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Interaction of S-Adenosylhomocysteine with Isolated Rat Hepatocytes

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SUMMARY

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The time course of the association of radioactivity with isolated hepatocytes incubated in the presence of *S*-[^{14}C]adenosylhomocysteine was characterized by an initial burst phase lasting for less than 15 sec (Phase I) followed by a slow linear phase (Phase II). Phase I was due to association of *S*-[^{14}C]adenosylhomocysteine with the hepatocytes. The possibility of intracellular resynthesis of *S*-[^{14}C]adenosylhomocysteine from [^{14}C]adenosine was ruled out. The process showed slight temperature dependence, and there was no effect of 2,4-dinitrophenol. These data argue against the possibility that Phase I was related to active uptake of *S*-[^{14}C]adenosylhomocysteine, but rather suggest binding of this compound to an acceptor on the cell surface. Competition studies showed that *S*-adenosylhomocysteine was the preferred ligand of this acceptor. Phase II corresponded to intracellular accumulation of ^{14}C-labeled adenine nucleotides and was nearly completely abolished by inhibitors of enzymatic hydrolysis of *S*-adenosylhomocysteine, such as adenosine and homocysteine. This process was explained by extracellular hydrolysis of *S*-[^{14}C]adenosylhomocysteine catalyzed by *S*-adenosylhomocysteine hydrolase leaking out of the cells, followed by rapid uptake of [^{14}C]adenosine. Trapping of adenosine formed from *S*-adenosylhomocysteine directed the extracellular enzyme catalysis towards hydrolysis. Extracellular hydrolysis of *S*-adenosylhomocysteine was the rate-limiting step in this sequence of events. It is concluded that *S*-adenosylhomocysteine was not taken up by rat hepatocytes as an intact molecule.

INTRODUCTION

AdoHcy[1] is both a product from (1) and a potent inhibitor of a number of transmethylation reactions using AdoMet as a methyl donor (2–5). The inhibition of methyl transfer reactions by AdoHcy may be a factor in the control of cellular methylation reactions (2–5).

Some synthetic analogues of AdoHcy are inhibitors of enzymes catalyzing AdoMet-dependent transmethylations (6–9). This may be the molecular basis of the biological effects of these agents (6–9), which implies that these compounds cross the cell membrane to reach their target enzymes.

Few data exist on cellular transport of AdoHcy and its structural analogues. It has been stated that cells are impermeable to AdoHcy (10), and that this metabolite does not penetrate cells in culture whereas its analogues do (11). AdoHcy is metabolized by whole cells, but extracellular hydrolysis of AdoHcy to adenosine and homocysteine made it difficult to assess whether AdoHcy crosses the cell membrane as an intact molecule (12). The metabolic precursor of AdoHcy, AdoMet, is taken up by isolated rat liver by what seems to be a carrier-mediated process (13). Cellular transport of AdoMet has also been studied in erythrocytes (14) and in yeast (15, 16).

This study was undertaken to investigate the disposition of extracellular AdoHcy by isolated rat hepatocytes. Two processes could be distinguished: association of [^{14}C]AdoHcy with the cells consistent with specific binding of [^{14}C]AdoHcy to the cell surface, and [^{14}C]AdoHcy was hydrolyzed by the enzyme *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1) in the extracellular medium and [^{14}C]adenosine was taken up by the cells. [^{14}C]AdoHcy was not transported into the cells as an intact molecule.

MATERIALS AND METHODS

Chemicals. AdoHcy, *S*-adenosyl-D-homocysteine, adenosine, inosine, adenine, DL-homocysteine, L-homocysteine thiolactone, thymidine, cytidine, uridine, amino acids, collagenase (Type I), albumin (Fraction V, defatted), and Hepes were obtained from Sigma Chemical Company, St. Louis, Mo. L-Homocysteine was prepared

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[1] The abbreviations used are AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; Hepes, 4(2-hydroxyethyl)-2-piperazineethanesulfonic acid; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; SIBA, 5′-deoxy-5′-isobutyl-thioadenosine; PEI-cellulose, polyethyleneimine-cellulose; ara-A, arabineadenosine.

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from L-homocysteine thiolactone by treatment of this compound with 5 N NaOH for 5 min at room temperature (17). AdoMet was purchased from Koch-Light Laboratories, London, England. The following reagents were gifts from the producers: EHNA, Burroughs Wellcome Company, Research Triangle Park, N. C.; dipyridamole, Boehringer Mannheim, Mannheim, Federal Republic of Germany; Sinefungin, Lilly Laboratories, Indianapolis, Ind.; and SIBA, provided by Dr. E. Lederer, Centre National de la Recherche Scientifique, Gif sur Yvette, France). [8-^{14}C]Adenosine (59 mCi/mmole) was a product of the Radiochemical Centre, Amersham, England, and *S*-[8-^{14}C]adenosylhomocysteine (59 mCi/mmole) was synthesized enzymatically and purified as described elsewhere (18). PEI-cellulose thin-layer sheets (0.25 mm) on glass plates (20 × 20 cm) were prepared as described by Randerath and Randerath (19).

Preparation of isolated hepatocytes. Liver cells were prepared essentially by the method of Berry and Friend (20). Following collagenase perfusion, the livers were perfused for 1 min with a collagenase-free solution at 20°. Hepatocytes were separated from nonparenchymal cells by centrifugation (20), and the cells were washed twice with the incubation medium.

Viability of cells. Cell viability and cell counts were determined as in ref. 20. Storage of the cells for 1.5 hr at 0° did not decrease significantly cell viability. The viability was 92 ± 7% (mean ± standard deviation) at the start of the experiments, and was always higher than 80% at the end of the incubations (except after incubation of the cells with 1 mM dinitrophenol, when the viability was about 50%).

Incubation conditions. Freshly isolated hepatocytes were incubated in a solution containing 137 mM NaCl, 5.37 mM KCl, 0.81 mM $MgSO_4$, 4 mM $CaCl_2$, 0.34 mM Na_2HPO_4, 0.35 mM KH_2PO_4, 2% bovine serum albumin, and 20 mM Hepes. The solution was adjusted to pH 7.4 at 37° by addition of 0.1 N NaOH (20). EHNA (10 μM) was included in the incubation mixture when not otherwise indicated. Hepatocytes (2.0 ml, 2×10^6–5×10^6 cells/ml) were pipetted into 10-ml Erlenmeyer flasks which were placed on ice for less than 1.5 hr until the start of the experiment. The incubation was performed at 37°.

Thin-layer chromatography. AdoHcy and its metabolites were separated on PEI-cellulose plates. Two solvent systems were used. The chromatograms were developed in glacial acetic acid/water/1-butanol (1:1:2) as described by Fain and Shepherd (21). AdoHcy (R_f = 0.40), adenosine (R_f = 0.74), adenine (R_f = 0.82), inosine (R_f = 0.45), hypoxanthine (R_f = 0.61), xanthine (R_f = 0.49), uric acid (R_f = 0.36), allantoin (R_f = 0.35), AMP (R_f = 0.05), and ADP (R_f = 0) were separated in this system. AdoHcy (R_f = 0.15), adenosine (R_f = 0.52), adenine (R_f = 0.61), inosine (R_f = 0.37), hypoxanthine (R_f = 0.52), uric acid (R_f = 0.10), and adenine nucleotides (R_f = 0) were also separated on PEI-cellulose plates using isobutanol/ethanol/water (2:1:1) as solvent.

Uptake experiments. Suspensions of hepatocytes (2 ml) were incubated for 10 min at 37°. The incubation was started by the addition of 0.5 ml containing [^{14}C]AdoHcy or [^{14}C]adenosine and potential effectors. Samples of 250 μl were removed from the incubation mixture at various times, layered onto a mixture (100 μl) of dinonyl phthalate and dibutyl phthalate (1:3) in 400-μl polyethylene microcentrifuge tubes, and centrifuged at 7000 rpm for 15 sec in a Beckman 152 microfuge. The cells were separated from the medium in less than 10 sec. The bottom of the tubes, containing the cell pellet, was cut off, wiped dry of oil, and placed in a scintillation vial containing 1 ml of 2% sodium dodecyl sulfate. After 18 hr at 23° (overnight), 6 ml of Dilusolve (Packard) were added.

Determination of AdoHcy and its metabolites associated with the cell. The experimental design was similar to that described for the uptake experiments except that samples of the incubation mixture were placed in 1500-μl tubes which were layered with 60 μl of 0.8 N perchloric acid in the bottom of the tubes and 200 μl of oil above the acid. The cells were centrifuged into the perchloric acid, the supernatant and oil were removed by suction, and the pellet obtained was resuspended in the acid. The perchloric acid was neutralized by the addition of 80 μl of 0.36 M KOH containing 0.30 M $KHCO_3$. The solution was left at 0° for 30 min to allow precipitation of perchloric acid. Samples from the solution were then subjected to thin-layer chromatography in the systems described above. The values obtained for the association of radioactive metabolites with the cells were not corrected for radioactivity due to adherent extracellular water.

Measurement of enzyme activities. Lactate dehydrogenase activity was determined by recording the decrease in optical density at 340 nM resulting from oxidation of NADH in the presence of pyruvate and enzyme (22). *S*-Adenosylhomocysteine hydrolase activity was determined in the extracellular medium (from which the cells were removed by centrifugation) by making the medium 1 μM in [^{14}C]AdoHcy and 50 units/ml in adenosine deaminase. The reaction was terminated and the reaction products were separated as described previously (18).

RESULTS

Association of Radioactive Metabolites with Rat Hepatocytes Incubated with [^{14}C]AdoHcy

The time course of the association of radioactivity with hepatocytes incubated at 37° with [^{14}C]AdoHcy (1 μM) was characterized by an initial burst phase (Phase I) lasting for less than 15 sec followed by a slow linear phase (Phase II). The curve then leveled off (Fig. 1A). Essentially the same results were obtained over a wide concentration range (0.2–50 μM) of [^{14}C]AdoHcy (data not shown).

In another experiment the amount of [^{14}C]AdoHcy and its metabolites associated with the cells incubated at 37° for various periods of time with [^{14}C]AdoHcy was determined by centrifugation of the cells into perchloric acid (see Materials and Methods for details) and analysis of the cell extract by thin-layer chromatography. The rapid initial phase (Phase I, Fig. 1A) corresponded to a rapid association of [^{14}C]AdoHcy with the hepatocytes. During incubation, the amount of [^{14}C]AdoHcy gradually declined (Fig. 1B). The slow phase of association of radioactivity with the cells (Phase II, Fig. 1A) paralleled a time-dependent accumulation of adenine nucleotides

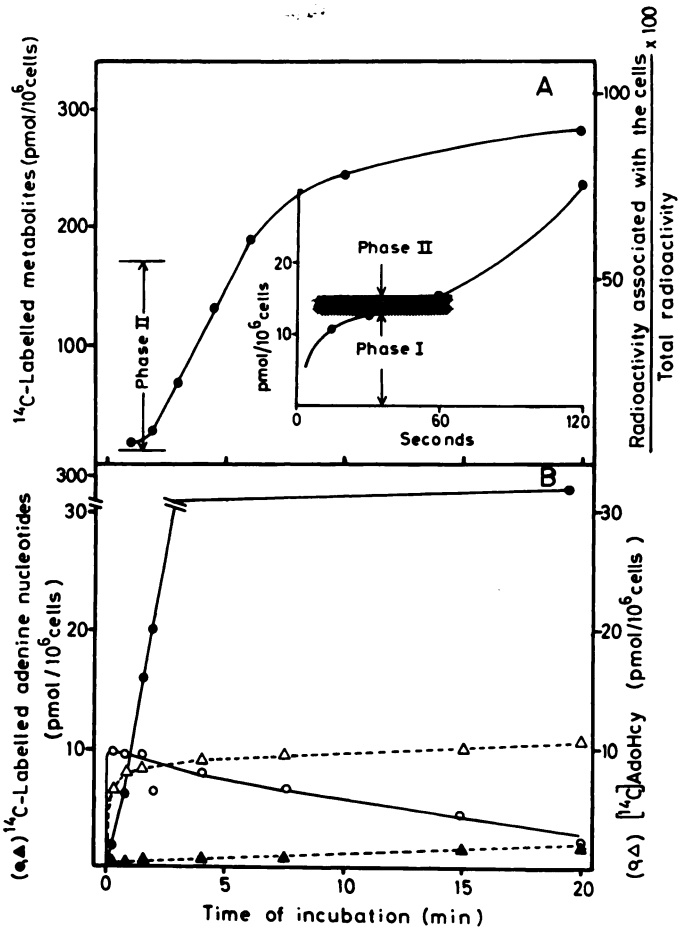

FIG. 1. *Progress curves for the association of radioactive metabolites with rat liver cells incubated with [^{14}C]AdoHcy*

A. Isolated rat liver cells (3.2 × 10^6/ml) were incubated at 37° with 1 μM [^{14}C]AdoHcy (59 mCi/mmole). At times indicated, cells were separated from the medium by centrifugation as described under Materials and Methods, and the total amount of radioactivity associated with the cells was determined. The amount of ^{14}C-labeled metabolites recovered in the cell pellet is plotted versus time of incubation. *Inset* is a replot of data obtained at the short time of incubation. Two components of the progress curve are defined, Phase I and Phase II.

B. Cells (5.3 × 10^6/ml) were incubated with 1 μM [^{14}C]AdoHcy (59 mCi/mmole). The temperature was 37° (●, ○) or 0° (▲, △). At times indicated, cells were separated from the medium and centrifuged into perchloric acid as described under Materials and Methods. The acid-soluble extract from the cells was analyzed by thin-layer chromatography. The amount of [^{14}C]AdoHcy (○, △) or adenine nucleotides (●, ▲) associated with the cells is plotted versus time of incubation.

within the hepatocytes (Fig. 1B). Less than 5% of the total cellular radioactivity chromatographed as adenosine at any time of incubation under the conditions described in the legend to Fig. 1B (data not shown).

Characterization of the Association of [^{14}C]AdoHcy with Hepatocytes (Phase I).

Test for intracellular synthesis of [^{14}C]AdoHcy. The possibility existed that [^{14}C]AdoHcy was hydrolyzed to [^{14}C]adenosine in the extracellular medium and that [^{14}C]adenosine was taken up by the cells and reincorporated into [^{14}C]AdoHcy. This possibility was tested by the following experiments. Unlabeled AdoHcy (1 μM) was incubated with the liver cells under the conditions described in the legend to Fig. 1B. After 2 min of incubation, the incubation mixture was made 1 μM in [^{14}C]adenosine. Formation of [^{14}C]AdoHcy associated with the cells could not be demonstrated. In another experiment, [^{14}C]-adenosine (1 μM) was incubated with liver cells in the absence and presence of 2 μM DL-homocysteine. The experimental design was otherwise as described in the legend to Fig. 1B. No cell-associated [^{14}C]AdoHcy could be demonstrated (data not shown). These data showed that association of [^{14}C]AdoHcy with the hepatocytes could not be explained by intracellular synthesis of [^{14}C]AdoHcy.

Effect of temperature and metabolic inhibitor. The association of [^{14}C]AdoHcy with hepatocytes incubated with 1 μM [^{14}C]AdoHcy at 0° was compared with the curve obtained at 37° (Fig. 1B). The progress curve at 0° was characterized by an initial burst phase followed by a slow increase in the amount of [^{14}C]AdoHcy. After about 10 min of incubation the cell-associated [^{14}C]AdoHcy equaled the maximal amount of cellular [^{14}C]AdoHcy observed at 37° (Fig. 1B).

Incubation of the hepatocytes with 1 mM 2,4-dinitrophenol (at 37°) for 15 min prior to the addition of [^{14}C]AdoHcy did not affect the assocation of [^{14}C]AdoHcy with the cells (data not shown).

Competition studies. [^{14}C]AdoHcy (1 μM) was incubated with hepatocytes in the presence of unlabeled AdoHcy or various analogues of AdoHcy (Table 1). Among the compounds tested, unlabeled AdoHcy was the most potent inhibitor of the association of [^{14}C]AdoHcy with the liver cells. Sinefungin was also an effective inhibitor and AdoMet was slightly inhibitory,

TABLE 1

Association of [^{14}C]AdoHcy with liver cells in the presence of various potential inhibitors

[^{14}C]AdoHcy (1 μM) was incubated for 30 sec at 37° with hepatocytes (4.5 × 10^6 cells/ml), and the amount of [^{14}C]AdoHcy associated with the cells was determined by centrifugation of the cells into perchloric acid as described under Materials and Methods. The acid-soluble extract from the cells was analyzed by thin-layer chromatography. The experiment was performed in the presence of the compounds listed at left.

Potential inhibitors	Concentration	Amount of [^{14}C]AdoHcy associated with cells[a]
	μM	*pmoles/10^6 cells*
Control	—	7.5 ± 0.21
AdoHcy	10	4.3 ± 0.30
	100	3.1 ± 0.40
AdoMet	100	6.0 ± 0.25
Adenosine	100	7.6 ± 0.22
Adenine	100	7.5 ± 0.52
L-Homocysteine	100	7.7 ± 0.35
L-Methionine	100	7.5 ± 0.42
SIBA	100	7.0 ± 0.28
Sinefungin	10	5.3 ± 0.22
	100	4.2 ± 0.28
D-AdoHcy	100	7.4 ± 0.52
Ara-A	100	7.6 ± 0.64

[a] Mean of four values ± standard deviation.

whereas adenosine, ara-A, homocysteine, methionine, SIBA, and D-AdoHcy were slightly inhibitory or essentially without effect (Table 1).

Characterization of the Slow Association of Radioactivity with Hepatocytes (Phase II)

Kinetics. The slow rate of association of radioactivity with the liver cells corresponding to Phase II (Fig. 1A) made determination of the kinetic parameters of this process possible. Cells (3.4×10^6 cells/ml) were incubated for 2, 4, and 6 min with various concentrations (0.1–200 μM) of [^{14}C]AdoHcy. The progress curves were linear in this time interval. From this part of the curves the rates of association of radioactivity with the liver cells were determined. The double reciprocal plot based on data obtained was linear and consistent, with a K_m of 3 μM for AdoHcy. The rate of association of radioactivity with the cells corresponding to phase II varied up to 2-fold from one cell preparation to another. Essentially the same results were obtained in the absence and presence of 10 μM EHNA (data not shown).

Temperature dependence. The temperature dependence of the process was determined by performing the same experiment as above at a saturating concentration (100 μM) of [^{14}C]AdoHcy and various temperatures (15°–40°). The Arrhenius plot based on these data was linear, and the energy of activation was 7.4 kcal/mole (data not shown). The high temperature dependence of this process was in accordance with nearly no accumulation of adenine nucleotides within the hepatocytes incubated with [^{14}C]AdoHcy at 0° (Fig. 1B).

Competition studies. The rate of association of radioactivity with hepatocytes incubated with [^{14}C]AdoHcy was determined in the presence of various purines, nucleosides, and amino acids, including L-homocysteine. Among the compounds tested, adenosine and homocysteine were the most potent inhibitors. In the presence of 100 μM adenosine, the process was almost completely inhibited (Table 2). A high concentration of dipyridamole (100 μM) inhibited the rate of association of radioactivity with the cells incubated with 1 μM [^{14}C]AdoHcy by 50%. The adenosine deaminase inhibitor, EHNA 6 μM, was without effect (data not shown).

Relationship between extracellular hydrolysis of AdoHcy and Phase II. Samples from the extracellular medium were subjected to analysis by thin-layer chromatography at various times (1–30 min) during incubation of liver cells ($2–4 \times 10^6$ cells/ml) with [^{14}C]AdoHcy (1–100 μM). At any time during incubation more than 87% of the radioactivity remaining in the extracellular medium was identified as AdoHcy.

A suspension of hepatocytes was incubated at 37° for 30 min and then divided into two parts. One part was rapidly centrifuged, and the cell-free supernatant was used for determination of extracellular hydrolysis of [^{14}C]AdoHcy. The other part (containing cells) was used for determination of the association of radioactivity with the cells. The time courses of these two processes were compared at the same initial concentration of [^{14}C]-AdoHcy. At a high concentration of [^{14}C]AdoHcy (100 μM), only a small fraction of the total [^{14}C]AdoHcy was hydrolyzed in the (cell-free) extracellular medium. Thus, after 20 min of incubation, about 20% of the total radioactivity added to the cell suspension was associated with the cells whereas 4% of the total amount of [^{14}C]AdoHcy was hydrolyzed in the cell-free medium (data not shown). The same experiment was performed at a low concentration of [^{14}C]AdoHcy (1 μM). Under these conditions the progress curves for the association of radioactivity with the cells and the hydrolysis of [^{14}C]AdoHcy in the corresponding cell-free medium followed each other closely for the first few minutes (Fig. 2). The curve for the association of radioactive metabolites with the cells proceeded until 100% of the total radioactivity was recovered in the cell pellet, whereas hydrolysis of [^{14}C]AdoHcy in the cell-free medium leveled off to reach a plateau (Fig. 2).

TABLE 2

Effect of purines and amino acids on the rate of association of radioactive metabolites with hepatocytes incubated with [^{14}C]Ado Hcy

[^{14}C]AdoHcy (1 μM, 0.59 mCi/mmole) was incubated with hepatocytes (2.2×10^6 cells/ml) for 2, 4, and 6 min and the rate of uptake of radioactive material was determined from the linear part of the uptake curves. The experiment was performed in the presence of the purines and amino acids listed at left.

Potential inhibitor	Concentration	Rate of uptake of radioactive material	
		Amount	% of control
	μM	*pmoles/min/10^6 cells*	
Control	—	41	100
Adenosine	10	4.5	11
	100	0.9	2.3
Adenine	100	16	40
AdoHcy	100	4.9	12
AdoMet	100	23	56
Ara-A	100	15	36
Inosine	100	18	44
Guanosine	100	28	68
Thymidine	100	27	67
Uridine	100	23	56
Cytidine	100	33	80
L-Homocysteine	100	9.4	23
L-Cysteine	100	42	103
L-Methionine	100	40	97
L-Proline	100	43	106

Leakage of enzyme from the hepatocytes. No *S*-adenosylhomocysteine hydrolase activity could be detected in the incubation medium not exposed to liver cells (data not shown). *S*-Adenosylhomocysteine hydrolase activity and lactate dehydrogenase activity in the extracellular medium increased as a function of time of incubation of the cells in the medium. The time course of the appearance of these enzymes in the medium followed each other closely and showed an initial rapid phase for the first 10 min followed by slow leakage of enzyme (Fig. 3).

Adenosine uptake. Uptake of adenosine (1–20 μM) into the liver cells was not affected by the presence of the adenosine deaminase inhibitor, EHNA (10 μM). In the presence of EHNA (10 μM) no deamination of adenosine to inosine in the extracellular medium could be demonstrated (data not shown). In the presence of EHNA,

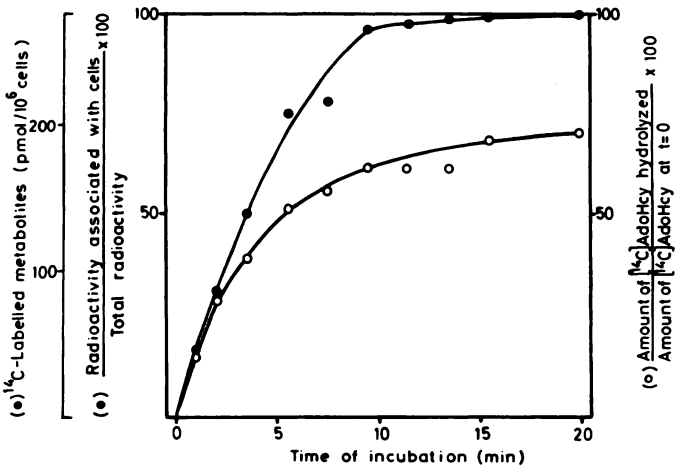

FIG. 2. *Comparison of the association of radioactive metabolites with rat liver cells incubated in the presence of [^{14}C]AdoHcy with the hydrolysis of [^{14}C]AdoHcy in the extracellular medium*

A suspension of rat liver cells (3.7 × 10^6/ml) was incubated for 30 min and then divided into two portions. One portion was centrifuged for 0.5 min at 9000 × *g*. The cell-free supernatant was made 1 μM in [^{14}C]AdoHcy (59 mCi/mmole), and the time course of the hydrolysis of [^{14}C]AdoHcy (O) was determined. The other portion (the cell suspension) was made 1 μM in [^{14}C]AdoHcy. At times indicated, the cells were separated from the medium by centrifugation. The amount of radioactive metabolites in the cell pellet is plotted versus time of incubation (●).

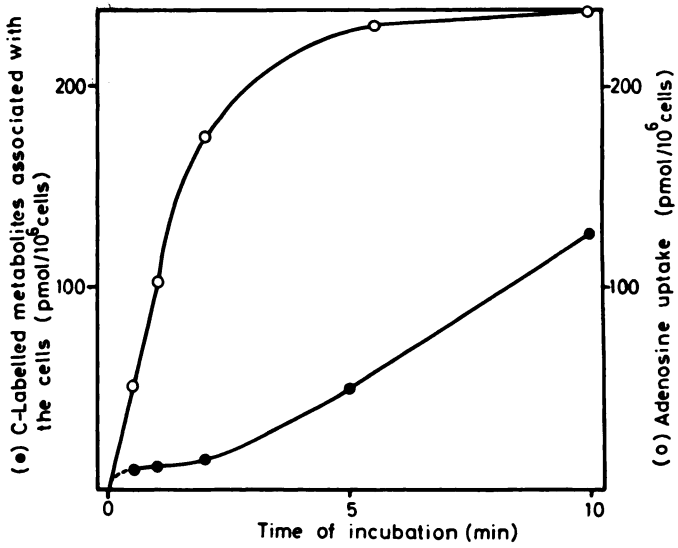

FIG. 4. *Comparison of the rate of association of radioactivity with rat liver cells incubated with [^{14}C]AdoHcy with the rate of adenosine uptake*

Rat liver cells (4.4 × 10^6 cells/ml) were incubated with either 1 μM [^{14}C]AdoHcy (0.59 mCi/mmole) or 1 μM [^{14}C]adenosine (59 mCi/mmole). At times indicated, the cells were separated from the medium as described under Materials and Methods. The amount of radioactive metabolites associated with the cells incubated with [^{14}C]AdoHcy (O) or [^{14}C]adenosine (●) is plotted versus time of incubation.

uptake of [^{14}C]adenosine (initial concentration 1 μM) was inhibited by about 50% when 100 μM dipyridamole was included in the incubation mixture (data not shown).

The association of radioactive metabolities with hepatocytes incubated with 1 μM [^{14}C]AdoHcy was compared with the adenosine uptake from the medium containing [^{14}C]adenosine at an initial concentration of 1 μM. The rates of these processes were determined from the linear part of the curves presented in Fig. 4. Adenosine uptake proceeded at a rate which was about 10 times the rate of association of radioactivity with the cells incubated with [^{14}C]AdoHcy.

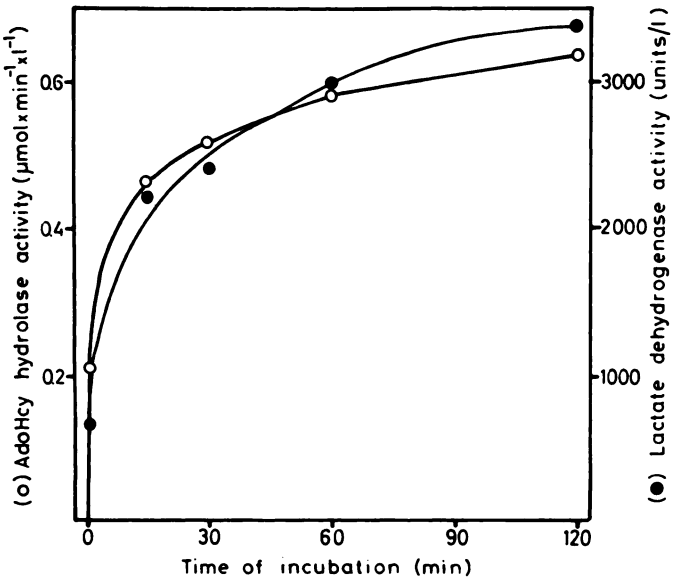

FIG. 3. *Leakage of enzyme from the hepatocytes*

Liver cells were washed three times in the incubation medium and finally resuspended in the medium to a final concentration of 3.1 × 10^6 cells/ml. The medium did not contain EHNA. At times indicated, aliquots of 1 ml from the cell suspension were centrifuged (for 15 sec at 9000 × *g*). *S*-Adenosylhomocysteine hydrolase activity (O) and lactate dehydrogenase activity (●) were determined in the supernatant. The activities are plotted versus time of incubation.

DISCUSSION

The association of radioactive metabolites with hepatocytes incubated with [^{14}C]AdoHcy could be separated into at least two components. These processes are visualized by two phases in the progress curve for the association of radioactivity with the cells incubated with [^{14}C]AdoHcy. The initial burst phase is termed Phase I and the slow linear phase is referred to as Phase II (Fig. 1A). These two components could be distinguished with respect to kinetic properties, temperature dependence, and inhibitory power of AdoHcy analogues, homocysteine and purines. The nature of the two processes is discussed below.

The initial rapid phase (Phase I) corresponds to a rapid association of [^{14}C]AdoHcy with the liver cells. Cell-associated [^{14}C]AdoHcy does not stem from intracellular synthesis of [^{14}C]AdoHcy as demonstrated by the lack of incorporation of [^{14}C]adenosine into [^{14}C]AdoHcy under the conditions of the experiment. Cellular uptake of [^{14}C]AdoHcy from a medium containing a 1 μM concentration of this compound is expected to be an active process, since the cellular content of AdoHcy (23) probably is 10-fold higher than the extracellular concentration used in this experiment. The low temperature dependence of the association of [^{14}C]AdoHcy with the cells

and the lack of effect of 2,4-dinitrophenol argue against the possibility of an active transport of AdoHcy. Furthermore, ara-A, which has been shown to increase the cellular level of AdoHcy several-fold (24), is also without effect (Table 1). This would not be expected if the association of [^{14}C]AdoHcy with the cells represents a rapid exchange between extracellular and intracellular AdoHcy. On this basis it is suggested that AdoHcy binds to an acceptor on the cell surface. The binding of AdoHcy is a reversible process as judged by the decrease in the amount of [^{14}C]AdoHcy as AdoHcy is metabolized (Fig. 1B). Competition studies (Table 1) show that AdoHcy is the preferred ligand of this acceptor.

The possibility exists that specific acceptor(s) for AdoHcy on the cell membrane is a hitherto unrecognized target for AdoHcy and its analogues and could be the molecular basis for some of their biological effects. Further investigation of this hypothesis requires elucidation of the relationship of this acceptor to membrane components participating in the metabolism of AdoMet and AdoHcy (25–27). Further characterization of this acceptor requires studies on isolated plasma membranes, and these are now in progress in our laboratory.

The second slow phase (Phase II, Fig. 1A) of the association of radioactivity with the liver cells incubated with [^{14}C]AdoHcy corresponds to intracellular accumulation of adenine nucleotides (Fig. 1B). This could be explained either by extracellular hydrolysis of AdoHcy to adenosine (and homocysteine) and cellular uptake of these metabolites, or by cellular uptake of AdoHcy and subsequent intracellular metabolism of this compound. Thus, the essential question is whether metabolism of AdoHcy takes place in the extracellular or intracellular medium.

[^{14}C]AdoHcy is hydrolyzed to [^{14}C]adenosine (and homocysteine) in the extracellular medium (Fig. 2). The hydrolysis is enzymatic in nature, as [^{14}C]AdoHcy is not decomposed in the medium not exposed to liver cells. The reaction is catalyzed by the soluble (28) enzyme *S*-adenosylhomocysteine hydrolase (29) which leaks out of the hepatocytes (Fig. 3). The leakage parallels a progressive appearance of cytosol constituents in the extracellular medium as judged by the activity of a marker enzyme for the soluble fraction, lactate dehydrogenase, in the medium (Fig. 3).

The question which remains is whether extracellular metabolism may totally account for the uptake of radioactive metabolites (Phase II). Knowledge of the catalytic properties of *S*-adenosylhomocysteine hydrolase is required for the design of experiments attempting to give an answer to this question. The enzyme catalyses a reversible hydrolysis of AdoHcy to adenosine and homocysteine, and the reaction is characterized by an equilibrium constant, K_{eq}, defined by the equation (29)

$$K_{eq} = \frac{[\text{adenosine}] \times [\text{L-homocysteine}]}{[\text{AdoHcy}]} \simeq 10^{-6}\ \text{M}$$

At high concentrations of AdoHcy (> 1 μM), the reaction favors synthesis of AdoHcy. Furthermore, adenosine and homocysteine are potent inhibitors of the hydrolytic reaction. The enzyme catalysis may be directed toward hydrolysis by removal of adenosine or homocysteine (29).

The association of radioactive metabolites with liver cells incubated with [^{14}C]AdoHcy (Phase II) is nearly completely inhibited by adenosine (Table 2). Adenine, unlabeled AdoHcy, and ara-A are also inhibitory. Among the amino acids tested, only homocysteine inhibited Phase II. Thus, this process is inhibited by structurally unrelated agents in a manner which closely parallels their inhibitory power toward *S*-adenosylhomocysteine hydrolase (18). The slight inhibition by nucleosides other than adenosine may be explained by competition between adenosine and these compounds for the nucleoside uptake mechanism.

The fact that phase II could be almost abolished by inhibitors of the enzymatic hydrolysis of AdoHcy indicates that extracellular AdoHcy does not cross the cell membrane as an intact molecule. This suggestion is supported by the observation that the rate of hydrolysis in the extracellular medium closely follows the cellular uptake of radioactive material for the first few minutes (Fig. 2). The lack of correlation after long periods of incubation may be explained by further leakage of enzyme and the fact that the enzyme catalysis in the cell-free medium (in the absence of an adenosine trap) will proceed toward equilibrium of the reaction. The slight effect of dipyridamole on Phase II correlates with the slight inhibition of the adenosine uptake into rat hepatocytes by this agent. The latter observation should be related to the interspecies variation (30) in the effect of dipyridamole as an inhibitor of nucleoside uptake.

To summarize, in Phase II, AdoHcy is hydrolyzed extracellularly to adenosine and homocysteine through the action of *S*-adenosylhomocysteine hydrolase, and adenosine is rapidly taken up by the cells (Fig. 4). The uptake of adenosine formed from AdoHcy traps adenosine and thereby relieves the inhibition of the hydrolytic reaction by this nucleoside. The hydrolytic reaction is the rate-limiting step in this sequence of events. This statement is supported by the observations that the rate of adenosine uptake is several-fold higher than the rate of uptake of radioactive metabolites derived from [^{14}C]AdoHcy (Fig. 4), and that adenosine or its metabolites do not accumulate extracellularly. It is concluded that extracellular AdoHcy is not transported across the plasma membrane of rat hepatocytes as an intact molecule.

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