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Decreased Transmethylation of Biogenic Amines After *In Vivo* Elevation of Brain *S*-Adenosyl-L-Homocysteine

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Abstract: The ability of *S*-adenosyl-L-homocysteine (AdoHcy) to inhibit biologic transmethylation reactions *in vitro* has led us to explore the possibility of pharmacologically manipulating AdoHcy levels *in vivo* and examining the consequences of these alterations on the transmethylation of some biogenic amines. Swiss-Webster mice were injected intraperitoneally with different doses of adenosine (Ado) and D,L-homocysteine thiolactone (Hcy) and were killed at various times thereafter. *S*-Adenosyl-L-methionine (AdoMet) and AdoHcy concentrations were determined by using a modified isotope dilution-ion exchange chromatography-high pressure liquid chromatography technique sensitive to less than 10 pmol. Increasing doses of Ado + Hcy (50–1000 mg/kg of each) produced a dose-related increase in blood, liver, and brain AdoHcy levels. At a dose level of 200 mg/kg Ado + Hcy, AdoHcy levels were markedly elevated, with minimal concomitant perturbations of AdoMet. This elevation was maximal 40 min after giving Ado + Hcy, returning to control values within 6 h. Ado + Hcy treatment resulted in decreased activities of catechol-*O*-methyltransferase, histamine-*N*-methyltransferase, and AdoHcy hydrolase *in vitro*. The cerebral catabolism of intraventricularly administered [^{3}H]histamine (HA) was decreased in a dose-related manner by Ado + Hcy treatment as evidenced by higher amounts of nonutilized [^{3}H]HA in brain, concurrent decreases in [^{3}H]methylhistamine formation, and decreases in the transmethylation conversion index. Steady state levels of HA also showed dose-related increases after Ado + Hcy treatment. It is concluded that injections of Ado + Hcy can markedly elevate AdoHcy levels *in vivo*, which can, in turn, decrease the rate of transmethylation reactions. **Key Words:** Transmethylation—Biogenic amine—*S*-Adenosylhomocysteine—*S*-Adenosylmethionine—HPLC. **Schatz R. A. et al.** Decreased transmethylation of biogenic amines after *in vivo* elevation of brain *S*-adenosyl-L-homocysteine. *J. Neurochem.* **36**, 1739–1748 (1981).

It is well established that *S*-adenosyl-L-homocysteine (AdoHcy) is a potent inhibitor, *in vitro*, of transmethylation reactions utilizing *S*-adenosyl-L-methionine (AdoMet) as the methyl donor. These enzymes include: tyramine-*N*-methyltransferase (EC 2.1.1.27) (Mann and Mudd, 1963), catechol-*O*-methyltransferase (EC 2.1.1.6) (COMT), phenylethanolamine-*N*-methyltransferase (EC 2.1.1.28) and acetylserotonin methyltransferase (EC 2.1.1.4) (Deguchi and Barchas, 1971), histamine-*N*-methyltransferase (HMT) (EC 2.1.1.8) (Zappia et al., 1969; Baudry et al., 1973; Borchardt et al., 1978), histone

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Abbreviations used: Ado, Adenosine; AdoHcy, *S*-Adenosyl-L-homocysteine; AdoMet, *S*-Adenosyl-L-methionine; COMT, Catechol-*O*-methyltransferase; DA, Dopamine; 3-deazaAdo, 3-Deazaadenosine; HA, Histamine; Hcy, Homocysteine; HMT, Histamine-*N*-methyltransferase; HPLC, High pressure liquid chromatography; MD, 3-*O*-Methyldopamine; MeHA, 3-Methylhistamine; MeIAA, 3-Methylimidazoleacetic acid; MN, Normetanephrine; NE, Norepinephrine; VMA, 3-Methoxy-4-hydroxymandelic acid.

methyltransferase (EC 2.1.1.42) (Hoffman et al., 1979), phosphatidylethanolamine methyltransferase (EC 2.1.1.17) (Chung and Law, 1964; Schneider and Vance, 1979), tRNA methyltransferase (EC 2.1.1.29–36) (Leboy et al., 1978; Salas and Sellinger, 1978), mRNA methyltransferase (Borchardt and Pugh, 1979), DNA methyltransferase (EC 2.1.1.37) (Cox et al., 1977), and protein methyltransferases I–III (EC 2.1.1.23, 2.1.1.24, 2.1.1.43) (Kim, 1974). In addition, AdoHcy inhibits N^5-methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13) (Burke et al., 1971) and spermine synthase (EC 2.5.1.16) (Hibasami and Pegg, 1978). Further interest in AdoHcy and the enzyme responsible for its hydrolysis, AdoHcy hydrolase (EC 3.3.1.1) (Schatz et al., 1979), has been spurred by the finding that some synthetic AdoHcy analogues prevent the oncogenic transformation of chick embryo fibroblasts infected with Rous sarcoma virus or Gross murine leukemia virus (Robert-Gero et al., 1975; Legraverend et al., 1977; Pierre et al., 1977; Chiang et al., 1978). Furthermore, elevated AdoHcy levels have been implicated in adenosine toxicity seen in T and B lymphoma cells (Kredich and Martin, 1977; Palella et al., 1980).

Recent pharmacologic studies have shown that AdoHcy administration increases the urinary excretion of dopamine (DA) and norepinephrine (NE) without concurrent alterations in urinary levels of 3-*O*-methyldopamine (MD) or normetanephrine (MN) (Bidard et al., 1979). In brain, AdoHcy administration increases NE synthesis and decreases serotonin (5-HT) synthesis and levels without altering DA metabolism (Fonlupt et al., 1979). After intracisternal [^{14}C]DA or [^{3}H]NE, brain levels of [^{14}C]MD and [^{3}H]NM were (unexpectedly) elevated in AdoHcy-treated rats (Bidard et al., 1979). Previously, Baudry et al. (1973) have shown that AdoHcy treatment had no effect on the catabolism of intracisternally administered [^{3}H]histamine (HA) in rat brain. Definitive interpretation of the findings of the aforementioned studies, however, is hampered by the fact that no attempt was made to correlate the biochemical changes with tissue levels of AdoHcy and AdoMet.

Recently, it has also been shown that the administration of the AdoHcy hydrolase inhibitor, 3-deazaadenosine (3-deazaAdo) to rats increased tissue AdoMet and AdoHcy levels and decreased liver creatinine levels, the urinary excretion of 3-methoxy-4-hydroxymandelic acid (VMA), and hepatic phospholipid methylation (Chiang and Cantoni, 1979; Chiang et al., 1980).

It was the objective of our studies to find an effective means of elevating brain AdoHcy levels, without severely perturbing those of AdoMet, and to determine the consequence of such high AdoHcy levels on several transmethylation reactions.

MATERIALS AND METHODS

Animals

Adult male Swiss-Webster mice (24–32 g) were from Charles River Laboratories (Portage, Michigan). The mice were fasted overnight prior to the experimental procedures, which were conducted between 8:00 and 11:00 a.m. to minimize possible diurnal variation.

Drug Treatment and Tissue Collection

Mice were injected intraperitoneally with different doses of *S*-adenosyl-L-homocysteine (AdoHcy) or of an equimolar mixture of adenosine (Ado) plus D,L-homocysteine thiolactone (Hcy) (20 ml/kg) suspended in saline containing Tween 80 (2.5%). At various times after drug administration, the mice were decapitated and the heads dropped into liquid nitrogen, after which the brains were removed and refrozen in liquid nitrogen. Trunk blood was collected directly into liquid nitrogen and livers were rapidly excised after blood collection and frozen, as above. All tissues were stored at −80°C until time of assay. In experiments where *in vitro* enzyme activity determinations were made, brains and livers were rapidly excised and placed into ice-cold, tared beakers containing the respective buffers required for assay.

Determination of AdoMet and AdoHcy Levels

AdoHcy and AdoMet disulfate di-*p*-toluenesulfonate were generously donated by Dr. Giorgio Stramentinoli (BioResearch Laboratories, Liscate, Italy). Amberlite CG-50 was from Accurate Chemical (Hicksville, New York), and Dowex 50W-X8 (200–400 mesh), from BioRad (Richmond, California). [Methyl-^{14}C]AdoMet (specific activity 57 mCi/mmol) and [8-^{14}C]Ado (specific activity 54 mCi/mmol) were from New England Nuclear (Boston, Massachusetts). Radioactive AdoHcy (8-^{14}C) was prepared from L-Hcy and [8-^{14}C]Ado as previously described (Schatz et al., 1977*a*). The AdoHcy was purified with a Waters high pressure liquid chromatography (HPLC) instrument on a μBondapak C18 column. AdoHcy was eluted with 0.04 M-ammonium formate (10% methanol) (2 ml/min), after which the sample was lyophilized, dissolved in water, and stored in small aliquots at −80°C until use. AdoMet and AdoHcy levels were determined by using a modified isotope-dilution method (Schatz and Sellinger, 1975*a*; Schatz et al., 1977*b*). To weighed, frozen tissues were added [^{14}C]AdoMet and AdoHcy ($1-7 \times 10^5$ d.p.m./μmol) in a sufficient quantity to dilute the normal, endogenous tissue concentrations by about 40%. The tissues were homogenized in 5 ml ice-cold 0.4 M-perchloric acid and centrifuged (10,000 *g* for 10 min). The supernatants were adjusted to pH 5.5–6 by stepwise addition of KOH (10 M, 1 M, 0.1 M) in an ice bath, and the volume was adjusted to 8 ml with water. After centrifugation as above, the supernatant was divided into two 4-ml fractions. For AdoMet determination, 4 ml of supernatant was applied to a Dowex-50 (Na^+) column (0.7 × 3 cm) previously equilibrated with 0.1 M-NaCl. The column was washed with 15 ml of 0.1 M-NaCl and 5 ml water, and AdoMet was eluted with 5 ml of

6 M-HCl. The HCl fraction was lyophilized and dissolved in 0.5 ml water, after which 25–50 μl was injected onto a μBondapak C-18 column. AdoMet was eluted with 0.04 M-monobasic ammonium phosphate (1% methanol) (2 ml/min). The AdoMet peak was collected and a 0.5-ml aliquot counted in 5 ml of ACS (a tissue solubilizer-scintillation mixture—Amersham-Searle, Arlington Heights, Illinois). Tissue concentrations of AdoMet were calculated by using the isotope-dilution equation (Wang and Willis, 1965).

For AdoHcy determination, the remaining 4 ml was applied to an Amberlite CG-50 (H^+) column (0.7 × 3 cm) previously equilibrated with water. The column was washed with 15 ml of 0.03 M-ammonium hydroxide, and AdoHcy was eluted with 5 ml of 3 M-ammonium hydroxide. The AdoHcy fraction was lyophilized and dissolved in 0.5 ml water, after which 100 μl was injected onto a μBondapak C-18 column. AdoHcy was eluted with 0.04 M-monobasic ammonium phosphate (7.5% methanol) (2 ml/min). The rest of the experimental procedure was identical to that used for determination of AdoMet. This assay technique is capable of detecting as little as 10 pmol AdoMet or AdoHcy.

In Vivo *Histamine Methylation and Endogenous Histamine Levels*

Histamine dihydrochloride was from Sigma (St. Louis, Missouri); 3-methylhistamine and 3-methylimidazoleacetic acid were from Calbiochem (LaJolla, California); silica gel thin-layer chromatography (TLC) plates from New England Nuclear (Boston, Massachusetts) or EM Laboratories (Elmsford, New York); and [2,5-^{3}H]histamine dihydrochloride (specific activity 7.7 Ci/mmol) from Amersham-Searle (Arlington Heights, Illinois). For intraventricular (i.vt.) histamine injections, the mice were lightly anesthetized with ether, a small piece of scalp removed for ease in identification of the injection site, and [^{3}H]histamine was injected into the lateral cerebral ventricle in 10 μl of artificial cerebrospinal fluid (Merlis solution) 5 min prior to death, which occurred following immersion of the whole animal (head first) into liquid nitrogen. After overnight storage at −20°C, whole brains were rapidly removed and immediately immersed in liquid nitrogen, and then stored at −80°C. The determinations of [^{3}H]histamine (HA), [^{3}H]methylhistamine (MeHA), and [^{3}H]methylimidazoleacetic acid (MeIAA) were as described by Schatz et al. (1978). Steady state histamine levels were measured by the single isotope procedure of Kobayashi and Maudsley (1972) as modified by Schatz et al. (1978).

Determination of AdoHcy Hydrolase, HMT, and COMT

Tissues were homogenized in 10 volumes (w/v) ice-cold 0.32 M-sucrose. In some cases, homogenates were dialyzed overnight against three changes of 0.005 M-potassium phosphate buffer (pH 7.4). AdoHcy hydrolase was measured in the hydrolytic direction by HPLC on a μBondapak column according to Schatz et al. (1977*a*), the only exception being the use of 0.04 M-monobasic ammonium phosphate (10% methanol) for the separation of AdoHcy and its catabolites. The assays of HMT and COMT were as described by Schatz and Sellinger (1975b) and Porcher and Heller (1972), respectively. Protein was determined according to Lowry et al. (1951).

RESULTS

AdoMet and AdoHcy Levels After AdoHcy or Ado + Hcy

In preliminary studies, intraperitoneal injections of AdoHcy (1 g/kg) slightly increased cerebral AdoHcy (29%) and had no significant effect on hepatic AdoHcy levels (Table 1). Co-administration of Ado and Hcy (Ado + Hcy) (1 g/kg of each), however, markedly elevated AdoHcy levels in both brain (970%) and liver (3200%) (Table 1). As co-administration of Ado + Hcy was capable of producing such large perturbations in AdoHcy levels (compared with AdoHcy), the balance of our investigation was directed toward determining the biochemical consequences of administering Ado + Hcy. In brain, increasing doses of Ado + Hcy resulted in dose-related increases in AdoHcy, reaching maximal levels (1000%) at 1 g/kg (Fig. 1). Cerebral AdoMet levels, however, were not significantly altered by doses of Ado + Hcy of up to 500 mg/kg (Fig. 1). Administration of increasing doses of Ado + Hcy resulted in concurrent, dose-related, increases in hepatic levels of both

TABLE 1. *Effect of AdoHcy and Ado + Hcy on mouse brain and liver AdoHcy levels*

	Brain			Liver		
Treatment[a]	nmol/g ± S.E.M. (*N*)	% Change[b]	*p*[c]	nmol/g ± S.E.M. (*N*)	% Change[b]	*p*[c]
Vehicle	6.89 ± 0.57 (7)	—	—	31.23 ± 2.57 (7)	—	—
AdoHcy	8.88 ± 0.96 (7)	+29	<0.05	37.47 ± 7.60 (6)	+20	NS
Ado + Hcy	73.82 ± 3.27 (6)	+970	<0.005	1031.0 ± 152.70 (6)	+3200	<0.005

[a] Mice were injected intraperitoneally with saline/Tween 80 (20 ml/kg), *S*-adenosyl-L-homocysteine (1 g/kg), or a combination of adenosine + D,L-homocysteine thiolactone (1 g/kg of each) 90 min prior to killing.

[b] % Change values are compared with vehicle-treated animals.

[c] *P* values were determined by Student's *t*-test (two-tailed); *P* values greater than 0.05 were considered not significant (NS).

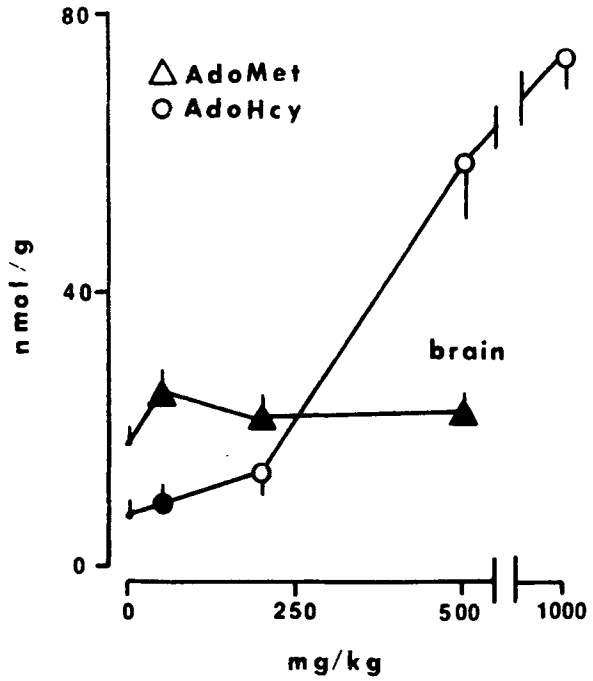

FIG. 1. Effect of increasing doses of Ado + Hcy on mouse brain AdoHcy and AdoMet. Mice were injected intraperitoneally with different doses of Ado + Hcy 90 min prior to death. AdoHcy and AdoMet levels are expressed as means ± S.E.M. in from four to seven mice. Open symbols denote significant differences compared with vehicle-treated controls at the 0.05 level, using Student's *t*-test (two-tailed).

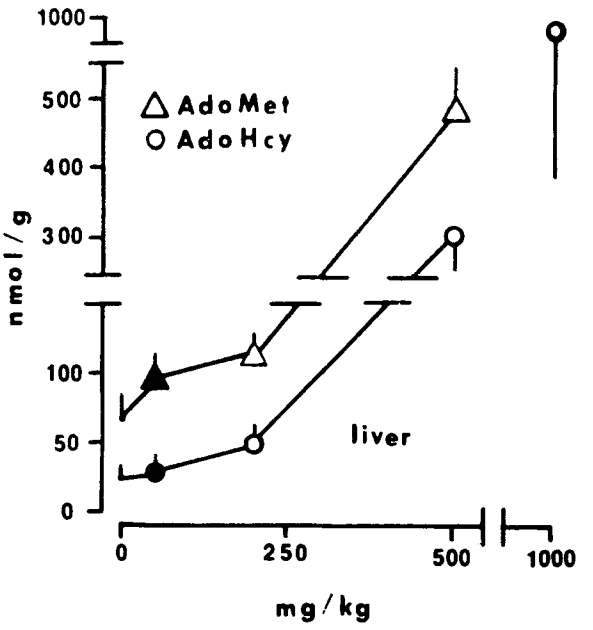

FIG. 2. Effect of increasing doses of Ado + Hcy on mouse liver AdoHcy and AdoMet. For details of drug treatment, expression of results, and statistical analysis, see Fig. 1.

AdoMet (650% at 500 mg/kg) and AdoHcy (2000% at 500 mg/kg) (Fig. 2). Blood levels of AdoMet were significantly increased (48%) only after 500 mg/kg of Ado + Hcy while AdoHcy levels progressively increased, reaching a maximal elevation (105%), also at a dose of 500 mg/kg (Fig. 3). Use of 200 mg/kg of Ado + Hcy, the dose chosen in most subsequent experiments, resulted in a 600% increase in brain AdoHcy at 40 min and a return to control levels within 6 h (Fig. 4). As noted previously, brain AdoMet levels were unaltered by the Ado + Hcy treatment (Figs. 1 and 4).

Effect of Ado + Hcy on AdoHcy Hydrolase, HMT, and COMT

After Ado + Hcy administration, COMT and HMT activity were significantly decreased in

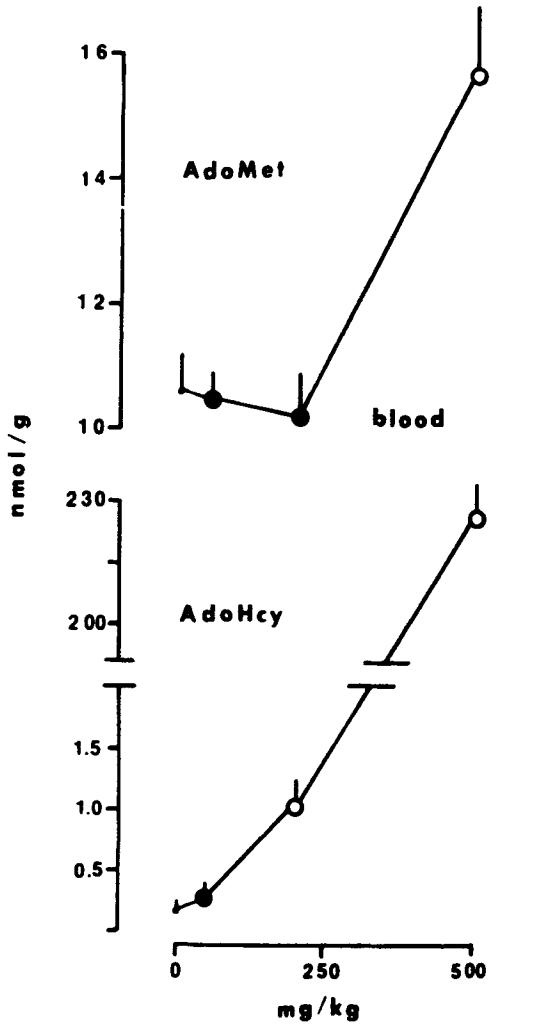

FIG. 3. Effect of increasing doses of Ado + Hcy on mouse blood AdoHcy and AdoMet. For details of drug treatment, expression of results, and statistical analysis, see Fig. 1.

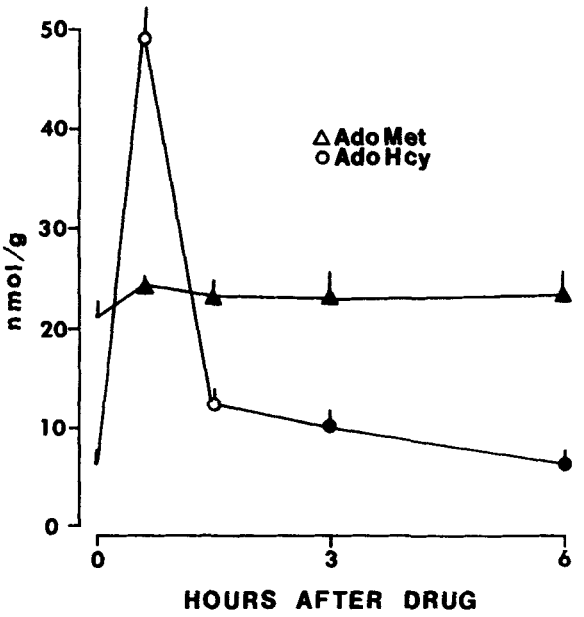

FIG. 4. Time course of altered mouse brain AdoHcy and AdoMet levels after Ado + Hcy. Ado + Hcy (200 mg/kg of each) were injected intraperitoneally into mice which were killed at the indicated times. For expression of results and statistical analysis, see Fig. 1.

homogenates of brain and liver, the hepatic enzymes being inhibited to a greater extent than those of brain (Fig. 5). Dialysis of brain and liver homogenates from Ado + Hcy-treated mice effectively removed this inhibition in the case of HMT (Fig. 6). The presence of high levels of AdoHcy in Ado + Hcy-treated mice prevented an accurate determination of AdoHcy hydrolase activity in undialyzed tissue homogenates using HPLC. After dialysis, however, brain homogenates from Ado + Hcy-treated mice were found to have significantly decreased AdoHcy hydrolase activity, the maximal decrease occurring concurrently with the maximal elevation in cerebral AdoHcy content (40 min after Ado + Hcy) (Fig. 7). The activity of AdoHcy hydrolase, like the levels of AdoHcy (Fig. 4), returned to control values within 6 h (Fig. 7).

Effect of Ado + Hcy Administration on Brain Histamine Levels and [^{3}H]Histamine Catabolism

Since *in vitro* enzyme activity measurements are usually carried out at optimal substrate concentrations, using tissue preparations whose cellular integrity has been drastically altered, we undertook to determine the effects of Ado + Hcy treatment on [^{3}H]histamine catabolism *in vivo*, which, in brain, occurs primarily via transmethylation (Schwartz et al., 1971; Schatz et al., 1978). Brains of Ado + Hcy-treated mice contained 68% more [^{3}H]HA at 50 mg/kg and 215% more [^{3}H]HA at 200 mg/kg compared with vehicle-treated controls (Fig. 8). Concurrently, significant decreases (24% and 33%) in [^{3}H]MeHA formation were noted. [^{3}H]MeIAA levels were also slightly, although not significantly, decreased (Fig. 8). Further, the endogenous levels of brain histamine were increased in a dose-related fashion after Ado + Hcy (30% at 50 mg/kg and 66% at 200 mg/kg) (Table 2), while the MeHA conversion index, used as an estimate of the MeHA transmethylation flux (Schatz et al., 1978; 1981), was decreased by 42% at 50 mg/kg and by 66% at 200 mg/kg of Ado + Hcy (Table 2).

DISCUSSION

There are a variety of techniques presently used to measure tissue levels of AdoMet and AdoHcy. AdoMet levels in rat or mouse liver range from as low as 37 nmol/g (Floridi et al., 1979) to as high as 123 nmol/g (Lombardini and Talalay, 1973), while those of AdoHcy range from 10 nmol/g (Hoffman et

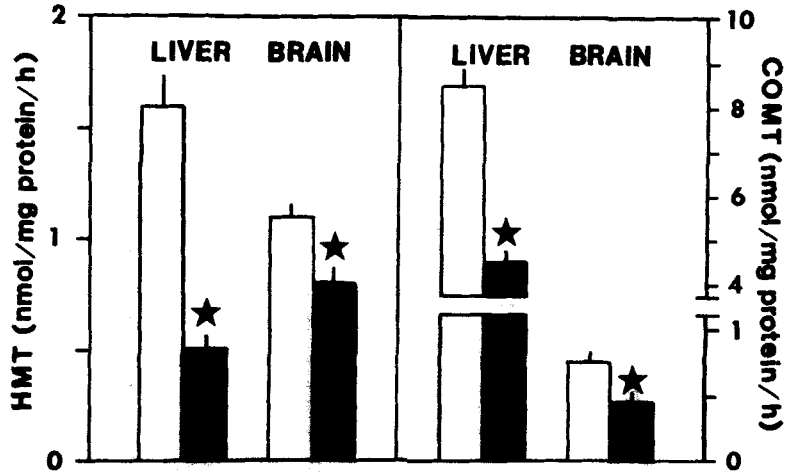

FIG. 5. Effect of Ado + Hcy on mouse brain and liver histamine-*N*-methyltransferase (HMT) and catechol-*O*-methyltransferase (COMT). Enzyme activity values are expressed as nmol/mg protein/h as means ± S.E.M. in homogenates from six to seven animals. Mice were injected intraperitoneally with saline/Tween 80 (20 ml/kg) (open bars) or Ado + Hcy (1 g/kg of each) (closed bars) 90 min prior to death. Stars indicate values significantly different from vehicle-treated controls at the 0.05 level, using the Student's *t*-test (two-tailed).

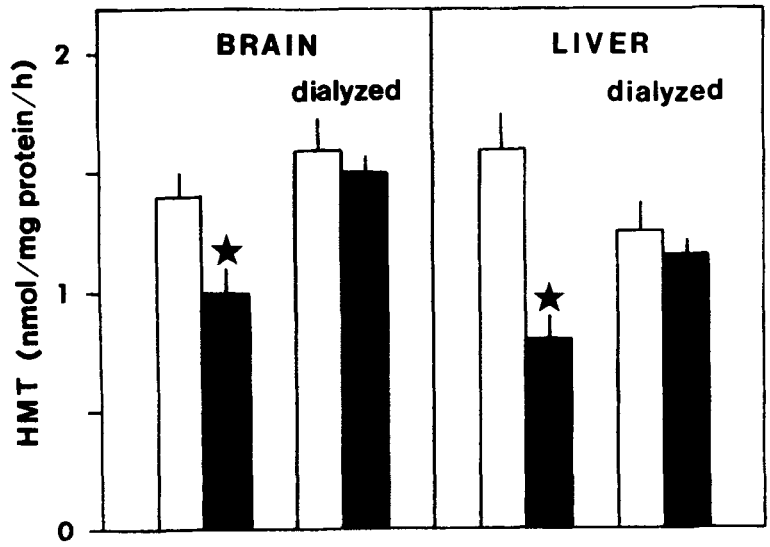

FIG. 6. Effect of dialysis on the inhibition of mouse brain and liver histamine-*N*-methyltransferase (HMT) by Ado + Hcy. For drug treatment, expression of results, and statistical analysis, see Fig. 5.

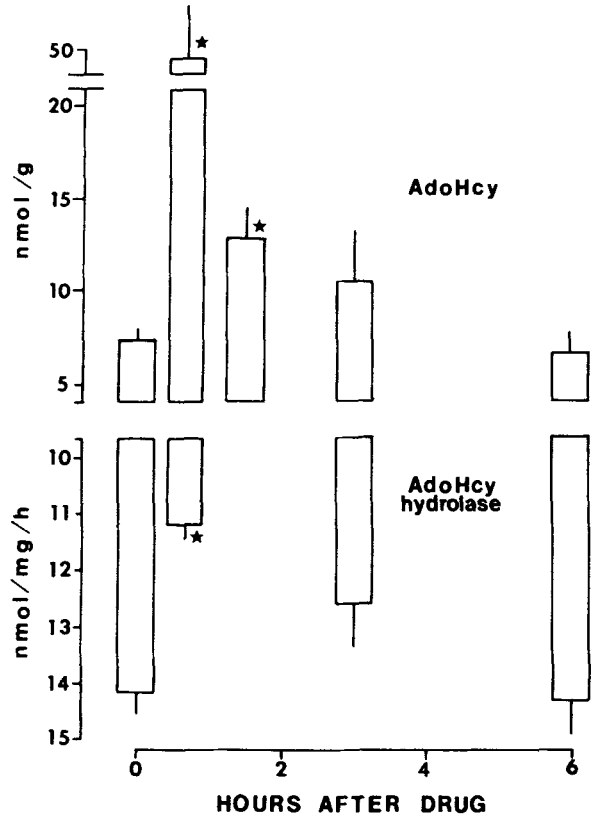

FIG. 7. Time course of altered mouse brain AdoHcy levels and AdoHcy hydrolase activity after Ado + Hcy. Mice were injected intraperitoneally with saline/Tween 80 (20 ml/kg) or Ado + Hcy (200 mg/kg of each) and killed at the times indicated. Values are expressed as means ± S.E.M. in five to seven mice. Enzyme activity was determined in dialyzed tissue homogenates. Stars denote values significantly different from vehicle-treated controls (zero time) at the 0.05 level, using Student's *t*-test (two-tailed).

al., 1979) to 60 nmol/g (Salvatore et al., 1971; Floridi et al., 1979). Brain levels of both AdoMet (15–72 nmol/g) (Salvatore et al., 1971; Taylor and Randall, 1975) and AdoHcy (2.5–72 nmol/g) (Eloranta, 1977; Schatz et al., 1977*b*) are generally lower than those of liver. AdoMet levels in human blood range from 15 to 50 nmol/ml (Illiano et al., 1971; Salvatore et al., 1971); and one investigation reports blood AdoHcy levels to be 32 nmol/ml (Salvatore et al., 1971). AdoMet and AdoHcy tissue levels reported herein (Table 1; Figs. 1–3) were determined by

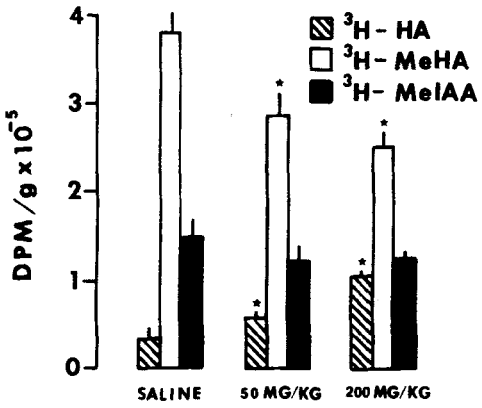

FIG. 8. Effect of Ado + Hcy on [^{3}H]histamine catabolism in mouse brain. Mice were injected intraperitoneally with saline/Tween 80 (20 ml/kg) or Ado + Hcy (50 or 200 mg/kg of each) 40 min prior to death. [^{3}H]Histamine (1 μCi/10 μl) was injected into the lateral cerebral ventricle 5 min prior to death. Results are expressed in d.p.m./g × 10^{-5} ± S.E.M. in six to seven mice. Stars denote a significant difference from corresponding vehicle-treated controls at the 0.05 level, using Student's *t*-test (two-tailed). Total radioactivity ([d.p.m./g] × 10^{-5}) of control, Ado + Hcy (50 mg/kg), and Ado + Hcy (200 mg/kg) groups was 5.67 ± 0.53, 4.83 ± 0.41, and 4.96 ± 0.42, respectively. There was no significant difference between either drug-treated group and the controls ($p > 0.05$).

TABLE 2. *Effect of Ado + Hcy on mouse brain histamine levels and the methylhistamine conversion index*

	Histamine			Methylhistamine conversion index[b]	
Treatment[a]	ng/g ± S.E.M. (*N*)	% Change[c]	*p*[d]	nmol/g	% Change
Saline	69.9 ± 9.6 (6)	—	—	7.3	—
50 mg/kg	90.9 ± 9.6 (6)	+30	NS	4.2	−42
200 mg/kg	116.0 ± 15.0 (6)	+66	<0.02	2.5	−66

[a] Mice were injected intraperitoneally with adenosine and D,L-homocysteine thiolactone (50 or 200 mg/kg of each) or saline/Tween 80 (20 ml/kg) 40 min before killing.

[b] Conversion index: (d.p.m./g brain [^{3}H]methylhistamine)/(histamine specific activity in d.p.m./nmol).

[c] % Change values compared with saline-treated mice.

[d] *P* values were determined by Student's *t*-test (two-tailed); *P* values greater than 0.05 were considered not significant (NS).

using an isotope dilution-ion exchange-HPLC assay system sensitive to less than 10 pmol. These levels are at the lower limit of the range of values reported in the literature, the only exception being the very low levels of AdoHcy in blood (0.15 nmol/ml; Fig. 3). Our data confirm that tissue AdoMet levels are generally higher than those of AdoHcy (Eloranta, 1977; Cantoni et al., 1979).

Administration of Ado + Hcy was much more effective in elevating AdoHcy levels than was an equimolar dose of the intact molecule, AdoHcy (Table 1), indicating that AdoHcy can effectively be synthesized from Ado and Hcy *in vivo* by AdoHcy hydrolase. Others have suggested that the main biologic function of AdoHcy hydrolase is the hydrolysis of AdoHcy, hence—indirectly—the regulation of transmethylation reactions (De la Haba and Cantoni, 1959; Deguchi and Barchas, 1971). It is known, however, that *in vitro* synthesis of AdoHcy is thermodynamically favored (Walker and Duerre, 1975; Schatz et al., 1979).

The inability (compared with Ado + Hcy) of exogenously administered AdoHcy to increase tissue AdoHcy levels suggests that cells are relatively impermeable to AdoHcy (Miller and Duerre, 1969; Duerre et al., 1969; Knudsen and Yall, 1972), but readily allow passage of Ado and Hcy individually (Walker and Duerre, 1975; Knudsen and Yall, 1972). Another, and less likely, possibility is that AdoHcy is rapidly hydrolyzed *in vivo;* however, the intravenous administration of [^{3}H]AdoHcy resulted in urinary excretion of essentially all of the radioactivity as unaltered AdoHcy (Walker and Duerre, 1975).

The dramatic increase in hepatic AdoHcy levels (Fig. 2) indicates that the liver possesses greater AdoHcy synthetic capability than blood or brain (Figs. 1 and 3), which is in agreement with previous observations that AdoHcy hydrolase activity is much higher in liver than in other tissues tested (Finkelstein and Harris, 1973; Walker and Duerre, 1975; Eloranta, 1977; Schatz et al., 1977*a*). Additionally, intraperitoneal injection favors the initial passage of a large portion of the drugs through the hepatic portal system (Lukas et al., 1971). The AdoHcy in blood may be due to the spillover of excess AdoHcy from other organs or from direct synthesis by blood AdoHcy hydrolase. The existence of AdoHcy hydrolase in blood remains unreported; however, blood has been shown to contain AdoMet synthetase (EC 2.5.1.6) (Baldessarini, 1975), as well as several methyltransferases (Axelrod and Cohn, 1971; Kim, 1974; Prozialeck et al., 1978; Cheng and Kazazian, 1978; Weinshilboum et al., 1979; Strittmatter et al., 1979). Interestingly, AdoMet synthetase and AdoHcy hydrolase generally exhibit a parallel tissue distribution, AdoHcy hydrolase being more active than AdoMet synthetase (Baldessarini, 1975; Eloranta, 1977; Finkelstein, 1979).

The administration of Ado + Hcy markedly elevated AdoMet levels in blood and liver (Figs. 2 and 3), while brain AdoMet appeared to be resistant (Fig. 1). The peripheral increase in AdoMet is presumably due to inhibition of transmethylation caused by the high levels of AdoHcy, a finding that is in agreement with the observation of Chiang and Cantoni (1979), who showed that the *in vivo* elevation of tissue AdoHcy after 3-deazaAdo resulted in increases of AdoMet and decreases in the methylation of liver phospholipids and guanidoacetic acid and in the urinary excretion of VMA. Conversely, brain levels of AdoMet were unchanged after doses of Ado + Hcy as high as 500 mg/kg (Fig. 1), indicating that cerebral transmethylations may be more resistant to inhibition by AdoHcy. Despite that, HMT and COMT activities in brain were inhibited after 1 g/kg of Ado + Hcy (Fig. 5). Initial experi-

ments using lower doses of Ado + Hcy (200 mg/kg) (data not shown) provided inconsistent results in terms of HMT or COMT inhibition. This is not particularly surprising, in consideration of the fact that *in vitro* enzyme determinations disrupt cellular integrity, thus potentially diluting the effects of enzyme inhibitors administered *in vivo*. Further, the greatest observed increase in AdoHcy levels (in liver after 1 g/kg Ado + Hcy), extrapolates to a concentration of about 1.5 μM, assuming a 70% volume of distribution (Cizek, 1954), a concentration which is at the lower end of the range of K_i values determined for AdoHcy toward various methyltransferases *in vitro* (Cantoni et al., 1979).

Dialysis of brain and liver homogenates from Ado + Hcy-treated mice effectively alleviated the inhibition of HMT (Fig. 6), further indicating that its inhibition was due to the presence of high endogenous concentrations of AdoHcy. Since an accurate determination of the activity of AdoHcy hydrolase using our HPLC assay system was confounded by the high tissue levels of AdoHcy after Ado + Hcy, the tissue preparations were dialyzed prior to the assay. The finding that AdoHcy hydrolase activity remained significantly decreased after dialysis (Fig. 7) is therefore of particular interest. Additionally, this inhibition was evident at a much lower dose of administered Ado + Hcy than that required to inhibit COMT or HMT. Since the kinetics of AdoHcy hydrolase are complex and, at present, poorly understood, the reason for the persistent inhibition of AdoHcy hydrolase is unclear. Recent evidence (Hershfield and Kredich, 1978; Ueland and Saebo, 1979) indicates that AdoHcy hydrolase binds adenosine tightly, and perhaps the inhibition of the cerebral enzyme in dialyzed homogenates from Ado + Hcy-treated mice is the result of such binding.

The maximal, and short-lived, elevation of brain AdoHcy levels after 200 mg/kg of Ado + Hcy was at 40 min, with no concurrent alterations of brain AdoMet (Fig. 4). To produce an elevation of liver AdoHcy levels, minimally two injections of 3-deazaAdo (100 mg/kg) were required (Chiang and Cantoni, 1979). These findings indicate that the body is capable of rapidly and effectively coping with increased tissue levels of AdoHcy. Indeed, as the enzymes of transsulfuration (Finkelstein, 1979) and the adenosine deamination pathways (Cortese et al., 1974; Hoffman et al., 1979; Schatz et al., 1977*a*) are even more active than AdoHcy hydrolase (Eloranta, 1977), there exists an overall homeostatic capacity to counteract long-lasting changes in AdoHcy levels.

The dose-related decreases in [^{3}H]HA methylation in mouse brain after Ado + Hcy (Fig. 8) are in agreement with similar observations of Chiang and Cantoni (1979), who noted that the *in vivo* methylation of hepatic catechols, guanidoacetic acid, and phospholipids decreased after elevation of liver AdoHcy levels. Although the inhibition of methylation noted by Chiang and Cantoni (1979) was undoubtedly, at least in part, due to elevated tissue AdoHcy, other factors may confound the interpretation of the results of experiments in which deazaAdo was administered: (1) deazaAdo is a substrate as well as an inhibitor of AdoHcy hydrolase (Chiang et al., 1977; Cantoni et al., 1979); (2) 3-deazaAdoHcy formed from deazaAdo inhibits methyltransferases (Borchardt, 1975; Leboy et al., 1978; Cantoni et al., 1979); and (3) some methylation reactions are inhibited *in vivo* by elevated AdoMet levels alone (Reilly and Schayer, 1978; Schatz et al., 1981).

In addition to inhibiting the catabolism of exogenous [^{3}H]HA, the administration of Ado + Hcy led to a dose-related increase in endogenous HA levels and a decrease in the transmethylation conversion index, which is an estimate of the transmethylation "flux" (Table 2) (Schatz et al., 1978; 1981). Additionally, as work in progress has revealed, cerebral protein carboxymethylation and phospholipid methylation are also inhibited after Ado + Hcy (data not shown).

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