UPTAKE OF ADENOSYLMETHIONINE AND RELATED SULFUR COMPOUNDS BY ISOLATED RAT LIVER

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1. Introduction

The biochemical roles of *S*-adenosylmethionine as donor of methyl group, propylamine moiety and aminobutyryl side chain in a variety of reactions are well established [1,2]. Although the biological importance of SAM is widely recognized, the mechanisms of transport of this highly charged molecule in mammalian cells have not yet been investigated.

An active transport system with high affinity towards SAM has been described in yeast cells, which accumulate very peculiarly the sulfonium compound into the vacuoles [3,4]; furthermore mutants lacking this transport system have been isolated [3]. The uptake of SAM by rat pancreas isolated cells [5] as well as rabbit erythrocytes [6] has also been studied.

The aim of the present work was to investigate the transport of circulating SAM into hepatic cells. Liver has been selected since it plays a critical role in SAM metabolism, particularly with respect to methyl transfer reactions as well as polyamine biosynthesis [1].

Abbreviations: SAM, *S*-adenosylmethionine; MTA, methylthioadenosine; decarboxylated SAM, *S*-adenosyl-L-(5')-3-methylthiopropylamine

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Isolated and perfused rat liver, which duplicates most of the important functions played by this organ in vivo, was used in our study in order to avoid the interference of exta-hepatic factors.

The rate of incorporation of differently labeled SAM has been compared with that of methionine in normal liver as well as in fatty liver. The nature of the transport system has been investigated by saturation kinetics analysis and by evaluating the effect of 2,4-dinitrophenol. The specificity of the system has been tested with several SAM analogs and derivatives.

Preliminary results of this investigation have been presented [7].

2. Materials and methods

2.1. *Chemicals and preparation of SAM analogs*

S-Adenosyl-L-methionine was prepared from cultures of *Saccharomyces cerevisiae* [8] and isolated by ion-exchange chromatography [9]; L-[*methyl*-^{14}C]methionine, *S*-adenosyl-L-[*carboxy*-^{14}C]- and *S*-adenosyl-L-[*methyl*-^{14}C]methionine were obtained from the Radiochemical Centre, Amersham; 5'-[*methyl*-^{14}C]methylthioadenosine was prepared by acid hydrolysis of labeled SAM [10,11]; 5'-di-[*methyl*-^{14}C]methylthioadenosine sulfonium salt was prepared by methylation of 5'-methylthioadenosine with $^{14}CH_3I$ [12]; the new sulfonium compound was

separated from MTA by ion-exchange chromatography on Dowex-50 after removal of the excess of CH_3I by evaporation under reduced pressure [11]. *S*-Adenosyl-L-(5')-3-[*methyl*-^{14}C]methylthiopropylamine was prepared by enzymatic decarboxylation of labeled SAM and isolated by ion-exchange chromatography [13]. SAM decarboxylase was purified from *E. coli* by the method in [14] and assayed according to [15]. The chemical and radiochemical purity of the above compounds was checked by thin-layer chromatography and high-voltage electrophoresis [16]. The sulfonium compounds were stored at –20°C, at pH 3.5, to prevent decomposition.

2.2. *Liver perfusion*

Male rats of Sprague-Dawley strain, 270–350 g, were used as liver donors. They were maintained on a standard laboratory diet and fasted 18 h before liver isolation. Experimental liver steatosis was induced by feeding normal rats with a choline-deficient diet (Nutritional Biochem. Corp., USA) for 20 weeks. The animals were anesthetized with ether and the liver was isolated by the usual surgical technique; portal vein and biliary duct were cannulated [17]. The perfusate contained 100 ml Krebs-Ringer bicarbonate buffer with 3% bovine serum albumin, 0.2% glucose and 15 000 units heparin (Liquemin, Roche). The pH, adjusted to 7.4, was monitored throughout perfusion and the labeled sulfur compounds were added to the perfusate at the initial concentrations reported in section 3. The absence of non-enzymatic alterations of SAM in the perfusion apparatus was tested in preliminary experiments with the perfusate circulating in a closed system without liver. The isolated organ was perfused for 1 h at 37°C according to the procedure in [18]. Samples, 1 ml, of the prefusate were taken at different time intervals and analyzed. At the end of perfusion a bile sample was taken and the liver was washed with 50 ml saline and frozen. $^{14}CO_2$ released from the liver during perfusion was trapped in 100 ml 40% KOH.

2.3. *Determination of radioactivity*

Radioactivity was measured in a Tri-Carb liquid-scintillation spectrometer (Packard, model 3380) equipped with an absolute radioactivity analyzer; 0.4% solution of 2,5-diphenyloxazole in a mixture (20 ml) of equal volumes of toluene and ethanol was employed. The quenching was corrected by external standardization. Liver samples (100 mg) were digested with 1 ml Soluene-350 (Packard) for 1 h at 60°C before radioactivity determination.

3. Results

The uptake of labeled methionine and SAM by the isolated rat liver has been monitored by following the disappearance of radioactivity in the perfusate. The data reported in fig.1 indicate that the rate of methionine incorporation exceeds by about 3 times that of SAM; 1 h perfusion results in 80% incorporation of the thioether and 20% of SAM. No significant difference has been found between the uptake of methyl-labeled and carboxyl-labeled SAM.

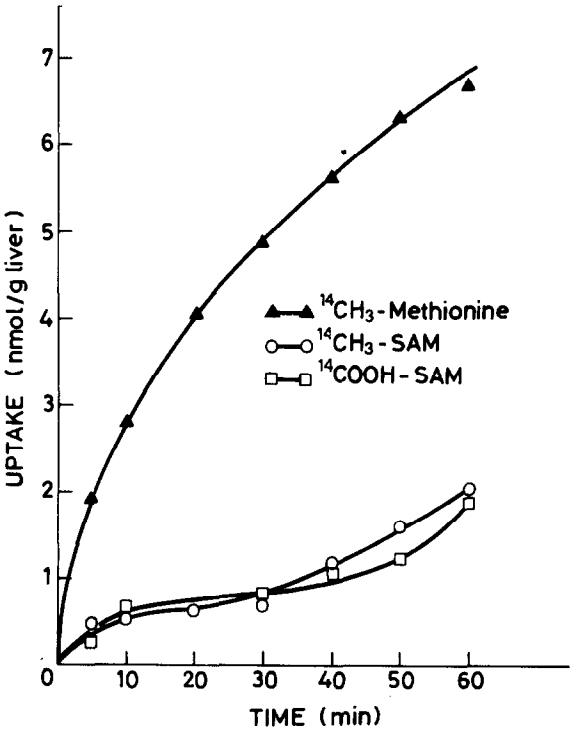

Fig.1. Time course of SAM and methionine uptake by isolated rat liver. The initial concns in the medium were 0.9 μM SAM and 1.35 μM methionine. The uptake was calculated on the basis of the decrease of radioactivity in the perfusing fluid. Each curve gives mean values from 3 (SAM) or 2 (methionine) separate perfusions.

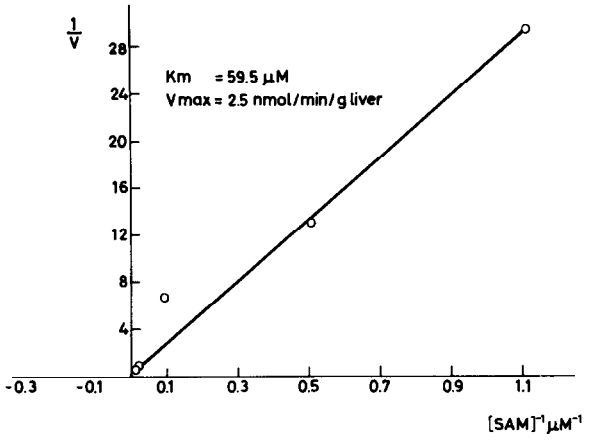

Fig.2. Lineweaver-Burk plot of SA[*methyl*-^{14}C]M uptake.

Samples of liver and bile were analyzed for radioactivity at the end of the perfusion with SAM: 99% of the radioactivity of the SA[*methyl*-^{14}C]M disappeared from perfusate is recovered in the liver and about 1% in bile. When carboxyl-labeled SAM is used, 83% of the disappeared radioactivity is associated to the liver and about 16% is detected as $^{14}CO_2$ diffused from the perfusate.

The kinetics of SAM uptake as a function of its concentration in the perfusate has also been studied. The data reported in fig.2 show that the system is saturable, with an app. K_m 59.5 μM.

In order to evaluate the influence of fatty liver infiltration on the transport system, the uptake of methionine and SAM has been compared in normal and in fatty acid liver for a choline-deficient diet. The data of fig.3 indicate that fatty degeneration of liver results in a marked decrease of methionine incorporation and in a moderate decrease of SAM uptake, detectable only after 60 min.

The specificity of the system has been investigated with the analogs listed in fig.4. The thioethers methionine and methylthioadenosine were incorporated at a rate higher than the 3 sulfonium compounds employed. It is worth noting that decarboxylated SAM, a regulatory intermediate of polyamine biosynthesis [1], is incorporated less efficiently than its parent sulfonium compound.

The addition of non-radioactive methionine to the perfusate at a concentration exceeding 10 times that

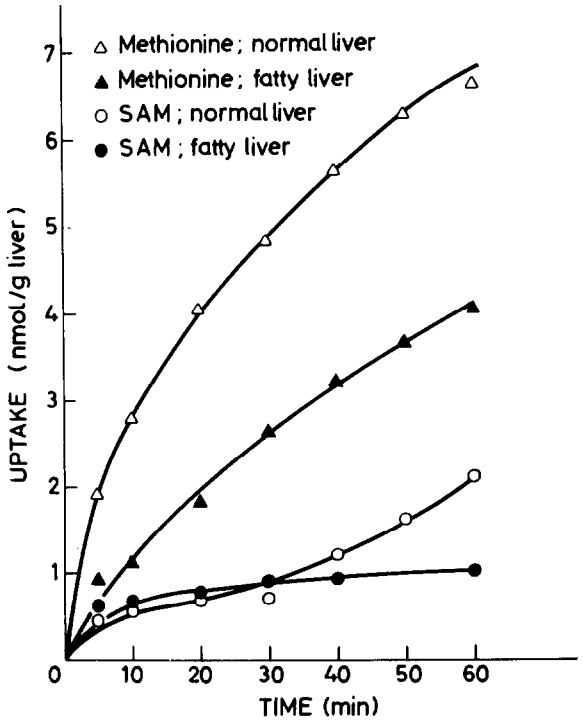

Fig.3. SAM and methionine uptake by normal and fatty liver. Experimental liver steatosis was induced by feeding normal rats with a choline-deficient diet for 20 weeks.

of labeled SAM did not modify significantly the rate of uptake of the sulfonium compound.

The addition of 1 mM 2,4-dinitrophenol to the perfusate, either at the beginning, or after 20 min perfusion, resulted in a quantitatve inhibition of SAM uptake (data not reported).

4. Discussion

The results reported here show that methione and SAM are incorporated by isolated rat liver at a different rate, the uptake of the amino acid being about 3 times higher than that of the sulfonium compound.

The liver SAM transport system appears to be saturable and shows an app. K_m 59.5 μM. This value is of the same order of magnitude of SAM concentration in rat liver [19,20] and in human blood [20], thus suggesting that the system is not saturated in physiological conditions. Therefore, it is conceivable

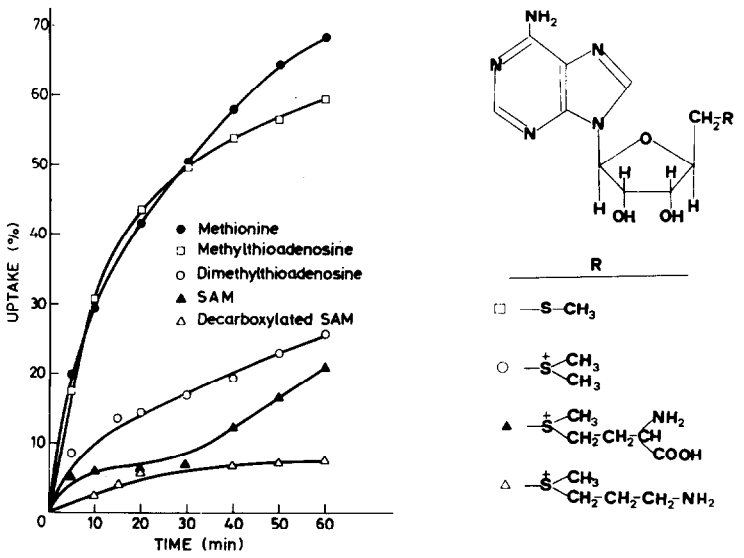

Fig.4. Uptake of SAM, related thioethers and sulfonium compounds by isolated rat liver. All compounds were $^{14}CH_3$-labeled. The initial concentrations and specific radioactivities were as follows: methionine 1.35 μM, 93 X 10^3 cpm/nmol; methylthioadenosine 0.88 μM, 27 X 10^3 cpm/nmol; dimethylthioadenosine 11.6 μM, 0.185 X 10^3 cpm/nmol; SAM 0.9 μM, 109 X 10^3 cpm/nmol; decarboxylated SAM 0.23 μM, 46 X 10^3 cpm/nmol.

that SAM levels in biological compartments contribute to the regulation of the transport of the molecule across biological membranes. It is worth noting, in this respect, that primitive eukaryotes, i.e., yeast cells, bear a similar transport system, but with higher affinity towards SAM [21].

The inhibition of SAM uptake observed in the liver by 2,4-dinitrophenol suggests that the system is energy-dependent, similarly to what has been already shown with spheroplasts of *Saccharomyces cerevisiae* [4].

A non-specific SAM-binding protein, stimulating phosphatidylethanolamine and RNA methylation, has been recently purified from rat liver [22]; whether this protein is also involved in SAM transport, deserves further investigation.

The results obtained with SAM analogs demonstrate that the rate of uptake of the thioethers is higher than that of the 3 sulfonium compounds investigated. The positively charged sulfonium pole probably limits the passage of the molecule into the cells: this is particularly evident when the incorporation of methylthioadenosine is compared to that of dimethylthioadenosine, 2 molecules mainly differing each other in the net charge. On the basis of the results reported it is possible to postulate the existence of a high-affinity permease, specific for thioethers, and a low-affinity system, specific for the sulfonium compounds.

The impairment of lipid metabolism due to choline-deficient diet affects the uptake of methionine more significantly than that of SAM; the decreased incorporation of the amino acid could be relevant with respect to the pathogenesis of liver steatosis.

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