Biochem. J. (1997) **327**, 383–389 (Printed in Great Britain)

383

Metabolism of exogenous *S*-adenosylmethionine in isolated rat hepatocyte suspensions: methylation of plasma-membrane phospholipids without intracellular uptake

Françoise BONTEMPS[1] and Georges VAN DEN BERGHE

Laboratory of Physiological Chemistry, International Institute of Cellular and Molecular Pathology and University of Louvain Medical School, Avenue Hippocrate 75, B-1200 Brussels, Belgium

Administration of *S*-adenosylmethionine (AdoMet), the main biological methyl donor, has been shown to exert favourable effects on liver disorders in man and animal models. The mechanism of action of AdoMet has, however, remained elusive, mainly owing to controversies with respect to its capacity to enter intact liver cells. Incubation of isolated rat hepatocytes with 2 or 50 μM [*methyl*-^{14}C]AdoMet showed that it was utilized predominantly to methylate cellular phospholipids, forming mainly phosphatidylcholine, although less than 0.2 % of labelled AdoMet was found inside the cells. The concentration of neither AdoMet nor *S*-adenosylhomocysteine (AdoHcy), its demethylation product, was significantly elevated inside the cells.

A slight elevation of intracellular AdoMet was only recorded on incubation with concentrations of AdoMet above 200 μM. AdoHcy, which does not penetrate cells, inhibited phospholipid methylation from [*methyl*-^{14}C]AdoMet but not from [*methyl*-^{14}C]Met. Elevation of intracellular AdoHcy by adenosine dialdehyde, an inhibitor of AdoHcy hydrolase, inhibited phospholipid methylation from [*methyl*-^{14}C]Met, but virtually not at all from [*methyl*-^{14}C]AdoMet. Taken together, these data indicate that exogenous AdoMet does not penetrate hepatocytes significantly but is utilized for phospholipid methylation on the outer surface of the plasma membrane.

INTRODUCTION

S-Adenosylmethionine (AdoMet) is produced from methionine (Met) and ATP by AdoMet synthetase, and is predominantly utilized in transmethylation reactions. Transfer of the methyl group of AdoMet to a large variety of acceptors results in the formation of, among others, creatine, sarcosine, and methylated phospholipids, nucleic acids and proteins (reviewed in [1–3]). These reactions also yield *S*-adenosylhomocysteine (AdoHcy), which is subsequently split into adenosine and homocysteine by AdoHcy hydrolase.

Numerous clinical studies in man have shown that the therapeutic administration of stable salts of AdoMet has favourable effects on liver disorders, including cholestasis, alcoholic and non-alcoholic cirrhosis and drug-induced hepatotoxicity (reviewed in [4–6]). Animal experiments have similarly produced evidence of protective effects of AdoMet against a variety of hepatotoxic agents [7–10]. The mechanism of action of AdoMet has, however, remained unclear, mainly owing to controversies with respect to its capacity to enter liver cells. Whereas uptake of AdoMet by isolated perfused rat liver [11], isolated rat hepatocytes [12–15], and rat [16] and baboon [8] liver *in vivo* has been reported, Hoffman et al. [17] have claimed that AdoMet does not penetrate the liver. On the other hand, Van Phi and Söling [18] have presented evidence that in isolated hepatocyte suspensions, the methyl group of exogenous AdoMet can be incorporated, without intracellular uptake, in phospholipids most probably situated on the outside of the plasma membrane.

In the present work we have reinvestigated in detail the metabolism of exogenous AdoMet added to suspensions of isolated rat hepatocytes.

EXPERIMENTAL

Materials

[*methyl*-^{14}C]AdoMet and [*methyl*-^{14}C]Met, both at 55 mCi/mmol, and inulin [^{14}C]carboxylic acid (9.4 mCi/ml) were purchased from Amersham International (Amersham, Bucks., U.K.). Collagenase A from *Clostridium histolyticum* was from Boehringer (Mannheim, Germany). Adenosine dialdehyde and BSA (fraction V) were from Sigma (St. Louis, MO, U.S.A.). AdoMet, in the stable form of 1,4-butane disulphonate, was from Knoll Farmaceutici (Liscate, Italy). Other compounds were the highest grade commercially available.

Preparation and incubation of isolated hepatocytes

Rat hepatocytes were isolated from fed, male Wistar rats as previously described [19], suspended in Krebs–Ringer-bicarbonate buffer supplemented with 1 % (w/v) dialysed BSA and 10 mM glucose, and gassed with O_2/CO_2 (19:1). Cells were diluted to a final concn. of 100 ± 10 mg of cells/ml (wet weight), except where otherwise stated, and incubated at 37 °C. A 15–20 min preincubation was performed before zero time. [*methyl*-^{14}C]AdoMet or [*methyl*-^{14}C]Met was added at 0.25 μCi/ml. Cell viability was assessed by measuring, by the method of Vassault [20], the proportion of lactate dehydrogenase in the extracellular medium. It reached 5–15 % of total lactate dehydrogenase at the end of the incubations.

Preparation of samples for analysis

When whole-cell suspensions were utilized, 500 μl aliquots were added to 125 μl of ice-cold 1 M $HClO_4$. When extracellular

---

Abbreviations used: AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; PEMT, phosphatidylethanolamine N-methyltransferase.
[1] To whom correspondence should be addressed.

and cellular media were studied separately, 500 μl aliquots of the suspension were transferred into 1.5 ml microcentrifuge tubes containing 500 μl of silicone AR 200 ($d = 1.04$) layered on top of 300 μl of 1 M HClO$_4$, followed by immediate centrifugation. Aliquots of extracellular medium, recovered above the silicone layer after centrifugation, were mixed with 0.25 vol. of 1 M HClO$_4$ and centrifuged to remove insoluble material. The cellular medium, recovered under the silicone layer, was resuspended in the acid phase after addition of 200 μl of water and centrifuged to separate the insoluble from the soluble cellular medium.

To determine intracellular AdoMet and AdoHcy without contamination by exogenous metabolites, 0.5 ml of the cell suspension was washed 3 times in 10 ml of ice-cold PBS before addition of 350 μl of 0.6 M ice-cold HClO$_4$ to the washed pellet. Extracts were kept at -20 °C. It was verified with labelled inulin, added to the suspension to assess adherent water, that approx. 0.1 % of extracellular inulin remained associated with the intracellular medium after three washes, and that this value could not be decreased by additional washes.

Analytical methods

AdoMet and AdoHcy were determined on HClO$_4$ extracts by HPLC with a Hewlett Packard 1090 apparatus and an Alltech AlphaBond C$_{18}$ column (300 mm × 3.9 mm), as described by Stet et al. [21]. A Berthold LB 507A HPLC radioactivity monitor was coupled to the HPLC apparatus for measurement of the radioactivity associated with AdoMet. The latter eluted at 15 min, and, separated from other HClO$_4$-soluble *methyl*-^{14}C-labelled products, eluted between 3 and 5 min. The radioactivity of the latter compounds, including presumably creatine and sarcosine, were added up and collectively termed HClO$_4$-soluble products. When the radioactivity of the sample was very low, as in the intracellular medium, fractions (1.25 ml), corresponding to an elution time of 1 min, were collected and counted. HClO$_4$-precipitated material was washed twice with 1 ml of 0.2 M HClO$_4$, dissolved at 37 °C in 1 ml of Soluene 350 (Canberra Packard, Meriden, CT, U.S.A.), and counted for radioactivity. Phospholipids were extracted by the method of Folch et al. [22]. For identification of radioactive phospholipids, the phospholipids were dissolved in 100 μl of methanol/chloroform (2:1, v/v) and 20 μl of the solution was applied to silica-gel TLC plates, after the addition of 10 μg of standard lipids (N-methylphosphatidylethanolamine, N,N-dimethylphosphatidylethanolamine and phosphatidylcholine). The chromatograms were developed in chloroform/methanol/water (55:25:4, by vol.). The spots were visualized by exposure to iodine vapour, cut out and counted.

Data analysis

All results of repeated experiments are given as means $\pm$ S.E.M. Significance was estimated by Student's t test (double tailed). Representative experiments, when shown, are typical of at least three studies that gave similar results.

RESULTS

Metabolism of [*methyl*-^{14}C]AdoMet added to hepatocyte suspensions

As depicted in Figure 1(A), labelled and unlabelled AdoMet disappeared at similar rates from the extracellular medium of isolated hepatocyte suspensions. Initial rates of disappearance were approx. 0.5 nmol/min per g of cells at 2 μM AdoMet, and 5.0 nmol/min per g of cells at 50 μM AdoMet. The major part of the radioactivity that had disappeared from the extracellular
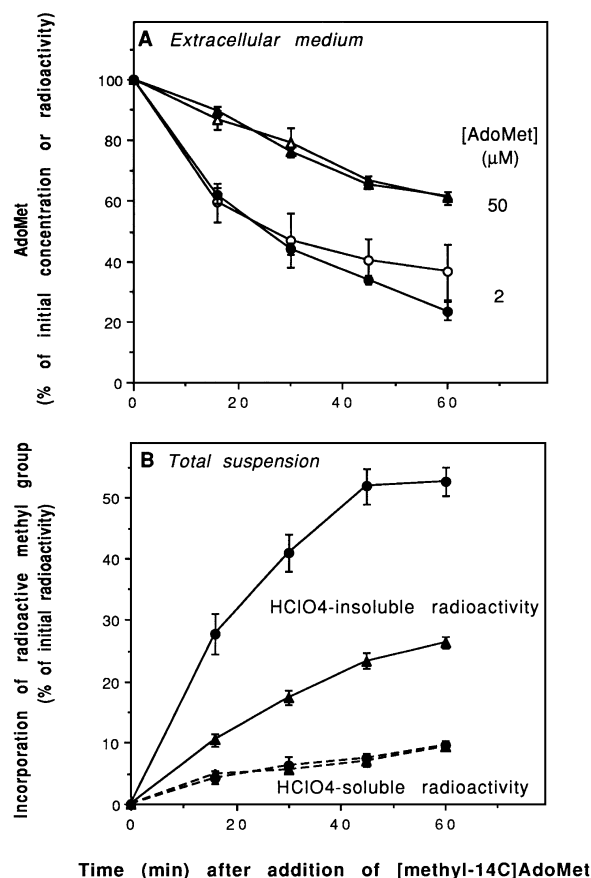

Figure 1   Metabolism of exogenous AdoMet in isolated hepatocyte suspensions

Incubations were performed in the presence of 2 μM (circles) or 50 μM (triangles) [*methyl*-^{14}C]AdoMet. Concentration (open symbols) and radioactivity (solid symbols) of AdoMet were determined in the extracellular medium (**A**). Incorporation of [^{14}C]methyl groups into HClO$_4$-insoluble (unbroken lines) and -soluble products (broken lines) was measured in the total suspension (**B**). Results are expressed as percentage of the initial radioactivity or concentration of extracellular AdoMet. Values are means $\pm$ S.E.M. of three separate experiments.

medium was recovered in HClO$_4$-insoluble products (Figure 1B, unbroken lines), whereas maximally 10 % of the initial radioactivity was recovered in HClO$_4$-soluble products (Figure 1B, broken lines). Initial rates of methylation of the HClO$_4$-insoluble products were approx. 0.4 and 3.0 nmol/min per g of cells in the presence of 2 μM and 50 μM AdoMet respectively. After centrifugation of the cell suspension through a silicone layer, 90 % of the HClO$_4$-insoluble radioactivity was found to be associated with the cells, whereas the HClO$_4$-soluble radioactivity was found to be nearly exclusively in the extracellular medium (results not shown).

The intracellular concentration of AdoMet, at the beginning of incubations without exogenous AdoMet, was approx. 30 nmol/g. It increased to approx. 40 nmol/g after 60 min; this increase was not modified by addition of 2 or 50 μM AdoMet (results not shown). The intracellular concentration of AdoHcy (approx. 5 nmol/g over the whole duration of the incubations), was also not modified by addition of AdoMet. Upon addition of 2 or 50 μM labelled AdoMet, a small amount of intracellular HClO$_4$-soluble radioactivity was detected. It increased progressively (Figure 2, unbroken lines), but did not exceed 2 % of the initial radioactivity after 60 min, with both 2 and 50 μM AdoMet.
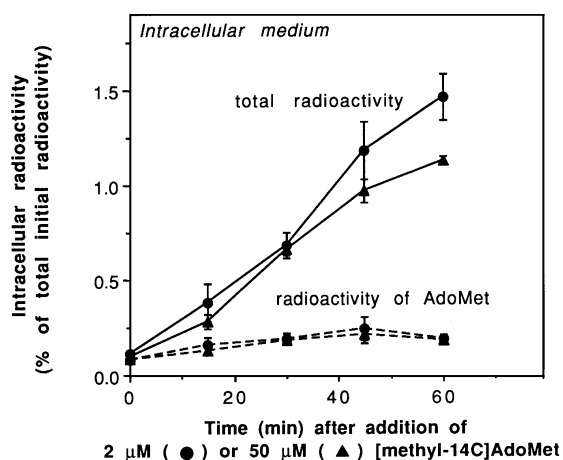
Figure 2 Appearance of HClO₄-soluble radioactivity inside hepatocytes after incubation with exogenous [*methyl*-¹⁴C]AdoMet

Incubations were performed in the presence of 2 μM (circles) or 50 μM (triangles) [*methyl*-¹⁴C]AdoMet. Radioactivity of the intracellular medium, obtained after elimination of the extracellular radioactivity, as described in the Experimental section, was analysed by HPLC. Total intracellular radioactivity (unbroken lines) and radioactivity associated to intracellular AdoMet (broken lines) are expressed as the percentage of initial radioactivity of extracellular AdoMet. Values are means ± S.E.M. of three separate experiments.
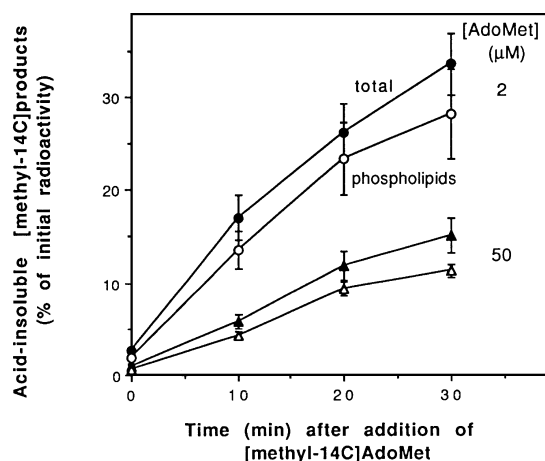

Figure 3 Incorporation of [¹⁴C]methyl groups of AdoMet into total HClO₄-insoluble products and phospholipids

Suspensions of isolated hepatocytes were incubated in the presence of 2 or 50 μM [*methyl*-¹⁴C]AdoMet, followed by centrifugation of the cells through a silicone layer. Incorporation of [¹⁴C]methyl into total HClO₄-insoluble products (solid symbols) and into phospholipids (open symbols) was measured in the cellular medium. Results are expressed as the percentage of the initial radioactivity of extracellular AdoMet. Values are means ± S.E.M. of three separate experiments.

A very small fraction of this radioactivity, not exceeding 0.25 % of the initial radioactivity, was associated with intracellular AdoMet (Figure 2, broken lines).

Suspensions of isolated hepatocytes inevitably contain some broken cells, from which intracellular methyltransferases and methyl acceptors are released. To assess the contribution of the latter components to the metabolism of exogenous AdoMet, the effects of 2 or 50 μM AdoMet were compared in a total cell suspension and in the extracellular medium, obtained by removal of the hepatocytes following a 30-min incubation. Prior
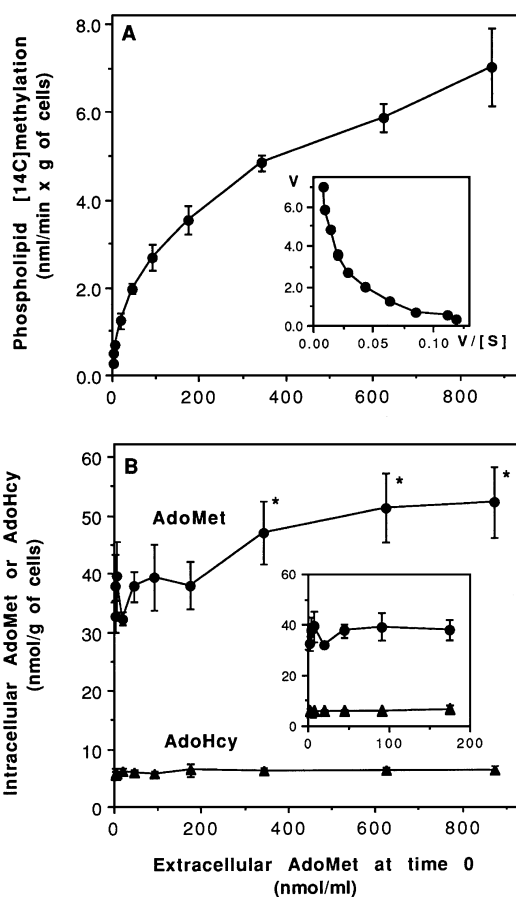

Figure 4 Dose–effect of extracellular AdoMet on the rate of phospholipid methylation (A) and on the intracellular concentration of AdoMet and AdoHcy (B)

Isolated hepatocytes were incubated for 15 min with various concentrations of [*methyl*-¹⁴C]AdoMet. Phospholipids were extracted from the cellular medium obtained by centrifuging the cells through a silicone layer. Intracellular concentrations of AdoMet and AdoHcy were measured after elimination of extracellular AdoMet by washing of the hepatocytes. Rates of phospholipid methylation were calculated from the specific radioactivity of extracellular AdoMet and are given in nmol/min per g of cells. Values are means ± S.E.M. (*n* = 3 or 4 separate experiments). In (**A**), the inset shows a Hofstee [23] representation of the results. In (**B**), the inset shows an enlargement of the *x*-axis; (*) a significant increase of intracellular AdoMet (*P* ≤ 0.05).

verification showed that no degradation of AdoMet occurred over a 60-min incubation at 37 °C in Krebs–Ringer-bicarbonate buffer. In contrast, AdoMet was metabolized in the extracellular medium from which the cells had been removed. However, rates of disappearance reached only 15–30 % of those recorded in total suspensions (results not shown). Furthermore, metabolism of AdoMet in extracellular medium resulted, for more than 90 %, in methylation of HClO₄-soluble products, which proceeded at a rate comparable with that measured in the total cell suspension. These results indicate that: (i) the methylation of HClO₄-soluble metabolites observed in total cell suspensions can be explained by the presence in the extracellular medium of methyltransferases and methyl acceptors, released from broken cells; (ii) the [¹⁴C]methylated HClO₄-insoluble compounds formed in total cell suspensions belong to intact hepatocytes. On the other hand, when the proportion of broken hepatocytes was enhanced by mixing intact cells with cells that had undergone freezing followed by thawing, metabolism of AdoMet increased commensurately
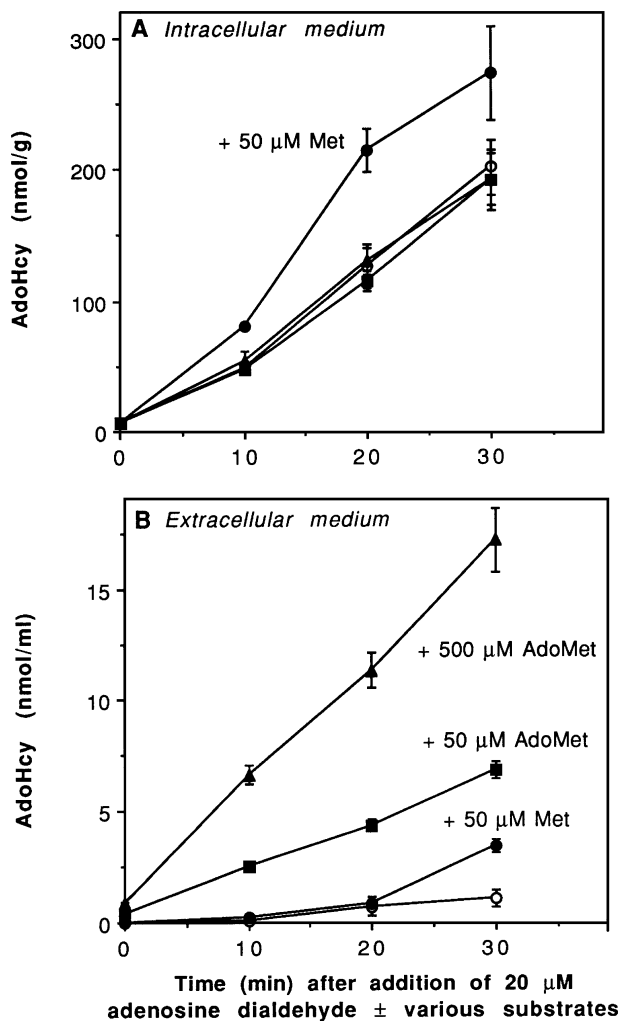
Figure 5   Effect of adenosine dialdehyde on the intracellular (A) and extracellular (B) accumulation of AdoHcy

Hepatocytes were incubated with 20 μM adenosine dialdehyde, without other addition, or with the substrates indicated. Intracellular concentrations of AdoHcy were determined after washing the hepatocytes. Values are means $\pm$ S.E.M. of three separate experiments.

Figure 6   Effect of extracellular AdoHcy on phospholipid methylation from 50 μM [*methyl*-14C]Met (A) or 50 μM [*methyl*-14C]AdoMet (B)

The initial extracellular concentration of AdoHcy was 50 μM. Phospholipids were extracted from the cellular medium obtained by centrifuging the cells through a silicone layer. Results are expressed as the percentage of the initial radioactivity of extracellular AdoMet.

(up to 7-fold) and yielded mainly methylated $HClO_4$-soluble compounds (results not shown).

Analysis of the acid-insoluble methylated cellular products formed from exogenous AdoMet showed that they corresponded mainly to phospholipids, both at 2 μM and 50 μM AdoMet (Figure 3). Furthermore, 80% of these phospholipids were identified as phosphatidylcholine, whereas phosphatidylmono-ethanolamine and phosphatidyldiethanolamine (results not shown) were nearly undetectable. A dose–effect of exogenous [*methyl*-14C]AdoMet on the methylation of cellular phospholipids showed an apparent K_m of 60–70 μM (Figure 4A), although saturation was not yet reached at 500–1000 μM AdoMet. A Hofstee [23] representation of the results (Figure 4A, inset), suggested the occurrence of two phospholipid methylation processes: the first with a V_{max} of 2.3 nmol/min per g of cells and a K_m of 17 μM, and the second with a V_{max} of 8.7 nmol/min per g of cells and a K_m of 240 μM.

Measurements of the intracellular concentrations of AdoMet and AdoHcy (Figure 4B) showed that a significant increase of
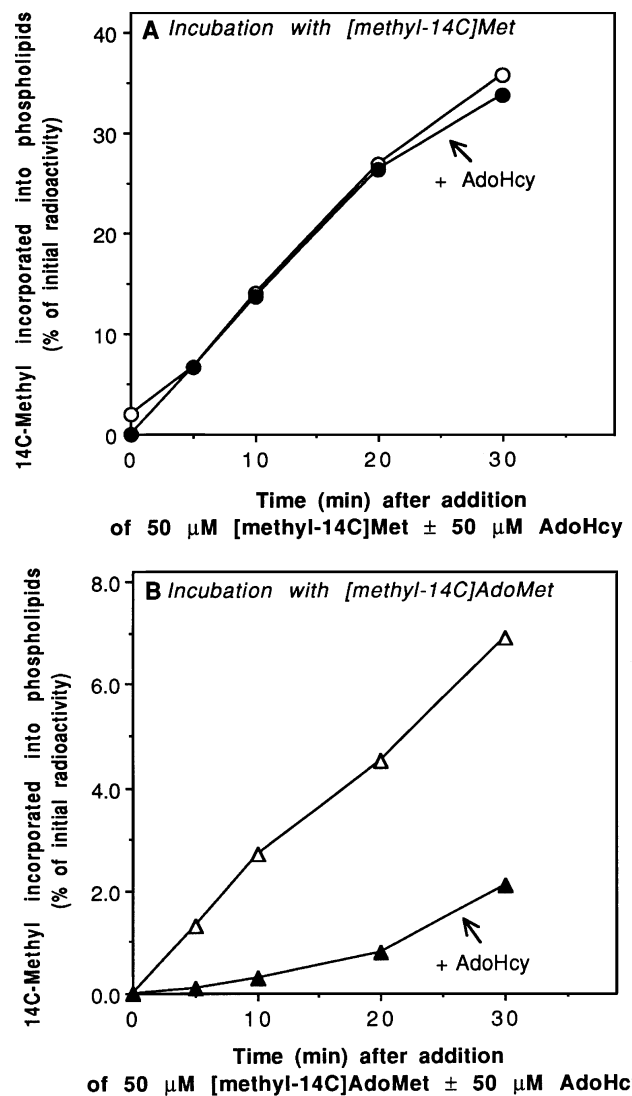
AdoMet was only observed at concentrations of exogenous AdoMet above 200 μM, whereas AdoHcy was not modified at any concentration of exogenous AdoMet. As in the experiments depicted in Figure 2, labelled AdoMet in the intracellular medium remained at the limit of detection: 0.25% of initial radioactivity at 2 μM AdoMet and 0.10% of initial radioactivity at 500 μM AdoMet (results not shown).

Effect of inhibition of AdoHcy hydrolase on intra- and extracellular accumulation of AdoHcy

To corroborate the conclusion that AdoMet does not penetrate easily inside hepatocytes, the effect of exogenous AdoMet on the intracellular accumulation of AdoHcy, its demethylation product, was assessed. To avoid metabolism of AdoHcy, adenosine
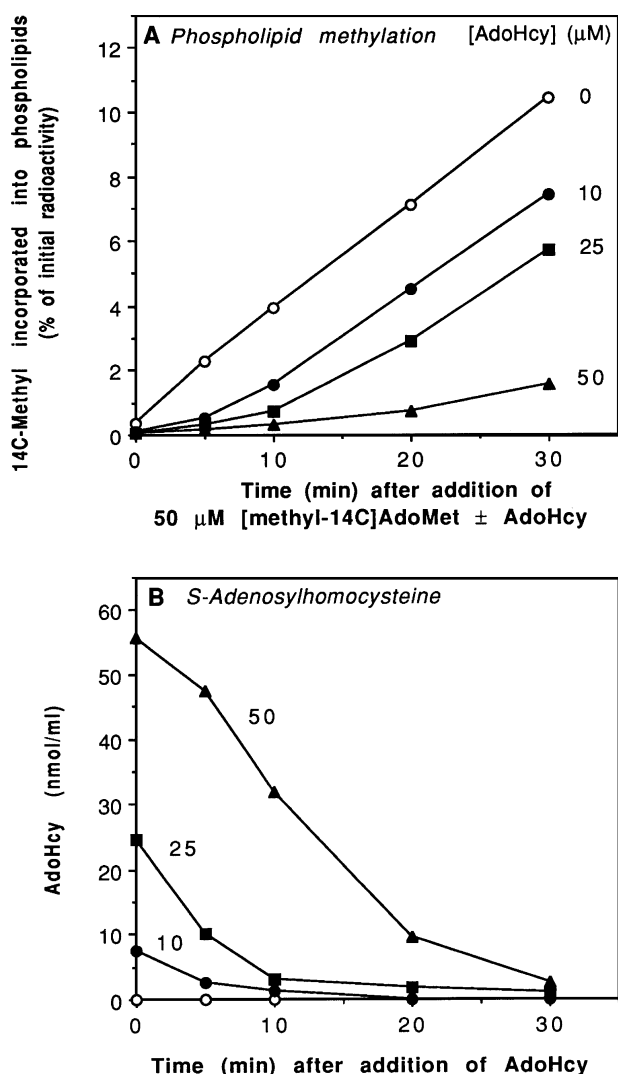
Figure 7   Dose–effect of extracellular AdoHcy on phospholipid methylation from [*methyl*-14C]AdoMet

(**A**) AdoHcy was added at the concentrations indicated. Radioactivity in phospholipids from the cellular medium was determined and expressed as the percentage of the initial radioactivity of extracellular AdoMet. (**B**) AdoHcy concentration was determined in the extracellular medium.

Figure 8   Effect of preincubation with adenosine dialdehyde on phospholipid methylation from 2 μM [*methyl*-14C]Met (A) or 2 μM [*methyl*-14C]AdoMet (B)

Hepatocytes (40 mg of cells/ml) were preincubated for 15 min in the absence (open symbols) or in the presence (solid symbols) of 20 μM adenosine dialdehyde. Radioactivity in phospholipids from the cellular medium was determined. Results are expressed as the percentage of the initial radioactivity of extracellular AdoMet. Values are means $\pm$ S.E.M. of three separate experiments.

dialdehyde, a potent inhibitor of AdoHcy hydrolase [24,25], which penetrates intact cells, was used. Addition of adenosine dialdehyde alone to a hepatocyte suspension (Figure 5A) provoked an accumulation of AdoHcy in the intracellular medium, indicating the occurrence of endogenous trans-methylations. Almost no accumulation of AdoHcy was observed in the extracellular medium (Figure 5B). Upon further addition to the cell suspension of 50 μM Met, which is converted into AdoMet after penetration of the cells, a clear additional increase of intracellular AdoHcy was observed (Figure 5A), and a minor elevation of AdoHcy took place in the extracellular medium (Figure 5B). In contrast, upon addition of 50 μM or 500 μM AdoMet to the cell suspension, no additional increase of intracellular AdoHcy occurred (Figure 5A), whereas AdoHcy increased markedly in the extracellular medium (Figure 5B). Taken together, these results indicate that, contrary to Met, exogenous AdoMet at the concentrations tested, is not a
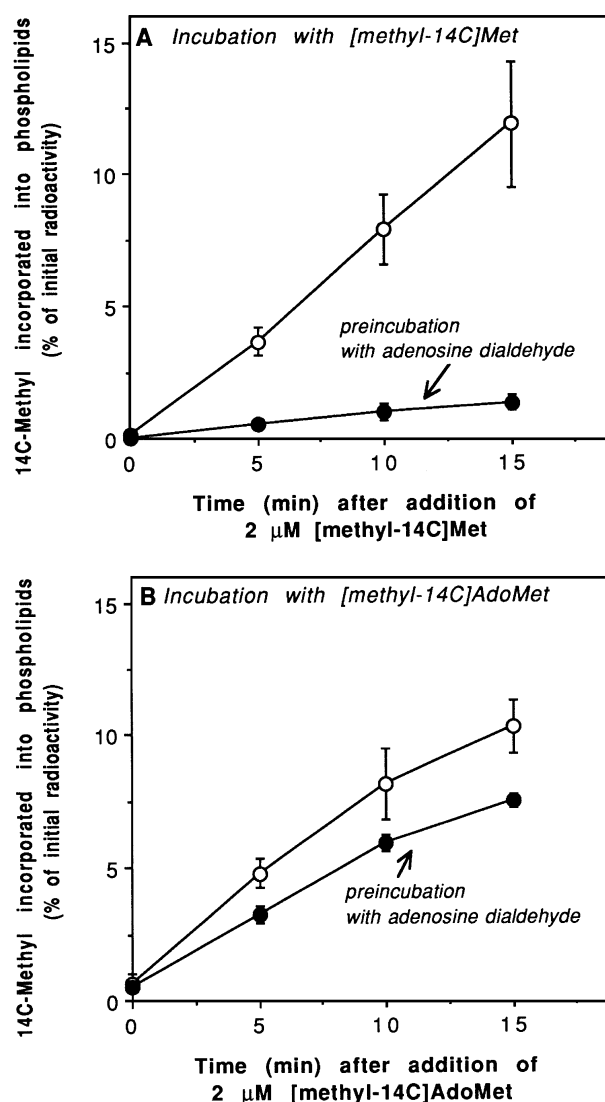
substrate for intracellular methylations in hepatocytes and, by the same token, AdoMet does not cross the plasma membrane of these cells.

Effect of AdoHcy on phospholipid methylation from exogenous AdoMet

The observation that hepatocyte phospholipid methylation occurred after addition of exogenous AdoMet, without entry of AdoMet into the cells, indicated that both the methylated phospholipids and a phospholipid methyltransferase activity are located on the outer cell surface. Phospholipid methyltransferase is known to be inhibited by AdoHcy [17,26–28]. On the other

hand, AdoHcy does not penetrate intact cells, including perfused rat liver [17] and isolated rat hepatocytes [29]. Accordingly, Schanche et al. [30] have shown that addition of AdoHcy to hepatocyte suspensions did not inhibit methylation of intracellular phospholipids by Met. Confirming these findings, addition of 50 μM AdoHcy to an isolated hepatocyte suspension had no effect on phospholipid methylation from [methyl-^{14}C]Met (Figure 6A). However, the same concentration of AdoHcy exerted a strong inhibitory effect on phospholipid methylation when extracellular [methyl-^{14}C]AdoMet was the methyl donor (Figure 6B). This inhibitory effect was even more pronounced at 2 μM than at 50 μM AdoMet (results not shown). Figure 7, depicts the dose–effect of AdoHcy on phospholipid methylation by 50 μM [methyl-^{14}C]AdoMet and shows that inhibition (Figure 7A) persisted as long as the concentration of AdoHcy in the external medium exceeded 5 μM (Figure 7B). The disappearance of AdoHcy from the external medium can be explained by the action of extracellular AdoHcy hydrolase released from broken cells (results not shown). Figure 8 shows an experiment similar to that depicted in Figure 7, but in which intracellular rather than extracellular AdoHcy was increased before addition of methyl-^{14}C-labelled Met or AdoMet. This was done by a 15 min preincubation in the presence of 20 μM adenosine dialdehyde, resulting in an increase in intracellular AdoHcy from 5.5 ± 0.8 nmol/g to 57.5 ± 8.0 nmol/g of cells ($n = 6$). Low concentrations of the methyl donors, and short times of incubation, were chosen to prevent an accumulation of AdoHcy, derived from extracellular methylations, in the extracellular medium. In the presence of adenosine dialdehyde, phospholipid methylation from exogenous Met was 90 % inhibited (Figure 8A), whereas phospholipid methylation from exogenous AdoMet (Figure 8B) was decreased by less than 30 %.

DISCUSSION

This study indicates that AdoMet, added at micromolar concentrations to suspensions of isolated rat hepatocytes, is utilized mainly to methylate phospholipids located on the outer face of the plasma membrane. It also shows that exogenous AdoMet does not penetrate hepatocytes significantly, except maybe at millimolar concentrations. The utilization of AdoMet by isolated hepatocytes, and the implications of our findings with respect to the mechanism of action of AdoMet in liver disorders, will be discussed separately.

Utilization of AdoMet by isolated hepatocytes

That AdoMet is metabolized in isolated hepatocyte suspensions is proven by its disappearance from the incubation medium (Figure 1A) and its appearance in acid-insoluble cellular material (Figure 1B). Despite the obvious metabolism of exogenous AdoMet by intact hepatocytes, we have no indication that AdoMet, at concentrations up to 200 μM, enters the cells. Firstly, labelled AdoMet inside the cells never exceeded 0.25 % of initial extracellular [methyl-^{14}C]AdoMet (Figure 2). Secondly, the experiments that showed that the rate of metabolism of exogenous AdoMet was greatly increased when hepatocytes were broken, provide a good indication that the plasma membrane is a barrier that limits the access of AdoMet to intracellular enzymes. Thirdly, the fact that the addition of 50 or 500 μM AdoMet did not modify the accumulation of intracellular AdoHcy induced by inhibition of AdoHcy hydrolase, whereas addition of Met had a marked elevating effect (Figure 5A), indicates that exogenous AdoMet, in contrast to Met, cannot serve as a precursor for intracellular transmethylations.

The HClO$_4$-soluble radioactive products formed upon addition

of [methyl-^{14}C]AdoMet (Figure 1B) are probably the result of the inevitable release of transmethylating enzymes and methyl acceptors by damaged cells. Indeed, their formation was observed in a medium from which cells had been removed, and this was enhanced by increasing the proportion of broken cells in the suspension. The methylated HClO$_4$-soluble metabolites could be creatine and/or sarcosine because the production of these compounds was increased by addition to the hepatocyte suspensions of their precursors, guanidinoacetate or glycine respectively (F. Bontemps, unpublished work).

As demonstrated by the analysis of the cellular acid-precipitable material (Figure 3), the metabolism of AdoMet resulted mainly in methylation of phospholipids. It is noteworthy that cellular phospholipids were readily methylated in the presence of 2 μM exogenous AdoMet, a concentration that is 15- to 20-fold lower than the intracellular concentration of AdoMet. That phospholipid methylation from exogenous AdoMet occurs on the external face of the plasma membrane of the hepatocytes was already suggested by the finding that, as discussed above, AdoMet does not cross the plasma membrane. This conclusion is corroborated by the observation that phospholipid methylation from exogenous AdoMet was strongly inhibited by exogenous AdoHcy (Figures 6 and 7), which also does not cross the plasma membrane [17,29]. To exert its inhibitory effect, exogenous AdoHcy should thus act on a phospholipid methyltransferase that is accessible from the outside of the cell. This implies that the outer face of the liver plasma membrane contains phospholipid methyltransferase activity. Two different forms of phosphatidylethanolamine N-methyltransferase (PEMT), which converts phosphatidylethanolamine to phosphatidylcholine using AdoMet as the methyl donor, have been shown to exist in liver: PEMT1, the major activity, is found on the endoplasmic reticulum; PEMT2 is recovered on mitochondria-associated membranes [31]. Moreover, phospholipid methyltransferase activity has been measured on rat-liver plasma membranes [28], although never purified. Further work is required to isolate, characterize and define the role of this membranous ecto-enzyme. Our study of the dose–effect relationship of AdoMet on the phospholipid methylation rate (Figure 4A) suggests that it has a K_m of approx. 17 μM. The second K_m, of approx. 240 μM, could reflect slow intracellular penetration of high concentrations of exogenous AdoMet, resulting in methylation of intracellular phospholipids.

A phospholipid methylation rate of 0.3 nmol/min per g at 2 μM AdoMet, as calculated from the data of Figure 4(A), should result in the conversion of 0.1 nmol/min of phosphatidylethanolamine into phosphatidylcholine, i.e. 6 nmol over 60 min. At 50 μM AdoMet, this conversion should reach 40 nmol over 60 min. Since rat liver has 2 mg of plasma membrane protein per g wet weight [32], containing approx. 100 nmol of phosphatidylethanolamine per mg of protein [33,34], one can calculate that these membranes contain approx. 200 nmol of phosphatidylethanolamine per g wet weight of hepatocytes. Although, to our knowledge, no precise data are available on the distribution of phosphatidylethanolamine between the internal and external surface of liver plasma membranes, it is generally surmised, as demonstrated for human erythrocyte membranes, that there is more phosphatidylethanolamine on the inner surface, and more phosphatidylcholine outside (see [35] and references therein). Nevertheless, even if only 20 % of total phosphatidylethanolamine of hepatocyte plasma membranes is located on the outside, 40 nmol per g are available for transmethylation. This value is sufficient to explain the rates of phospholipid methylation recorded over 60 min in our experiments.

Although our results lead to the conclusion that AdoMet does not penetrate hepatocytes, they are not fundamentally con-

tradictory to observations by others of isolated liver cells with respect to AdoMet uptake. The conclusion by Traver et al. [13] and Engstrom and Benevenga [15] that AdoMet enters the cells can be explained by their utilization of 0.5 mM concentrations of AdoMet. Other authors have concluded that transport of AdoMet occurs because they observed the disappearance of exogenous AdoMet from the medium [11] or accumulation of radioactivity from labelled AdoMet, associated with liver cells [12,14]. Only Van Phi and Söling [18] have proposed that exogenous AdoMet can serve as a methyl donor for hepatocyte plasma-membrane phospholipids without penetrating the cells, a result that we have confirmed and extended.

Mechanism of action of AdoMet in liver disorders

Decreased levels of AdoMet and glutathione, and reduced activity of AdoMet synthetase, have been documented in a variety of liver disorders (reviewed in [4–6]). Numerous clinical and experimental studies have shown that administration of AdoMet prevents or reduces these alterations. Isolated hepatocytes are an adequate model for the study of the therapeutic mechanisms of exogenous AdoMet, as shown by several recent reports that have confirmed its beneficial effects under various conditions: cold storage and preservation [36], bromobenzene- and D-galactosamine-induced toxicity [37] and deleterious effects of cytokines [38]. The beneficial effects of AdoMet are usually attributed to replenishment of liver AdoMet and glutathione, the latter allowing reactivation of AdoMet synthetase [5,6,36]. This theory implies that exogenous AdoMet by-passes the deficient synthesis of endogenous AdoMet, and thus presupposes that AdoMet can be transported across the liver plasma membrane. However, as shown by our and others' work, penetration of micromolar concentrations of AdoMet into liver cells is highly unlikely. Moreover, although active transport of AdoMet has been demonstrated in yeast [39], an equivalent system has, to our knowledge, not been found in mammalian cells.

Our observation, that in isolated hepatocyte suspensions exogenous AdoMet is mainly utilized to methylate plasma-membrane phospholipids, suggests that the protective effects of AdoMet are, at least initially, mediated by a direct effect on membrane structure and function. How these membrane changes result in protection and improvement of hepatic function remains to be determined.

REFERENCES

1 Cantoni, G. L. (1975) Annu. Rev. Biochem. **44**, 435–451

2 Cantoni, G. L. (1977) in The Biochemistry of Adenosylmethionine (Salvatore, F., Vorek, E., Zappia, V., Williams-Ashman, H. G. and Schlenk, F., eds.), pp. 121–143, Columbia University Press, New York

3 Chiang, P. K., Gordon, R. K., Tal, J., Zeng, G. C., Doctor, B. P., Pardhasaradhi, K. and McCann, P. P. (1996) FASEB J. **10**, 471–480

4 Friedel, H. A., Goa, K. L. and Benfield, P. (1989) Drugs **38**, 389–416

5 Chawla, R. K., Bonkovsky, H. L. and Galambos, J. T. (1990) Drugs **40**, 98–110

6 Mato, J. M., Alvarez, L., Corrales, F. J. and Pajares, M. A. (1994) in The Liver: Biology and Pathobiology (Arias, I. M., Boyer, J. L., Fausto, N., Jakoby, W. B., Schachter, D. A. and Shafritz, D. A., eds.), pp. 461–470, Raven Press, New York

7 Stramentinoli, G., Gualano, M. and Ideo, G. (1978) Biochem. Pharmacol. **27**, 1431–1433

8 Lieber, C. S., Casini, A., DeCarli, L. M. , Kim, C. I., Lowe, N., Sasaki, R. and Leo, M. A. (1990) Hepatology **11**, 165–172

9 Bray, G. P., Tredger, J. M. and Williams, R. (1992) Hepatology **15**, 297–301

10 Varela-Moreiras, G., Alonso-Aperte, E., Rubio, M., Gasso, M., Deulofeu, R., Alvarez, L., Caballeria, J., Rodes, J. and Mato, J. M. (1995) Hepatology **22**, 1310–1315

11 Zappia, V., Galletti, P., Porcelli, M., Ruggiero, G. and Andreana, A. (1978) FEBS Lett. **90**, 331–335

12 Pezzoli, C., Stramentinoli, G., Galli-Kienle, M. and Pfaff, E. (1978) Biochem. Biophys. Res. Commun. **85**, 1031–1038

13 Traver, J., Varela, I. and Mato, J. M. (1984) Biochem. Pharmacol. **33**, 1562–1564

14 Tsukada, K., Abe, T., Kuwahata, T. and Mitsui, K. (1985) Life Sci. **37**, 665–672

15 Engstrom, M. A. and Benevenga, N. J. (1987) J. Nutr. **117**, 1820–1826

16 Giulidori, P., Galli-Kienle, M., Catto, E. and Stramentinoli, G. (1984) J. Biol. Chem. **259**, 4205–4211

17 Hoffman, D. R., Marion, D. W., Cornatzer, W. E. and Duerre, J. A. (1980) J. Biol. Chem. **255**, 10822–10827

18 Van Phi, L. and Söling, H. D. (1982) Biochem. J. **206**, 481–487

19 Van den Berghe, G., Bontemps, F. and Hers, H. G. (1980) Biochem. J. **188**, 913–920

20 Vassault, A. (1983) in Methods of Enzymatic Analysis, 3rd edn., vol. 3 (Bergmeyer, H. U., ed.), pp. 118–126, Verlag Chemie, Weinheim

21 Stet, E. H., De Abreu, R. A., Bökkerink, J. P. M., Blom, H. J., Lambooy, L. H. J., Vogels-Mentink, T. M., de Graaf-Hess, A. C., van Raay-Selten, B. and Trijbels, F. J. M. (1994) Biochem. J. **304**, 163–168

22 Folch, J., Lees, M. and Sloane-Stanley, G. H. (1957) J. Biol. Chem. **226**, 497–509

23 Hofstee, B. H. J. (1959) Nature (London) **184**, 1296–1298

24 Hoffman, J. L. (1980) Arch. Biochem. Biophys. **205**, 132–135

25 Bartel, R. L. and Borchardt, R. T. (1984) Mol. Pharmacol. **25**, 418–424

26 Gibson, K. D., Wilson, J. D. and Udenfriend, S. (1961) J. Biol. Chem. **236**, 673–679

27 Hirata, F., Viveros, O. H., Diliberto, Jr., E. J. and Axelrod, J. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 1718–1721

28 Schanche, J. S., Schanche, T. and Ueland, P. M. (1981) Mol. Pharmacol. **20**, 631–636

29 Aarbakke, J. and Ueland, P. M. (1981) Mol. Pharmacol. **19**, 463–469

30 Schanche, J. S., Schanche, T. and Ueland, P. M. (1982) Biochim. Biophys. Acta **721**, 399–407

31 Cui, Z., Vance, J. E., Chen, M. H., Voelker, D. R. and Vance, D. E. (1993) J. Biol. Chem. **268**, 16655–16663

32 Lauter, C. J., Solyom, A. and Trams, E. G. (1972) Biochim. Biophys. Acta **266**, 511–523

33 Rovinski, B. and Hosein, E. A. (1983) Biochim. Biophys. Acta **735**, 407–417

34 Levy, J., Suzuki, Y., Avioli, L. V., Grunberger, G. and Gavin, J. R. (1988) Diabetologia **31**, 315–321

35 Bergelson, L. D. and Barsukov, L. I. (1977) Science **197**, 224–230

36 Vara, E., Arias-Diaz, J., Villa, N., Hernandez, J., Garcia, C., Ortiz, P. and Balibrea, J. L. (1995) Cryobiology **32**, 422–427

37 Wu, J., Söderbergh, H., Karlsson, K. and Danielsson, A. (1996) Hepatology **23**, 359–365

38 Arias-Diaz, J., Vara, E., Garcia, C., Villa, N., Rodriguez, J. M., Ortiz, P. and Balibrea, J. L. (1996) J. Surg. Res. **62**, 79–84

39 Murphy, J. T. and Spence, K. D. (1972) J. Bacteriol. **109**, 499–504