160°</td>
<td>0.3 ± 0.1°</td>
<td>10 ± 7°</td>
</tr>
<tr>
<td>Hcy (0.5 mM)</td>
<td>175 ± 65°</td>
<td>2050 ± 140°</td>
<td>0.6 ± 0.2°</td>
<td>55 ± 9°</td>
</tr>
<tr>
<td>Ado (4.0 mM)</td>
<td>1250 ± 190°</td>
<td>4000 ± 160°</td>
<td>0.3 ± 0.1°</td>
<td>10 ± 7°</td>
</tr>
</tbody>
</table>

* Significant differences between control values (50 μM methionine) and experimental values greater than p < 0.001.

Forty per cent of this was attributable to cystathionine biosynthesis, while the remainder could be accounted for in the liver and perfusate as AdoMet and AdoHcy. The presence of serine did not significantly alter the rate of formation of AdoMet or AdoHcy.

Discussion

In the normal rat liver, the intracellular concentration of AdoMet is dependent upon the availability of L-methionine. The maximum level of AdoMet obtainable in the presence of high concentrations of L-methionine was 300 nmol/g, which is 3- to 4-fold greater than that reported in rats fed a normal diet. Increases in the hepatic AdoMet levels following methionine administration have been reported previously (36-39). When the liver was perfused with high concentrations of radioactive methionine (2.25 μM), the specific activity of the resultant AdoMet only reached 45% of that of the exogenous methionine in 15 min and then decreased gradually to 25% at 90 min. Under these conditions it appears that homocysteine is conserved by the cell. As the AdoMet is utilized by a host of transmethylation reactions the resultant AdoHcy is rapidly degraded to adenosine and homocysteine. The homocysteine is recycled to methionine via the methylenetetrahydrofolate and/or betaine methyltransferases resulting in dilution of the specific activity of the intracellular AdoMet. Cystathionine synthase competes for the homocysteine and irreversibly removes it from the cell (Scheme I). The rate of removal of homocysteine by this mechanism appeared to be limited even in the presence of serine. These observations support the homocysteine-conserving cycle proposed by Mudd et al. (18, 19). In contrast to homocysteine, adenosine is metabolized rapidly. This can occur by several competing enzymes, i.e. adenosine deaminase, adenosine kinase, and S-adenosylhomocysteine hydrolase. The S-adenosylhomocysteine hydrolase from rat liver has a high affinity for adenosine; however, AdoHcy could be synthesized only if ample homocysteine were available since the Michaelis constant of the enzyme for this compound is much greater.

AdoHcy appears to act as a positive effector of S-adenosylmethionine synthase. Whenever the tissue level of AdoHcy increased, a concomitant increase in AdoMet occurred. The results obtained in liver differ from those obtained in yeast, where AdoHcy essentially had no effect on S-adenosylmethionine synthase (42). AdoHcy could possibly stimulate AdoMet synthesis via methionine biosynthesis; however, AdoHcy has been reported to suppress both the betaine homocysteine (43) and methylenetetrahydrofolate methyltransferases in liver (44). Regardless of the intracellular concentration of AdoHcy, AdoMet reached a plateau level of 1200 to 1400 nmol/g. This is consistent with the observations of Lombardini et al. (45, 46) that AdoMet acts as a negative effector of its own synthesis at high concentrations.

The liver is apparently impermeable to exogenous AdoMet since it was not utilized as a methyl donor (Fig. 1). Furthermore, the liver retained only trace amounts of exogenous [methyl-14C]AdoMet, which is contradictory to previous reports (47, 48). The exit of AdoMet from the cell also appears to be restricted as liver cells can accumulate large quantities of AdoMet without releasing it into the perfusate. In addition, the hepatocytes appear to be relatively impermeable to AdoHcy (21); however, a unidirectional flow of AdoHcy from...
the hepatocyte occurs when a concentration gradient develops.

Acknowledgment—The excellent technical assistance of Mary Briske-Anderson and Judy Haning is gratefully acknowledged.

REFERENCES