Protective Effects of S-Adenosyl-L-Methionine against Enzyme Leakage from Cultured Hepatocytes and Hypotonic Hemolysis

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Abstract—Effects of S-adenosyl-L-methionine disulfate tosylate salt (SAMe-ST) and L-methionine (L-Met) on rat erythrocytes and primary cultured hepatocytes were studied. SAMe-ST in concentrations of 0.2 to 5.0 mg/ml protected erythrocytes from hypotonic hemolysis. Almost an identical level of protection was provided by SAMe chloride, suggesting that this protective effect is due to the SAMe moiety itself but not its sulfate or tosylate moiety. L-Met also showed a slight protective effect, but at higher concentrations, it slightly enhanced hemolysis. When the cultured hepatocytes were treated with SAMe-ST, the leakage of enzymes from the hepatocytes were significantly decreased compared with that in the control. L-Met also showed similar protective effects, but to a lesser degree than in the case of SAMe-ST. SAMe-ST significantly increased Na+,K+-ATPase activity. The present results indicate that SAMe remarkably inhibits hypotonic hemolysis and enzyme leakage from cultured hepatocytes and that its mechanism is probably related to a change in the membrane property.

The importance of S-adenosyl-L-methionine (SAMe) in many biochemical processes is well-established (for review, see refs. 1 and 2). This compound is present in most tissues of various animal species, and it is a main methyl donor for biochemical transmethylation reactions in many biological systems (2). Many clinical studies show that SAMe treatment decreases free bile acid clearance in familial forms of intrahepatic cholestasis (ref. 3 and refs. cited therein). In nonfamilial intrahepatic cholestasis, which is induced by many causes, SAMe decreases both serum total bilirubin and glutamic-oxaloacetic transaminase (GOT) activity (4). In another clinical study, the treatment of depressed patients with SAMe resulted in marked clinical improvement (5). A recent study has shown that administration of SAMe to normal and ethinyl estradiol (EE)-treated rats markedly stimulated membranes, increasing both Na+,K+-ATPase activity and the fluidity of isolated liver plasma membrane (6). Therefore, the effect of the SAMe to inhibit cell damage will be clinically applied when cell membrane damage occurred, such as hepatic disorder. The purpose of the present study was to clarify whether SAMe or its particular salt form is responsible for the protection of erythrocytes from hypotonic hemolysis. Another study was performed with cultured hepatocytes to compare the effects of a SAMe salt with the effect of L-methionine, a precursor of SAMe, on the leakage of enzymes from hepatocytes.

Materials and Methods

Chemicals and animals: Two salt forms of SAMe, disulfate tosylate (SAMe-ST) and chloride (SAMe-Cl), were kindly provided by Sankyo Co., Ltd. Fetal bovine serum (FBS), penicillin, streptomycin and fungizone were obtained from Gibco Oriental Co., Ltd. N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES), collagenase type IV, L-methionine (L-Met), dexamethasone, adenosine-5'-triphosphate, tris(hydroxy-methyl) aminomethane salt, insulin and ouabain were obtained from Sigma Chemical Co. Williams' E medium was obtained from...
Flow Laboratories. All other chemicals used were of the purest grade commercially available. In this study, male Sprague-Dawley rats, weighing about 170 g, were used.

Studies on hypotonic hemolysis: Effects of the two SAMe salts and L-Met on hypotonic hemolysis of the isolated rat erythrocytes were studied according to Seeman’s method (7). Two different SAMe salts and L-Met were dissolved in a hypotonic buffer (NaCl in 10 mM phosphate buffer, pH 7.4, adjusted to 54 mM, which usually lyses about 50% of the erythrocytes). The erythrocyte suspension (2×10⁹ cells/ml) was incubated for 1 hr at 37°C with various doses in a range of 0.01 to 5.0 mg/ml of either SAMe salt or L-Met. After centrifugation, the free hemoglobin concentration in each of the supernatants, an index of hemolysis, was spectrophotometrically determined at 540 nm.

Preparation and cultures of hepatocytes: Rat hepatocytes were isolated by a slightly modified Seglen’s method (8). Briefly, the liver was perfused at 15 ml/min, via the portal vein, for 10 min with an oxygen saturated Ca²⁺-Mg²⁺-free Hanks balanced salt solution (HBSS) containing 0.5 mM EGTA and 10 mM HEPES (pH 7.5) at 37°C. The liver was then recirculated with oxygen saturated Ca²⁺-Mg²⁺-free HBSS with 0.05% collagenase, 5 mM CaCl₂ and 10 mM HEPES (pH 7.5) at the same flow rate. The liver was then removed from the body and shaken in the same collagenase-Ca²⁺-Mg²⁺-free HBSS solution. The resulting cell suspension was first passed through 150 μm mesh and then centrifuged. The supernatant fluid was removed, and the remaining cell pellet was resuspended in Williams’ E medium supplemented with 10% FBS, insulin (1×10⁻⁹ M), dexamethasone (1×10⁻⁸ M), penicillin (100 U/ml), streptomycin (100 μg/ml) and fungizone (0.25 μg/ml) at pH 7.4. This treatment was repeated twice. The test for exclusion of trypan blue showed approximately 90–98% viability of the hepatocytes. For the hepatocyte culture, the cell suspension (1×10⁶ cells/ml) was plated on plastic dishes (35 mm i.d.) and cultured as monolayers at 37°C under 5% CO₂ and 95% air. The medium was changed 3 hr after starting the culture, and hepatocytes were cultured for a further 21 hr. The cultured hepatocytes were then used in the following experiments.

Determinations of enzymes: To compare the effects of SAMe-ST with those of L-Met, various doses of these compounds in a range of 0.1 to 1.0 mg/ml were added to the culturing medium, and the hepatocytes were then cultured for a further 24 hr at 37°C. Activities of GOT and glutamic-pyruvic transaminase (GPT) that leaked from the cultured hepatocytes were determined by the method of Ohkawa et al. (9). Activities of ornithine carbamyl transferase (OCT) and lactate dehydrogenase (LDH) that leaked from the hepatocytes were determined by the method of Ceriotti (10) and Babson and Phillips (11), respectively.

Assay of ATPase activity: ATPase activity was measured by the method of Pressley et al. (12) with 3 mM ouabain, which completely inhibits the activity of this enzyme. The amount of released inorganic phosphate was determined by the method of Goldenberg and Fernandez (13). Protein contents were determined by the method of Bradford (14) with bovine serum albumin as the standard. Statistical significance of the difference between the control and test values was calculated by Student’s t-test.

Results

The comparative effects of the two salts, SAMe-ST and SAMe-Cl, and L-Met on hypotonic hemolysis of rat erythrocytes are shown in Fig. 1. Both SAMe salts to a similar extent protected erythrocyte hemolysis in a concentration range of 0.2 to 2.0 mg/ml (SAMe-ST, 0.3 to 6.4 mM; SAMe-Cl, 0.5 to 11.5 mM). L-Met at 1.0 mg/ml (6.7 mM) also showed a slight protective effect, but at higher concentrations, it slightly enhanced hemolysis. Since both SAMe salts produced similar protective effects, the SAMe moiety itself, but not the sulfate, tosylate or chloride moiety, seemed to cause the antihemolytic effect. Thus, for further studies, we used only stable SAMe-ST as a test compound.

A significant decrease in the leakage of GOT and OCT from the cultured hepatocytes, compared with that of the control, was observed after treatment with SAMe-ST at 0.5 and 1.0 mg/ml, but after treatment with L-
Met, only effects comparable with those found in the control were observed (Figs. 2 and 3). Similarly, a decrease in GPT leakage from the cultured hepatocytes was clearly observed after treatment with SAMe-ST or L-Met (Fig. 4); the prevention of its leakage by SAMe-ST was more remarkable. In the case of LDH leakage from the cultured hepatocytes, treatment with SAMe-ST caused a decrease; and treatment with L-Met at 0.1 and 0.5 mg/ml caused a similar decrease, although at 1.0 mg/ml, it caused an increase, compared with the LDH leakage observed in the control (Fig. 5).

The effects of 1.0 mg/ml of SAMe-ST or L-Met on Na⁺,K⁺-ATPase activity in the cultured hepatocytes were studied (Fig. 6). Both SAMe-ST and L-Met treatment induced an
Fig. 5. Effect of SAMe-ST and L-Met on leakage of LDH from the cultured rat hepatocytes. Culturing conditions were the same as those described in Fig. 2. Significance: *P<0.05, **P<0.01, ***P<0.001, control vs. SAMe-ST- or L-Met-treated group.

Fig. 6. Effect of SAMe-ST and L-Met on the activity of Na\(^+\),K\(^+\)-ATPase in the cultured rat hepatocytes. Culturing conditions were the same as in Fig. 2. Each value is expressed as \(\mu\)mol Pi released/mg protein/hr. Significance: *P<0.05, control vs. SAMe-ST-treated group.

The increase in Na\(^+\),K\(^+\)-ATPase activity in cultured hepatocytes, the increase due to the former treatment being more remarkable.

Discussion

In this study, the effect of SAMe and L-Met on the hypotonic hemolysis of erythrocytes and enzyme leakage from primary cultured hepatocytes were investigated. In the hypotonic hemolysis study, it is indicated that the SAMe moiety per se is responsible for the inhibitory effect on hypotonic hemolysis of erythrocytes, and that L-Met is not as inhibitory against hemolysis as SAMe salts. SAMe is a naturally occurring compound present in various animal tissues and other organisms such as bacteria and plants. This compound induces important physiological actions such as an increase in the turnover rate of membrane phospholipids (15), membrane fluidity (16) and the turnover rate of catecholamines (17), probably by mediating transmethylation reactions. Considering the importance of this compound in the transmethylation of various substrates as the most versatile methyl donor in various biological systems, its action on the membrane seems to be essential. In the present study, SAMe-ST showed membrane protective effects, as can be seen from the decrease in the leakage of enzymes. However, a high concentration of SAMe was not necessary to obtain better protection. Therefore it is possible that SAMe has an optimum concentration for stabilizing the cell membrane. L-Met also showed similar protective effects, but to a lesser degree than in the case of SAMe-ST. However, the L-Met at 1.0 mg/ml significantly increased the LDH leakage from hepatocytes. Reasons for the increase in the LDH leakage are unknown. A number of possible explanations for the underlying mechanism of these effects can be proposed. In recent studies, SAMe was found to possess the ability to increase the bulk lipid phase transition of the membrane (16, 18). This effect on the membrane would greatly regulate the compositions and translocation of membrane phospholipids. Changes in this membrane fluidity due to phospholipid methylation should produce pleiotropic changes in membrane function (16, 18). In the present study, an increase in the Na\(^+\),K\(^+\)-ATPase activity of the hepatic membrane was observed by the treatment with SAMe. Davis et al. also reported that changes in lipid composition altered Na\(^+\),K\(^+\)-ATPase activity (19). Therefore, SAMe was demonstrated to have the ability to protect the cell membrane from injury.

All data presented here indicate that hypotonic hemolysis and enzyme leakage from hepatocytes were greatly inhibited by SAMe and, to a lesser extent, by L-Met. Although the mechanisms of action of these compounds...
are unclear at present, the present study suggests that the membrane is the primary site(s) for the protective action of SAMe. However, further study is necessary to clarify the precise mechanism(s) of action of this compound on biomembranes.

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