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Inhibition of lymphocyte cyclic AMP phosphodiesterase and lymphocyte function by 5′-methylthioadenosine

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5′-Methylthioadenosine (MTA) is formed in mammalian cells chiefly as a by-product of polyamine biosynthesis and via the direct enzymatic cleavage of *S*-adenosylmethionine [Ref. 1 and references contained therein]. Under normal physiological conditions, tissue levels of MTA are maintained in the micromolar range [2] due to catabolism of this compound by MTA phosphorylase to adenine and 5-methylthioribose-1-phosphate [1], the latter of which is further metabolized to methionine [3]. While it is unclear whether MTA itself fulfills a requirement within cells, the addition of high concentrations of MTA to cells under a variety of experimental conditions has been reported to be inhibitory to lectin-induced lymphocyte transformation [4], virus-induced chick embryo fibroblast transformation [5], and lymphoid cell proliferation [6]. These biological effects of MTA have been ascribed to the inhibition of either a methyltransferase [1, 5] or spermine synthase [7]. The present report documents the novel finding that MTA is an inhibitor of cyclic AMP (cAMP) phosphodiesterase (3′, 5′-cyclic-nucleotide 5′-nucleotidohydrolase, EC 3.1.4.17) at concentrations of this nucleoside which are inhibitory to lymphocyte function.

Materials and methods

Reagents. MTA was a product of the Sigma Chemical Co., St. Louis, MO. Other materials were from sources identified previously [8–10].

Lymphocyte-mediated cytolysis (LMC). The *in vitro* assay for LMC has been described [10]. Briefly, this assay involves the determination of the ^{51}Cr released during a 70-min incubation at 37° of ^{51}Cr-labeled EL4 cells and cytolytic lymphocytes. Cytolytic lymphocytes were isolated from CD-1 mice 10 or 11 days after intraperitoneal injection of 3×10^7 C57BL leukemia EL4 cells. The EL4 cells were maintained, harvested, and labeled with $Na_2{}^{51}CrO_4$ as before [10]. Normal spleen cells were prepared from CD-1 mice as described [9]. Dulbecco's phosphate-buffered saline supplemented with 5% fetal calf serum (heated 56°/30 min) was used as the medium for all cell incubations except those involving protein carboxymethylation determinations, where Gey's medium (pH 7.2) was used.

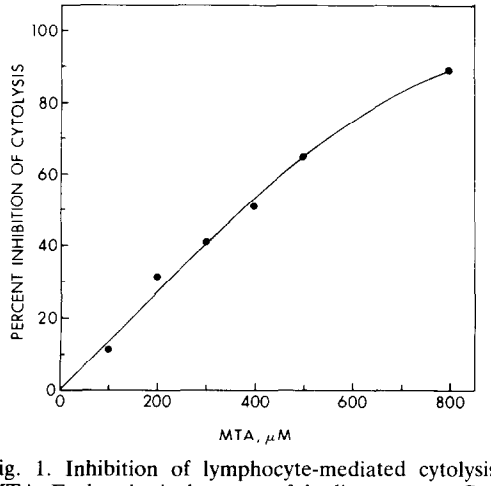

Fig. 1. Inhibition of lymphocyte-mediated cytolysis by MTA. Each point is the mean of duplicate assays. Control lysis was 11.1%

Enzyme determinations. Homogenates of normal spleen lymphocytes were used as the source of cAMP phosphodiesterase (PDE) and adenylate cyclase (ATP pyrophosphate-lyase [cyclizing], EC 4.6.1.1) [9]. cAMP phosphodiesterase was assayed by the two-step procedure of Thompson *et al.* [11]. 5′-Nucleotidase added during the second step of this assay was not inhibited by 500 μM MTA. Adenylate cyclase was assayed by the "Modified Method C" of Salomon *et al.* [12]. Protein was determined by the method of Lowry *et al.* [13].

Biochemical determinations. cAMP was quantitated by radioimmunoassay after purification of acid-soluble cell extracts on sequential columns of aluminum oxide and Dowex 1-X8 [8]. Acid-soluble extracts of cells were analyzed for ribonucleoside 5′-triphosphates by anion-exchange high-performance liquid chromatography (HPLC) [8] and for [^{35}S]adenosylhomocysteine (AdoHcy) by reversed-phase HPLC [14]. Protein carboxymethylation in cytolytic lymphocytes was determined using a slight modification of the method of O'Dea *et al.* [15].

Results and discussion

Lymphocyte-mediated cytolysis. MTA inhibited LMC in a dose-dependent manner, exhibiting a 50% inhibitory concentration of 375 μM (Fig. 1) and 89% inhibition at the highest concentration tested (800 μM). This inhibition was not increased when the lymphocytes were preincubated for 60 min with this agent prior to the start of the LMC assay and was reversed rapidly when the cytolytic lymphocytes were pretreated for 60 min with 500 μM MTA and then transferred to drug-free medium for the LMC assay. The activity of MTA in the LMC assay was unaffected by the addition of the adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) inhibitor *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA, 7.9 μM), the PDE inhibitor 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724, 25 μM), or L-homocysteine (200 μM).

Phosphodiesterase inhibition. The findings that other 5′-modified derivatives of adenosine, AdoHcy [9] and 5′-deoxy-5′-*S*-isobutylthioadenosine [16], inhibited PDE prompted us to examine the activity of MTA towards this enzyme. As shown in Fig. 2, MTA is a competitive inhibitor of the high-affinity PDE (K_m for cAMP = 0.64 μM) from mouse lymphocytes with a K_i value of 255 μM.

MTA added to intact lymphocytes was found to potentiate the cellular cAMP response to prostaglandin E_1 (PGE_1) and cholera toxin (Table 1). This enhancement of the cAMP response to PGE_1 was shown to be concentration dependent with respect to MTA, and significant ($P < 0.01$) potentiation was observed with an MTA concentration as low as 250 μM. In contrast to its effect with PGE_1 and cholera toxin, MTA partially reversed the effect of 2-chloroadenosine on cAMP levels. This latter result is attributed to antagonism by MTA of the binding of 2-chloroadenosine to the adenosine receptors [17].

The potentiation of PGE_1- and toxin-stimulated cAMP elevation by MTA appears to be due solely to inhibition of cAMP degradation and not to augmentation of cAMP production. MTA (250 and 500 μM) did not increase basal,

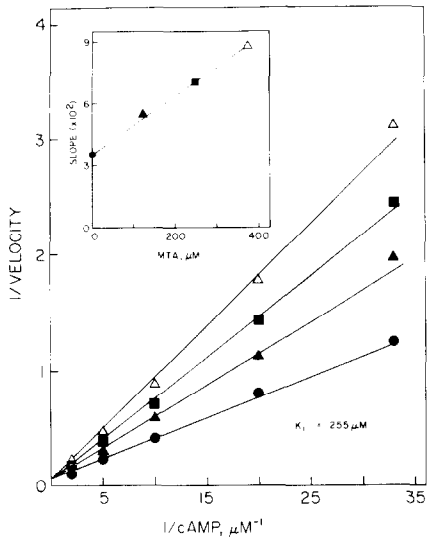

Fig. 2. Inhibition of cAMP phosphodiesterase activity in a homogenate of mouse spleen lymphocytes. Initial velocities are expressed as pmoles [^{3}H]adenosine formed·min^{-1}·(mg protein)$^{-1}$. Each point is the mean of duplicate determinations. Key: (●) no MTA, (▲) 125 μM MTA, (■) 250 μM MTA, and (△) 375 μM MTA.

PGE_1- or isoproterenol-stimulated activities of adenylate cyclase assayed in lymphocyte extracts. Furthermore, 60-min pretreatment of intact lymphocytes with MTA (250 and 500 μM) did not enhance the activity of adenylate cyclase assayed subsequently in cell homogenates (results not shown).

In other experiments, we have found that after a 60-min incubation of cytolytic lymphocytes with 500 μM MTA the pool sizes of CTP, UTP, ATP or GTP were 114, 93, 107 and 91% of control respectively. A 2.75-fold increase in AdoHcy and a 26% inhibition of protein carboxymethylation occurred under these conditions. However, these effects of MTA on AdoHcy and protein carboxymethylation appear to be unrelated to the inhibition of LMC since periodate-oxidized adenosine [18], which affects AdoHcy and protein carboxymethylation to a much greater degree than MTA (results not shown), is not inhibitory to LMC.

We are obviously not able to prove a direct relationship between inhibition of PDE and inhibition of LMC, although the present results indicate that these two effects are related. The cytolytic activity of sensitized lymphocytes is known to be modulated by cAMP levels within the effector cells [references cited in Ref. 8]. It has also been reported that virus-induced cell transformation, mitogen-stimulated lymphocyte transformation and cell proliferation, all of which are inhibited by MTA [2–4], are also inhibited by elevated cellular levels of cAMP [19–21]. It is pertinent to note that two other inhibitors of PDE, 1-methyl-3-isobutylxanthine and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone, are also inhibitors of LMC (IC_{50} values = 250 and 800 μM respectively).

The manner in which a PDE inhibitor, which causes only a minimal (< 2-fold, Table 1) elevation in basal cAMP, could inhibit LMC through a cAMP mechanism remains a matter of conjecture. There have been a number of reports that the interaction of lymphocytes and various agents leads to a transient rise in cellular cAMP. These stimulatory agents include polystyrene latex and zymosan particles [22], antibody [23] and mitogenic lectins [24]. It is possible that the extensive contact between the cytolytic lymphocyte and its target cell leads to an activation of adenylate cyclase which causes a normally modest and transient rise in cAMP levels. This putative target cell-stimulated rise in cAMP may be magnified by MTA in a manner similar to the effect of MTA on the PGE_1-stimulated elevation in cAMP (Table 1).

In summary, we have shown that MTA inhibited lymphocyte function, potentiated the lymphocyte cAMP response to prostaglandin E_1 and cholera toxin, and inhibited the high-affinity cAMP PDE in lymphocyte homogenates at similar concentrations. These novel findings should be considered in the interpretation of the various biological activities of MTA.

Table 1. Effect of MTA on lymphocyte cAMP response to various activators of adenylate cyclase*

MTA (μM)	Stimulant	cAMP (pmoles/10^7 cells)
0	None	1.59 ± 0.04
250		1.39 ± 0.02
500		1.67 ± 0.05
1000		2.43 ± 0.29
0	Prostaglandin E_1 (2.0 μM)	5.31 ± 0.51
250		7.68 ± 0.32 ($P < 0.01$)
500		10.3 ± 0.4 ($P < 0.001$)
1000		18.3 ± 1.8 ($P < 0.001$)
0	2-Chloroadenosine (5.0 μM)	4.25 ± 0.23
500		2.48 ± 0.22 ($P < 0.01$)
0	Cholera toxin (10 μg/ml)	64.2 ± 3.8
500		150 ± 12 ($P < 0.001$)

* Cytolytic lymphocytes (1.0×10^7 cells/2.0 ml medium) were pretreated for 30 min at 37° with the specified concentration of MTA. The cell suspensions were then supplemented with prostaglandin E_1 or 2-chloroadenosine and incubated for a further 30 min at 37° prior to acid-extraction of the cells. Cholera toxin was added to the cells at the start of the MTA pretreatment period and cells were in contact with this agent for a total of 60 min. This experiment was performed in duplicate and each column-purified extract was radioimmunoassayed in duplicate for cAMP. Each value is the mean ± S.E.M. for four determinations. The statistical comparisons between stimulant-induced levels of cAMP found in cells pretreated in the absence or presence of MTA were calculated by means of the two-tailed Student's *t*-test.

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Blockade by haloperidol of the increase in tryptophan hydroxylase activity induced by incubation of slices of brain stem with dibutyryl cyclic AMP

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A discrepancy exists in the literature concerning the role of cyclic AMP in the regulation of the activity of tryptophan hydroxylase [tryptophan-5-monooxygenase, L-tryptophan, tetrahydropterin: oxygen oxidoreductase (5-hydroxylating), EC 1.14.16.4] from rat brain. Low speed supernatant preparations of this enzyme are reversibly activated under phosphorylating conditions by a process that is enhanced by the addition of micromolar amounts of calcium and requires the presence of calmodulin, but which is unaffected by cyclic AMP or any other cyclic nucleotides [1–5]. On the other hand, the *in vivo* conversion of tryptophan to 5-HTP* in the presence of an aromatic amino acid decarboxylase inhibitor is increased markedly following administration of dibutyryl cyclic AMP via the intracerebroventricular route [6]. This action of dibutyryl cyclic AMP cannot be explained by changes in tryptophan availability and may, therefore, arise from an activation of tryptophan hydroxylase [6]. Consistent with this suggestion is the finding that incubation of slices of rat brain stem with dibutyryl cyclic AMP produces an activation of tryptophan hydroxylase which can be demonstrated by kinetic measurements made on low speed supernatant fractions of the enzyme prepared from the pretreated slices [7]. The occurrence of this activation is surprising in view of the failure of cyclic AMP to increase tryptophan hydroxylase activity when added to low speed supernatant fractions in the presence or absence of phosphorylating conditions. To determine whether endogenous cyclic AMP has any action on tryptophan hydroxylase, a study was made in which slices of rat brain stem were exposed to substances which increase endogenous cyclic AMP levels in nervous tissue (phosphodiesterase inhibitors [8], cholera toxin [9], adenosine and 2-chloroadenosine [10]). In addition, another cyclic AMP analogue, 8-bromo cyclic AMP, and the antipsychotic drug,

* Abbreviations: 5-HTP, 5-hydroxytryptophan; $6MPH_4$, DL-6-methyl-5,6,7,8-tetrahydropterin; brocresine (NSD 1055), 3-hydroxy-4-bromobenzyloxyamine dihydrogen phosphate; dibutyryl cyclic AMP, N^6,$O^{2'}$-dibutyryladenosine 3′:5′-cyclic monophosphate; 8-bromo cyclic AMP, 8-bromoadenosine 3′:5′-cyclic monophosphate; and EGTA, ethylene glycol bis(β-aminoethylether)-*N*,*N*′tetraacetic acid.