EFFECT OF 5'-DEOXY-5'-*S*-ISOBUTYL-THIOADENOSINE (SIBA) ON THE DISPOSITION OF 5'-METHYLTHIOADENOSINE BY ISOLATED RAT HEPATOCYTES

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1. Introduction

5'-Deoxy-5'-*S*-isobutyl-thioadenosine (SIBA) was synthesized as in [1] for the purpose of providing a biologically active analogue of the endogenous transmethylase inhibitor, AdoHcy [2]. SIBA has been shown to exert numerous biological effects which include antiviral, oncostatic, antimitotic and antiparasitic activities (review [3]). However, the mechanism(s) of action of this structural analogue of AdoHcy still remain(s) obscure. SIBA is an inhibitor of tRNA methylation and some protein methyltransferases, but is a weak inhibitor of these enzymes in vitro [4,5]. Cellular transport of nucleosides and sugar is inhibited by SIBA, suggesting interaction with membrane components [6]. A membrane site of action is also indicated by the finding that this agent stimulates membrane-bound adenylate cyclase in *Xenopus laevis* oocytes [7].

Several papers have been published on the function of SIBA as a substrate for the enzyme responsible for the metabolic degradation of MTA, namely MTA phosphorylase [8–10]. In the light of these reports we investigated the effect of SIBA on the disposition of endogenous MTA by isolated rat hepatocytes. The amount of cellular MTA increased in the presence of SIBA, and the increase was associated with export of MTA from the cells into the extracellular medium.

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine *N'*-2-ethane-sulfonic acid; SIBA, 5'-deoxy-5'-*S*-isobutyl-thioadenosine; MTA, 5'-methylthioadenosine (5'-deoxy-5'-*S*-methyl-thioadenosine); AdoHcy, *S*-adenosyl-L-homocysteine; AdoMet, *S*-adenosyl-L-methionine

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2. Experimental

2.1. *Materials*

Sources of most reagents used are in [11]. μBondapak C18 column (30 × 0.39 cm) was purchased from Waters Associates. ODS Hypersil 3 μm microparticle medium for reverse-phase chromatography was from Shandon Southern Products and microparticle medium for cation-exchange chromatography, Partisil 10 SCX, from HPLC technology. ODS Hypersil columns (0.5 × 10 cm, 9000–11 000 theoretical plates) and Partisil 10 SCX columns (0.46 × 25 cm) were packed by Dr Solheim at our institute, using a Shandon column packer.

2.2. *High-pressure liquid chromatography*

Reverse-phase liquid chromatography was performed on either a μBondapak C18 column, or a ODS Hypersil column, which were eluted isocratically at ambient temperature at a flow rate of 2 ml/min or 1 ml/min, respectively. The ODS Hypersil column was eluted in a constant pressure mode. The mobile phases were 7–12% acetonitril or 20–25% methanol in 9 mM potassium phosphate buffer (pH 6.0).

A Partisil 10 SCX column was used for cation-exchange chromatography. The column was eluted isocratically at a flow rate of 1.5 ml/min. The mobile phases were 210–600 mM ammonium formate buffer, pH 2.6, 3.5 and 4.0, and all buffers contained 1% isopropanol, which somewhat improved the efficiency of the column.

The solvent delivery system was a Spectra-Physics model SP 8000B liquid chromatograph. The absorbance of the effluent was recorded at 254 nm, using a Spectra-Physics UV-detector, model SP 8300.

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2.3. *Preparation and incubation of isolated rat hepatocytes*

Hepatocytes were prepared by a slight modification [12] of the collagenase perfusion method [13]. The cells were incubated in an isotonic salt solution containing bovine serum albumin and antibiotics, as in [11]. The temperature was 37°C.

2.4. *Determination of cellular MTA*

Samples of 1.5 ml from the cell suspension (4.4 X 10^6 cells/ml) were centrifuged at 9000 X *g* for 15 s in a Beckman type B microfuge, the supernatant carefully removed by suction, and the cell-pellet immediately homogenized in 150 μl ice-cold, 5% sulfosalicyclic acid. The homogenate was centrifuged for 5 min at 100 000 X *g*, using a Beckman Air-fuge. The supernatant was immediately neutralized to pH 7 by addition of 2 N Tris. The neutralized extracts were stored at −80°C, and analyzed by HPLC on a ODS Hypersil column within 12 h. The injection volume was 20 μl.

2.5. *Determination of extracellular MTA*

Samples of 225 μl from the extracellular medium (from which the cells had been removed by centrifugation) were mixed with 25 μl of 50% sulfosalicylic acid. The extracts were processed and analyzed as in section 2.4.

3. Results

3.1. *Identification and measurement of MTA in hepatocytes exposed to SIBA*

Hepatocytes exposed to SIBA accumulated UV-absorbing material which showed the same retention time as MTA on a μBondapak C18 column (fig.1 A–C). This material was separated into 2 distinct peaks on a ODS Hypersil column. The compound eluting at the lowest retention time (6.83 min) is referred to as compound X, and the second peak (retention time 7.22 min) co-chromatographed exactly with MTA (fig.1D–F).

Compound X was clearly separated from AdoHcy, AdoMet, adenosine, adenine and SIBA by reverse-phase chromatography on a ODS Hypersil column.

The other compound cochromatographed exactly with MTA on the ODS Hypersil column eluted with various concentrations of acetonitril (7–12%) or methanol (20–25%) in phosphate buffer (pH 6.0) (not shown). The effluent corresponding to this material was collected, lyophilized, and rechromatographed on a Partisil 10 SCX column. The MTA standard and the compound showed the same retention times in systems with the following mobile phases: 600 mM ammonium formate (pH 2.6) (retention time 8.56 min), 600 mM ammonium formate (pH 3.5) (4.33 min) and 210 mM ammonium formate (pH 4.0)

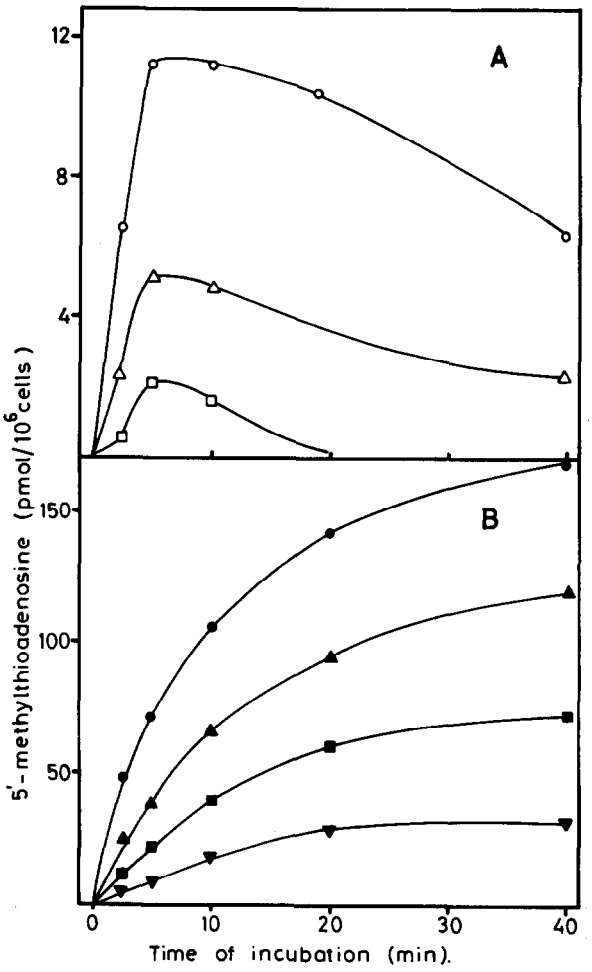

Fig.1. Reverse-phase liquid chromatographic analysis of MTA in extract from hepatocytes exposed to SIBA. MTA standard (A,D), extract from hepatocytes not exposed to SIBA (B,E) and extract from hepatocytes (4.4 X 10^6 cells/ml) incubated with 400 μM SIBA for 10 min (C,F) were analyzed on a μBondapak C18 column (A–C) or a ODS Hypersil column (D–F). The injection volumes were 50 μl (μBondapak column) or 20 μl (ODS Hypersil column). The chromatographic analysis was otherwise performed as in section 2. The mobile phase was 10% acetonitril in 9 mM potassium phosphate buffer (pH 6.0).

(4.71 min). Based on these data, it is concluded that MTA accumulates in hepatocytes exposed to SIBA.

Among the chromatographic systems tested, only reverse-phase chromatography on ODS Hypersil separated MTA from compound X and other interferring substances present in extract from hepatocytes exposed to SIBA.

3.2. *Accumulation of MTA in rat hepatocytes*

The amount of MTA in hepatocytes not exposed to SIBA was below the detection limit of the method used. Cellular-associated MTA increased in a dose-dependent manner in hepatocytes incubated with SIBA. The MTA-level increased for a few minutes, and then declined (fig.2A). In contrast, the cellular content of compound X progressively increased for at least 40 min (not shown).

3.3. *Egress of MTA from rat hepatocytes*

Increase in cellular level of MTA was associated with a massive secretion of MTA into the extracellular medium. The amount of extracellular MTA increased as a function of the concentration of SIBA and time of incubation (fig.2B). The total amount of MTA secreted into the medium was one order of magnitude higher than the amount associated with the cells (fig.2).

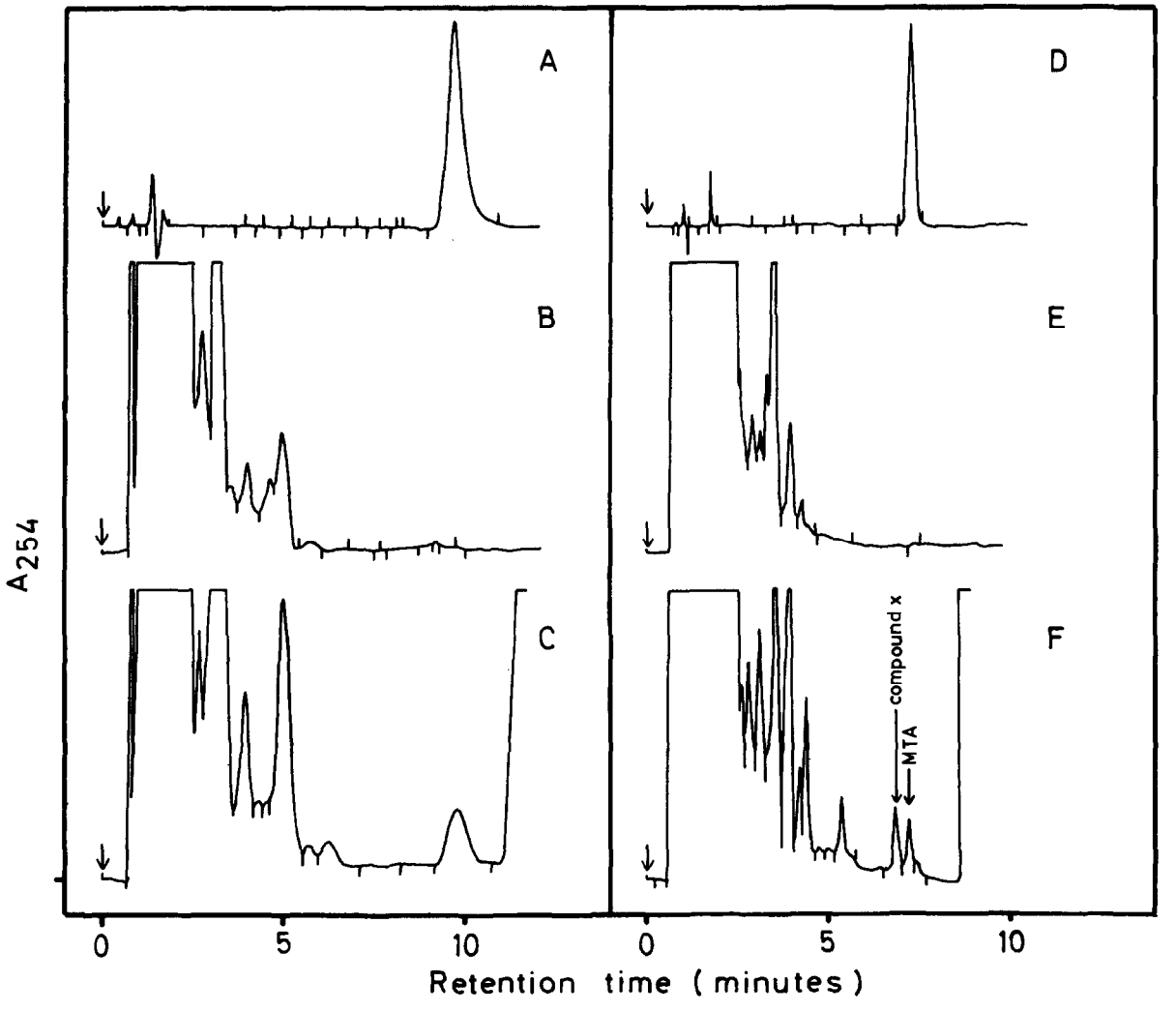

Fig.2. Disposition of MTA by isolated hepatocytes exposed to various concentrations of SIBA: (A) amount of MTA associated with the hepatocytes (4.4 × 10^6 cells/ml); (B) [MTA] in the extracellular medium. The initial concentrations of SIBA added to the cell suspensions were 50 μM (▼), 100 μM (■,□), 200 μM (▲,△) and 400 μM (●,○).

Only trace amounts of compound X were detected in the medium, even after 40 min of incubation with 400 μM SIBA (not shown).

4. Discussion

This is the first report on perturbation of MTA metabolism by SIBA in intact cells. The increase in cellular content of MTA induced by SIBA (fig.2) is in accordance with the observation that SIBA is a substrate for MTA phosphorylase [8–10]. MTA and SIBA are probably competing substrates for this enzyme in rat hepatocytes, and SIBA may reduce the capacity of the metabolic pathway leading to degradation of MTA, beyond the rate of production of this metabolite.

The amount of MTA secreted into the extracellular medium by hepatocytes exposed to SIBA, far exceeds the amount retained within the cells (fig.2). A pronounced egress of MTA from cultured human leukemic cells lacking MTA phosphorylase has been described [14]. Furthermore, the rate of uptake of extracellular MTA by human lymphocytes is low [15]. Thus, the disposition of MTA by intact cells shows similarities with cellular handling of another thio-ether compound, namely AdoHcy. AdoHcy is secreted into the extracellular medium by rat hepatocytes and some cultured cells when the metabolic degradation of this metabolite is blocked by 9-β-D-arabinofuranosyladenine [16]. Extracellular AdoHcy is not taken up by intact cells [11,17] and an unidirectional transport of AdoHcy has been suggested [18]. Cellular transport of *S*-adenosylamino acids and related compounds obviously deserves further attention.

MTA is a product formed from AdoMet during polyamine-synthesis [19]. MTA is a potent inhibitor of spermine synthase from bovine brain [20]. Some AdoMet-dependent transmethylation reactions are inhibited by MTA in vitro [21], and methylation of DNA in *E. coli* is in fact more sensitive to MTA than to AdoHcy [22]. MTA also exerts a growth inhibitory effect on human lymphocytes [15] and SV-3T3 fibroblasts [23]. One may speculate whether at least some effects of SIBA in biological systems [3] are mediated by MTA. This possibility is somewhat reinforced by the observation that the concentrations of SIBA inducing detectable increase in cellular content of MTA (fig.2) equal those required to obtain biological effects of SIBA [3].

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