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INHIBITION OF PHOSPHOLIPID METHYLATION IN ISOLATED RAT HEPATOCYTES BY ANALOGUES OF ADENOSINE AND *S*-ADENOSYLHOMOCYSTEINE

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Phospholipid methylation in isolated hepatocytes was inhibited in the presence of 3-deazaadenosine (ID_{50} = 1.7 μM) 9-β-D-arabinofuranosyladenine (ID_{50} = 6.0 μM), *S*-tubercidinylhomocysteine (ID_{50} = 30 μM), and 5′-deoxy-5′-isobutylthioadenosine (ID_{50} = 177 μM). A transient inhibitory effect was observed with adenosine, whereas *S*-adenosyl-L-homocysteine and Sinefungin were essentially without effect. The inhibition of phospholipid methylation by *S*-tubercidinylhomocysteine and 9-β-D-arabinofuranosyladenine showed a lag-phase, whereas the effect of the other inhibitors was apparent within a few minutes. Cells exposed to 9-β-D-arabinofuranosyladenine or 3-deazaadenosine accumulated large amounts of AdoHcy, and adenosine induced a transient increase in the AdoHcy level. In addition, 3-deazaadenosine served as a precursor for the formation of *S*-3-deazaadenosylhomocysteine, which accumulated rapidly in cells exposed to this agent. The inhibitory effects of 3-deazaadenosine, 9-β-D-arabinofuranosyladenine and adenosine could be explained by the increase in total nucleosidylhomocysteine induced by these agents. In contrast, only a slight (less than 2-fold) increase in *S*-adenosyl-L-homocysteine content was observed in hepatocytes treated with 5′-deoxy-5′-isobutylthioadenosine, and this metabolic effect could not explain the inhibition of phospholipid methylation induced by this agent. None of the compounds tested reduced the amount nor the specific radioactivity of *S*-adenosylmethionine. Biological processes determining the inhibitory effects of adenosine, *S*-adenosyl-L-homocysteine and their analogues on phospholipid methylation in intact cells are discussed.

Introduction

S-Adenosyl-L-homocysteine is a product and potent inhibitor of methyltransfer reactions using AdoMet as methyldonor [1]. These enzymatic reactions are involved in the metabolism and postsynthetic modifications of numerous biological compounds, including proteins, nucleic acids, phospholipids and various low molecular weight compounds. Recently, much interest has been devoted to the physiological regulation and pharmacological inhibition of AdoMet-dependent transmethylation reactions [1–3].

Inhibition of transmethylases in intact cells can be obtained by at least three different procedures.

(1) Inhibition or inactivation of *S*-adenosyl-L-homocysteine hydrolase, the enzyme catalyzing the reversible hydrolysis of AdoHcy to adenosine and

Abbreviations: AdoHcy, *S*-adenosyl-L-homocysteine; D-AdoHcy, *S*-adenosyl-D-homocysteine; c^3AdoHcy, *S*-3-deazaadenosyl-L-homocysteine; c^7AdoHcy, *S*-7-deazaadenosyl-L-homocysteine (*S*-tubercidinylhomocysteine); SIBA, 5′-deoxy-5′-isobutylthioadenosine; ara-A, 9-β-D-arabinofuranosyladenine; c^3Ado, 3-deazaadenosine; Sinefungin, 5′-deoxy-5′-(1,4-diaminopentanoic acid) adenosine; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; ID_{50}, the dose that gives 50% inhibition.

homocysteine [1], results in cellular build-up of AdoHcy [4–7]. Accumulation of AdoHcy is also induced by directing the *S*-adenosyl-L-homocysteine hydrolase reaction towards synthesis of AdoHcy, by supplementing exogenous adenosine and/or homocysteine [8–11].

(2) Analogues of AdoHcy may be synthesized intracellularly by exposure of cells to adenosine analogues serving as substrates for *S*-adenosyl-L-homocysteine hydrolase [4,6]. 3-Deazaadenosine is an even better substrate for the enzyme than adenosine itself [12], and this compound has been widely used as an inhibitor of biological methylation [1].

(3) Numerous analogues of AdoHcy have been synthesized, and some of these are potent inhibitors of transmethylases in cell-free systems [2,3]. Few data exist on the effect of these compounds on methyltransfer reactions in intact cells.

Phospholipid methylation involves a stepwise incorporation of three methyl groups from AdoMet into phosphatidylethanolamine to form phosphatidylcholine [13]. Whether these reactions are catalyzed by one or two enzymes is a matter of some controversy [14,15]. Phospholipid methylation has been assigned a role in numerous biological processes related to membrane function and structure [14]. This fact together with the consistent findings [9,15–19] that this methylation reaction is particularly sensitive to the effect of AdoHcy, led us to investigate the effect of analogues of adenosine and AdoHcy and their natural counterparts on phospholipid methylation in intact rat hepatocytes. Compounds were tested, which are believed to exert their inhibitory effect by each of the mechanisms listed above. In the light of the data obtained, factors determining the effects of methyltransferase inhibitors in intact cells are discussed.

Materials and Methods

Chemicals

AdoHcy, D-AdoHcy, ara-A, collagenase (Type I), albumin (fraction V, defatted), Hepes, L-α-phosphatidylethanolamine dipalmitoyl, L-α-phosphatidyl-*N*,*N*-dimethylethanolamine dipalmitoyl and phosphatidylcholine dipalmitoyl were obtained from Sigma Chemical Co., St. Louis, MO and L-α-phosphatidyl-*N*-monomethylethanolamine dipalmitoyl from Calbiochem-Behring Corporation, La Jolla, CA. c^3Ado was kindly supplied by Dr. J. Montgomery, Southern Research Institute, Birmingham, AL. The following reagents were gifts from the producers: Sinefungin (Lilly Laboratories, IN), SIBA (Dr. E. Lederer, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France) and c^7AdoHcy (Dr. J. Coward, Rensselaer Polytechnic Institute, Troy, NY). c^3AdoHcy was prepared from c^3Ado according to an enzymatic method published elsewhere [18]. L-[*methyl*-^{3}H]methionine (93 Ci/mmol) and *S*-adenosyl-L-[*methyl*-^{3}H]methionine (74 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, U.K. ODS Hypersil 3 μm microparticle material for reverse-phase chromatography was purchased from Shandon Southern Products Ltd, Cheshire, U.K., and Partisil 10 SCX microparticular material for cation exchange chromatography from HPLC Technology Ltd, Cheshire, U.K. ODS Hypersil columns (10 × 0.46 cm) and Partisil 10 SCX columns (25 × 0.46 cm) were packed at our institute using a Shandon column packer. The ODS Hypersil columns were equipped with 1.5 cm guard columns (Shandon) packed with 40 μm Pelliguard LC-18 (Supelco, Inc.).

Preparation and incubation of isolated rat hepatocytes

Hepatocytes were prepared by a slight modification [20] of the collagenase perfusion method described by Berry and Friend [21]. The cells were incubated in an isotonic solution containing salts, glucose, bovine serum albumin and antibiotics [20]. The temperature was 37°C. Cell viability was higher than 95% at the start of the experiments.

Preparation of rat liver microsomes

This was carried out by a procedure involving homogenisation of rat liver in an isotonic sucrose medium, followed by fractionation of the homogenate by differential centrifugation and sedimentation in a discontinuous sucrose gradient [22]. The specific activity of NADPH cytochrome *c* reductase in the preparation of microsomes (510 nmol/min per mg protein) was about 5-fold higher than in the liver homogenate (97 nmol/min per mg).

Determination of [^{3}H]methyl incorporation into phospholipids of rat hepatocytes

Hepatocytes ($3–7 \cdot 10^6$ cells/ml, 3–6 ml) were incubated in 10–25 ml Erlenmeyer flasks in a shaking water bath. The cell suspensions were supplemented with 50 μM [*methyl*-^{3}H]methionine (62.5 mCi/mmol). The incubation was terminated by centrifugation of samples (0.5 ml) for 15 s at $9000 \times g$, using a Beckman microfuge B. The pellets obtained were homogenized in 1 ml 1.2 M HCl, the precipitate collected on glassfiber filters, which were washed with acid and extracted with 1-butanol as described [18]. An aliquot of the butanol phase was dried and total phospholipid radioactivity determined by liquid scintillation counting.

Assay for phospholipid methyltransferase activity

The assay is based on the determination of [^{3}H]methyl groups incorporated into exogenous phospholipids and phospholipids in the microsomes in the presence of [^{3}H]AdoMet. The exogenous lipid substrates were prepared by suspending L-α-phosphatidyl-*N*-monomethylethanolamine (4 mg/ml) and L-α-phosphatidyl-*N*,*N*-dimethylethanolamine (4 mg/ml) in 0.06% Triton X-100 in 25 mM Tris-glycine, pH 7.4, by sonication at 35°C for 20 min. This suspension was diluted 4-fold in the incubation mixture. The incubation mixture contained 1 mM $MgCl_2$, 50 μM [^{3}H]AdoMet, exogenous phospholipids (1 mg/ml of each) and microsomes (1.5 mg microsomal protein/ml) in 25 mM Tris/glycine buffer, pH 7.4. The temperature was 37°C. The reaction was terminated by addition of samples of 100 μl to 1 ml of 1.2 M HCl, and the radioactive phospholipids were extracted and determined as described in the preceding paragraph. The specific activity of the phospholipid methyltransferase in the microsomes was 230 pmol/mg protein per min.

Identification of radioactive phospholipids

The identification and quantitation of radioactive phospholipids were carried out according to a modification of a published procedure [18]. Briefly, the incubation and extraction of rat hepatocytes and microsomes was done as described above. An aliquot of the butanol-phase was dried under a stream of N_2 and dissolved in 100 μl chloroform containing 0.25 mg/ml of the authentic phospholipid standards. This was applied to silica plates (Silica Gel 60 plates, E. Merck, Darmstadt, F.R.G.). using an Analytical Instrument Specialities thin-layer chromatography multispotter. The plates were developed in propionic acid/1-propanol/chloroform/water (2 : 2 : 1 : 1). The spots were visualized by exposure to iodine vapour. After visualization the plates were scraped in 0.5 cm bands and eluted with 1 ml chloroform. Radioactivity was determined by liquid scintillation counting. In hepatocytes more than 90% of the radioactive lipids chromatographed as phosphatidylcholine (Fig. 1, panel A). In isolated microsomes,

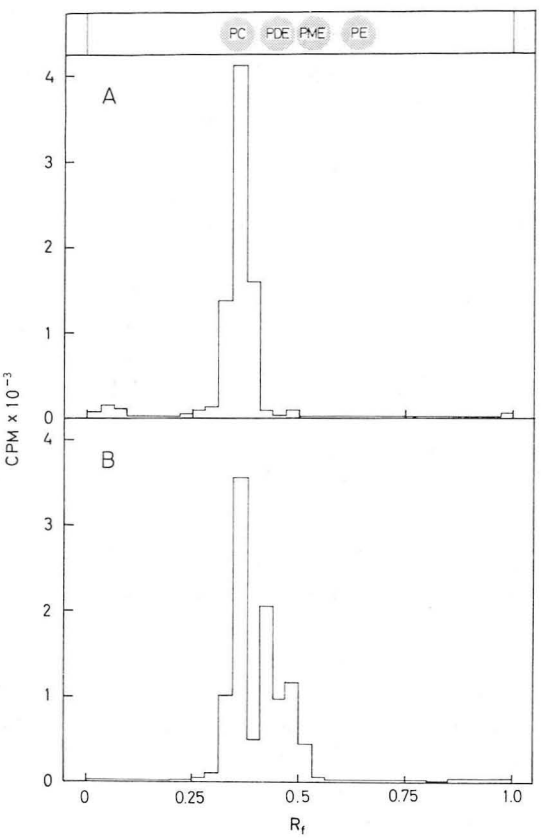

Fig. 1. Thin-layer chromatogram of radioactive methylated phospholipids from hepatocytes (A) or microsomes (B). Butanol-extracts from hepatocytes incubated with [^{3}H]methionine for 90 min or microsomes incubated with [^{3}H]AdoMet for 20 min were subjected to thin-layer chromatography on Silica gel plates. PC, phosphatidylcholine; PDE, phosphatidyl-*N*′,*N*-dimethylethanolamine; PME, phosphatidyl-*N*-monomethylethanolamine; PE, phosphatidylethanolamine.

radioactivity was more evenly distributed among the methylated species (Fig. 1, panel B).

Determination of cellular content of AdoHcy, c^3AdoHcy and AdoMet

Cell pellets containing $2–3 \cdot 10^6$ cells were extracted with 0.8 M $HClO_4$ (100 μl). The cellular extracts were centrifuged for 5 min at $100\,000 \times g$ using a Beckman Airfuge. The supernatants were stored at −80°C and analyzed within 48 h. AdoHcy, c^3AdoHcy and AdoMet were determined by HPLC using a Spectra-Physics SP 8000B Liquid chromatograph, a Partisil 10 SCX column, and a model SP 8300 ultraviolet detector, recording the absorbance at 254 nm. The column was eluted isocratically at ambient temperature at a flow rate of 1.5 ml/min in a constant pressure mode. The mobile phases were 40, 60 or 500 mM ammonium formate, pH 3.5, supplemented with 1% 1-propanol, for the determination of AdoHcy, c^3AdoHcy or AdoMet, respectively. AdoHcy was also determined by analysis of the perchloric acid extract, neutralized to pH 6.0 by addition of 0.36 M KOH/0.30 M $KHCO_3$, followed by removal of precipitated potassium perchlorate, on a 3 μm ODS Hypersil column. The same liquid chromatograph and detector were used as above. The column was eluted isocratically at a flow rate of 2 ml/min in a constant pressure mode. The mobile phase was 7% methanol in 10 mM potassium phosphate buffer, pH 6.0. AdoHcy eluted with a retention time of 3.63 min.

Determination of [^{3}H]methyl group incorporation into AdoMet

Perchloric acid extracts of hepatocytes exposed to [^{3}H]methionine was analyzed on the cation exchange column, as described above for the determination of AdoMet. The effluent was fractionated and the radioactivity in the fractions determined by liquid scintillation counting. A radioactive peak was observed which co-chromatographed exactly with AdoMet (data not shown). For routine analysis, the effluent corresponding to the retention time of AdoMet was collected, lyophilized, and the radioactivity determined by liquid scintillation counting. The relative specific activity of AdoMet in hepatocytes was determined by dividing the amount of radioactivity by the amount of AdoMet in the sample.

Results

Time course for the incorporation of [^{3}H]methyl groups from [^{3}H]methionine into phospholipids and AdoMet. The progress curve for the incorporation of radioactivity into phospholipids of hepatocytes incubated with [^{3}H]methionine was almost linear for at least 90 min incubation. In contrast, the incorporation of radioactivity into AdoMet increased for about 10 min, and then levelled off (Fig. 2). In the light of these results, hepatocytes were incubated for 10 min with [^{3}H]methionine before the addition of potential transmethylase inhibitors to the cell suspension.

Time course for the inhibition of phospholipid methylation. The progress curves for phospholipid methylation in the presence of various potential inhibitors were determined. The initial part of the curves obtained with ara-A and c^7AdoHcy followed the time course observed in the absence of inhibitor for some minutes, and the levelled off. Thus, the inhibition of phospholipid methylation was observed after a lag-phase. In contrast, the inhibitory effect of SIBA, c^3Ado and Ado was apparent after a few minutes of exposure of the hepatocytes to these agents (Fig. 3).

Dose-response curves for various inhibitors. c^3Ado ($ID_{50} = 1.7$ μM) was the most potent inhibitor of phospholipid methylation in rat hepatocytes (determined 60 min after addition of the inhibitor), followed by ara-A ($ID_{50} = 6.0$ μM), c^7AdoHcy ($ID_{50} = 30$ μM) and SIBA ($ID_{50} = 177$ μM). Adenosine was the weakest inhibitor when the degree of inhibition was determined 60 min after addition of adenosine to the cell suspension (Fig. 4). However, the inhibitory effect of adenosine was transient (Fig. 3), and this was particularly pronounced at low concentrations of this nucleoside. No inhibitory effect on phospholipid methylation was observed in the presence of Sinefungin, D-AdoHcy or AdoHcy (data not shown).

The same compounds or their metabolic products were tested as inhibitors of phospholipid methylation in a cell-free system. Physiological pH (7.4) and AdoMet concentrations (50 μM) were used. Phospholipid methylation was determined in the microsomal fraction of rat liver homogenate, because this fraction has been reported to be rich in phospholipid methyltransferase [13,23].

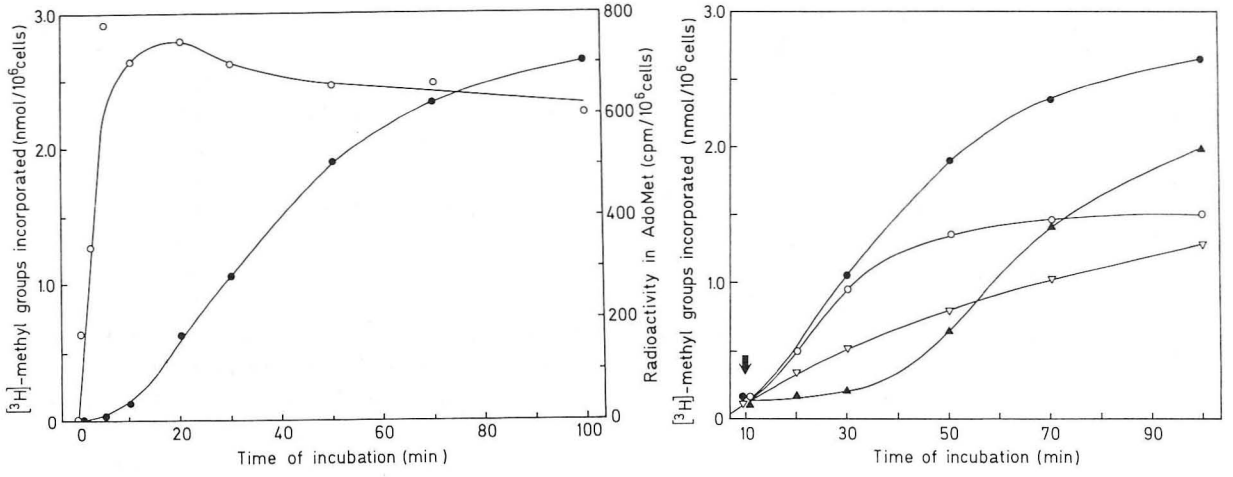

Fig. 2. Incorporation of [^{3}H]methyl groups from [^{3}H]methionine into AdoMet and phospholipids. The incorporation of [^{3}H]methyl groups into AdoMet (○——○) and phospholipids (●——●) of hepatocytes ($6 \cdot 10^6$ cells/ml) incubated in the presence of [^{3}H]methionine (50 μM) was determined.

Fig. 3. Progress curves for phospholipid methylation in hepatocytes exposed to various transmethylase inhibitors. Rat hepatocytes ($6 \cdot 10^6$ cells/ml) were incubated with 50 μM [^{3}H]methionine. After 10 min incubation, the cell suspensions were supplemented with (arrow); 500 μM Ado (▲——▲) or 5 μM ara-A (○——○) or 1.5 μM c^{3}Ado (▽——▽) or no inhibitor (●——●, control).

c^{3}AdoHcy was the most potent inhibitor of the phospholipid methyltransferase(s) in rat liver microsomes, followed by c^{7}AdoHcy = AdoHcy, D-AdoHcy and Sinefungin with decreasing efficiency in the order mentioned (Fig. 5). ara-A, adenosine c^{3}Ado and SIBA were without effect (data not shown).

Cellular content of AdoHcy and c^{3}AdoHcy. ara-A

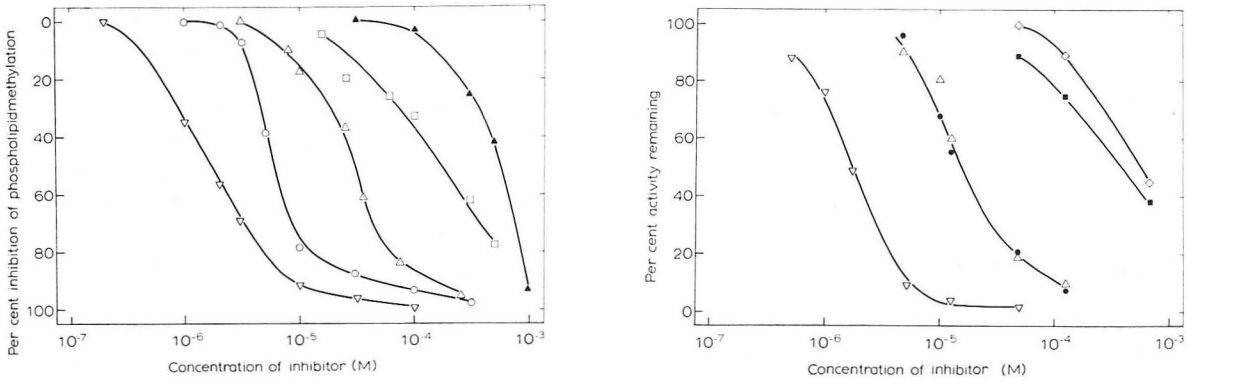

Fig. 4. Dose-response curves for inhibition of phospholipid methylation in isolated rat hepatocytes. Rat hepatocytes ($6 \cdot 10^6$ cells/ml) were exposed to 50 μM [^{3}H]methionine for 10 min, and at this time point one of the following potential inhibitors was added to the cell suspension: c^{3}Ado (▽——▽), ara-A (○——○), c^{7}AdoHcy (△——△), SIBA (□——□) and Ado (▲——▲). Phospholipid methylation was determined 60 min after addition of inhibitor.

Fig. 5. Dose-response curves for the inhibition of phospholipid methyltransferase activity in rat liver microsomes. The initial velocity of phospholipid methylation in rat liver microsomes incubated with 50 μM [^{3}H]AdoMet was determined in the presence of increasing concentrations of c^{3}AdoHcy (▽——▽) or AdoHcy (●——●) or c^{7}AdoHcy (△——△) or D-AdoHcy (■——■) or Sinefungin (◇——◇).

induced, in a dose-dependent manner, a massive accumulation of AdoHcy, which levelled off after about 1 h. The cellular build-up of AdoHcy in the presence of c^3Ado was less pronounced, but this adenosine analogue induced an accumulation of c^3AdoHcy. A transient increase in the AdoHcy level in hepatocytes exposed to adenosine was observed. Even when high concentrations (up to 1 mM) of adenosine were added to the cell suspension, the AdoHcy content in the cells reached the pretreatment level within 1 h. The cellular content of AdoHcy in cells exposed to SIBA increased less than 2-fold (Fig. 6). Concentration of SIBA (150 μM), which exerted a marked inhibitory effect of phospholipid methylation (Fig. 4), induced a minimal elevation of AdoHcy content (Fig. 6).

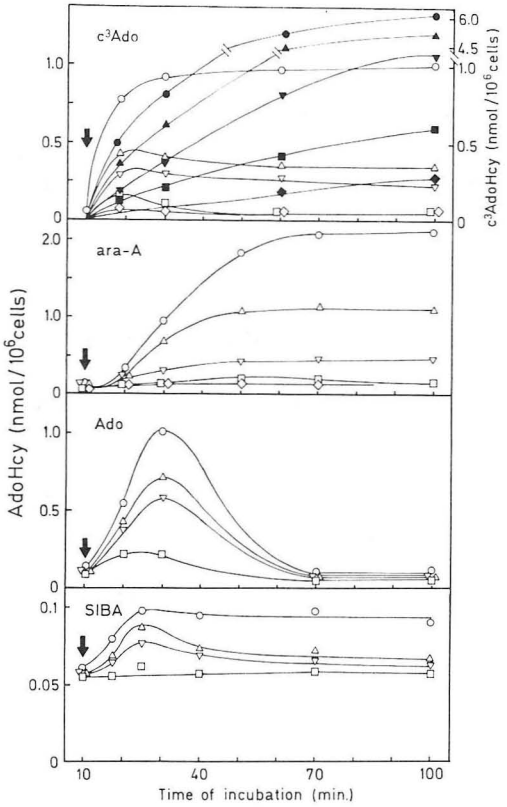

Fig. 6. Accumulation of AdoHcy and c^3AdoHcy in hepatocytes exposed to various transmethylase inhibitors. Rat hepatocytes ($6 \cdot 10^6$ cells/ml) were incubated with 50 μM methionine for 10 min. At this time point (arrow) the cell suspensions were supplemented with c^3Ado (1 μM, ◇; 3 μM, □; 30 μM, ▽; 100 μM, △; 300 μM, ○) or ara-A (3 μM, ◇; 12 μM, □; 25 μM, ▽; 50 μM, △; 100 μM, ○) or Ado (100 μM, □; 300 μM, ▽; 0.5 mM, △; 1 mM, ○) or SIBA (0, □; 150 μM, ▽; 300 μM, △; 600 μM, ○). Open symbols show the amount of cellular AdoHcy, and filled symbols the amount of c^3AdoHcy in cells exposed to c^3Ado.

Test for effect of transmethylase inhibitors on cellular content of AdoMet and specific radioactivity of this compound. The possibility existed that the decreased formation of radioactive phospholipids in the presence of transmethylase inhibitors was caused by interference with cellular disposition of [^{3}H]methionine or the synthesis of [^{3}H]AdoMet. To exclude this possibility the amount of AdoMet and the specific radioactivity of the methyldonor was determined in cells exposed to potential inhibitors. Compounds exerting an inhibitory effect (ara-A, c^3Ado, c^7AdoHcy, SIBA and high doses of adenosine) elevated the cellular level of AdoMet by less than 100%, and slightly increased (by less than 40%) the specific radioactivity of AdoMet. There is a correlation between this effect and inhibition of phospholipid methylation (data not shown), suggesting that these transmethylase inhibitors reduce the consumption of AdoMet.

Discussion

The transmethylase inhibitors tested in this work can be divided into two classes. (1) AdoHcy and some of its analogues are potent inhibitors of phospholipid methyltransferase(s) in cell-free systems (Fig. 5), but are relatively ineffective when added to intact rat hepatocytes. (2) Adenosine and adenosine analogues act via *S*-adenosyl-L-homocysteine hydrolase, and are without effect in broken cell preparations, but some of these compounds exert a pronounced inhibitory effect on phospholipid methylation in intact hepatocytes (Fig. 4). Thus, several biological processes mediate or modulate the inhibitory effects of these compounds on methyltransfer reactions in the intact cells, and this subject will be discussed below.

The inhibitory effect of various compounds used was tested on phospholipid methyltransferase(s) in rat liver microsomes (Fig. 5), because this subcellular fraction is particularly rich in this enzyme activity [23]. Somewhat unexpectedly, we observed that the effect of AdoHcy and its analogues on the enzyme(s) in microsomes (Fig. 5) differed from the effect on the enzyme(s) in purified plasma mem-

brane [18]. c^3AdoHcy is far the most potent inhibitor of the enzyme(s) in microsomes (Fig. 5) but is less effective than AdoHcy against the enzyme(s) in plasma membranes [18]. This finding was confirmed when the inhibitory effect of these compounds was determined with phospholipid methyltransferase(s) from these two sources under identical experimental conditions (unpublished data). Whether the difference is related to different properties of the enzyme(s) from microsomes and plasma membranes, or to modification of the enzyme(s) during preparation of the subcellular fractions, remains to be established.

The natural transmethylase inhibitor, AdoHcy, is a potent inhibitor of isolated phospholipid methyltransferase(s) (Refs. 15–19, Fig. 5), but exogenous AdoHcy is not an inhibitor of phospholipid methylation in intact rat hepatocytes (Fig. 4). This observation is in accordance with data provided by others, showing that exogenous AdoHcy does not inhibit methyltransfer reactions in intact cells [24–26]. Lack of effect of AdoHcy has been explained by extensive metabolism of AdoHcy by cells [27,28]. Furthermore, AdoHcy is not taken up by cells [29,30], including rat hepatocytes [31]. This can be explained by impermeability of the cell membrane to AdoHcy [32] or by export of this thio-ether compound by the cells [7].

AdoHcy analogues like Sinefungin (structure given in Ref. 18) and D-AdoHcy are essentially ineffective inhibitors of phospholipid methylation in intact rat hepatocytes, but are inhibitors of this enzymatic process in microsomes (Fig. 5) and plasma membranes [18] from rat liver. Lack of effect of Sinefungin should be related to the recent findings that Sinefungin is not an inhibitor of protein carboxymethylation in intact rat synaptosomes [33], but is a potent inhibitor of the isolated protein carboxymethyltransferase [34] and the enzyme in lyzed synaptosomes [33]. Extensive metabolism of Sinefungin by the synaptosomes could not be demonstrated [33]. Thus, cellular transport and disposition of Sinefungin and related compounds may be an important factor determining their effectiveness in vivo.

c^7AdoHcy may be a useful compound for studying the disposition of extracellular AdoHcy analogues and their effect on cellular transmethylation reactions. This AdoHcy analogue is a metabolically stable compound, which is not a substrate for *S*-adenosyl-L-homocysteine hydrolase. Furthermore, c^7AdoHcy is a potent inhibitor of numerous transmethylases in vitro [35], including phospholipid methyltransferase(s) (Fig. 5). The inhibitory effect of c^7AdoHcy on phospholipid methylation in vivo was observed after a lag-phase. This could be explained by a slow transport of this compound from the extracellular compartment to the intracellular compartment. However, the observation that c^7AdoHcy nearly completely blocks phospholipid methylation in rat hepatocytes (Fig. 4) and inhibits some transmethylation reactions in cultured cells [24,25] show that c^7AdoHcy is taken up by cells to reach the intracellular target enzymes. This is in accordance with the hypothesis of Trewyn and Kerr, suggesting that some AdoHcy analogues cross the membrane [32].

SIBA inhibits phospholipid methylation in rat hepatocytes (Fig. 4), but is not an inhibitor of phospholipid methyltransferase(s) in rat liver plasma membranes [18] and microsomes (Fig. 5). This finding is in agreement with the observation that SIBA is a weak inhibitor of methyltransferases in cell-free systems, but inhibits methyl transfer reactions in intact cells [3,35].

The cellular content of AdoHcy in hepatocytes increased only slightly in cells exposed to high concentrations of SIBA, which is in agreement with the observation that SIBA is a weak inhibitor of *S*-adenosyl-L-homocysteine hydrolase [3]. However, inhibition of phospholipid methylation by SIBA was observed at concentrations of this compound inducing no detectable increase in cellular AdoHcy content (Figs. 4 and 6). This suggests that SIBA inhibits phospholipid methylation in rat hepatocytes by mechanisms not involving AdoHcy. SIBA induces accumulation of 5′-deoxy-5′-methylthio adenosine in rat hepatocytes [36], but this thio-ether compound is not an inhibitor of phospholipid methyltransferase(s) from rat liver (unpublished data). Thus the mode of action of SIBA on phospholipid methylation and on other methyltransfer reactions in cells remains obscure.

c^3Ado is the most potent inhibitor of phospholipid methylation among the compounds tested, and essentially no lag-phase was observed for the inhibitory effect (Figs. 3 and 4). This should be

related to a rapid increase in AdoHcy content in cells exposed to c^3Ado caused by inhibition of AdoHcy hydrolase by this adenosine analogue [1]. Furthermore, intracellular synthesis of c^3AdoHcy from c^3Ado catalyzed by *S*-adenosyl-L-homocysteine hydrolase [1,12] contributes to the effect of c^3Ado. c^3AdoHcy is a potent inhibitor of phospholipid methyltransferase(s) (Ref. 18, Fig. 5). Besides, c^3Ado is a metabolically stable compound which is not a substrate for adenosine kinase and adenosine deaminase [1], and this ensures a long-lasting effect of c^3Ado.

ara-A inactivates AdoHcy hydrolase in rat hepatocytes at a rate of 0.7 min^{-1} at saturating concentrations of inactivator [7]. Thus, the inhibition of AdoHcy catabolism by ara-A is a time-dependent process, which explains the observation that the accumulation of AdoHcy (Ref 7, Fig. 6) and the inhibitory effect on phospholipid methylation requires about 5 min of cellular exposure to ara-A before the effect is observed (Figs. 3 and 4). The amount of AdoHcy in hepatocytes treated with ara-A decreases slightly after prolonged incubation (Fig. 6). This may be explained by egress of AdoHcy from the cells [7] and/or reactivation of AdoHcy hydrolase [37].

Adenosine is an equally effective inhibitor and substrate of *S*-adenosyl-L-homocysteine hydrolase as c^3Ado [1] but this naturally occurring compound is rapidly deaminated and phosphorylated in the intact cell [38]. Therefore, adenosine induces only a transient increase in the cellular AdoHcy content (Fig. 6), and the inhibition of phospholipid methylation even at high concentrations ($>$ 0.5 mM) of adenosine is overcome within 1 h (Fig. 3). Thus, metabolic consumption of adenosine and its analogues functioning as inhibitors or substrates for AdoHcy hydrolase, may be a major factor limiting their effect on methyl transfer reactions in intact cells.

In conclusion, AdoHcy and some of its analogues are potent inhibitors of methyltransferases in cell-free systems, but low permeability of the cell membrane to these compounds may reduce their inhibitory effect in vivo. In contrast, adenosine and adenosine analogues rapidly enter the intracellular compartment, and these compounds are probably taken up by the nucleoside transport system [39]. Inactivators of *S*-adenosyl-L-homocysteine hydrolase may exert a prolonged inhibitory effect on biological methylation, although they may be rapidly degraded in the cells. Metabolic consumption of nucleosides serving as substrates or reversible inhibitors of *S*-adenosyl-L-homocysteine hydrolase seems to be a major factor limiting their effectiveness as transmethylase inhibitors in the intact cells.

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