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SHORT COMMUNICATIONS

Inhibition of histamine release and phosphatidylcholine metabolism by 5′-deoxy-5′-isobutylthio-3-deazaadenosine

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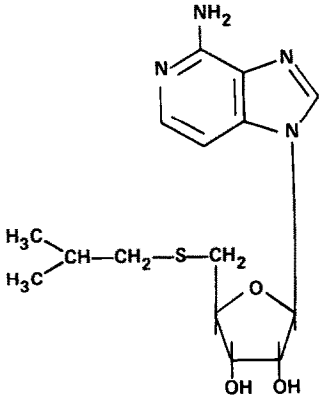
The rat basophilic leukemia cells (RBL-2H3) have IgE receptors and degranulate non-cytotoxically to release histamine through an IgE-mediated reaction as do normal mast cells [1]. The compound 5′-deoxy-5′-isobutylthio-3-deazaadenosine (3-deaza-SIBA), a postulated analogue of *S*-adenosylhomocysteine, has a variety of biological activities thought to be due to its inhibiting methylation reactions [2–6].



3-Deaza-SIBA

In the present experiments, we have shown 3-deaza-SIBA inhibition of IgE-mediated histamine release and, also, inhibition of choline incorporation into phosphatidycholine and choline uptake by RBL-2H3 cells. Unlike inhibitors of methylation which inhibit only IgE-mediated reactions, 3-deaza-SIBA also blocked the histamine release from mast cells induced by other secretagogues, such as compound 48/80, ionophore A23187, polymyxin B and ATP. 3-Deaza-SIBA may perturb membrane functions of basophilic leukemia cells and mast cells by inhibiting the biosynthesis of phosphatidylcholine via choline incorporation, the turnover of which is probably required for cellular degranulation and the release of histamine.

Materials and methods

Materials. 1,4-Piperazine bis-(ethanesulfonic acid) (Pipes), 1-homocysteine thiolactone (Hcy), ATP, ovalbumin, compound 48/80, and polymyxin B sulfate were obtained from the Sigma Chemical Co., St. Louis, MO. Dextran T 70 was purchased from Pharmacia Fine Chemicals Inc., Piscataway, NJ. Bovine serum albumin was obtained as a 36% solution from Miles Laboratories, Elkhart, IN. 3-Deazaadenosine (DZA) was synthesized by Dr. J. A. Montgomery of the Southern Research Institute, Birmingham, AL. The calcium ionophore A23187 was dissolved in dimethylsulfoxide (Fisher Scientific Co., Fair Lawn, NJ) at a concentration of 1 mg/ml and stored at −20°. Phosphatidylserine was from Analabs, Inc., North Haven, CT. L-[^{35}S]Methionine (792.75 Ci/mmole), L-[methyl-^{3}H]methionine (200 mCi/mmole) and [1,2-^{14}C]choline chloride (5.4 mCi/mmole) were purchased from the New England Nuclear Corp., Boston, MA.

Ascitic fluid containing ovalbumin-specific IgE antibody was obtained from mice as described [7]. Rabbit anti-mouse IgE antibody (anti-IgE) was prepared as described previously [7].

Buffers. A Pipes medium (Pipes A) contained 119 mM NaCl, 5 mM KCl, 25 mM Pipes, 40 mM NaOH, 5.6 mM glucose and 0.1% bovine serum albumin (pH 7.4). For histamine release reactions, Pipes A containing 1 mM Ca^{2+} and 0.4 mM Mg^{2+} (Pipes ACM) was used, unless otherwise stated.

Histamine release from rat basophilic leukemia cells (RBL-2H3 cells). The 2H3 cloned subline of the rat basophilic leukemia cells was grown in Eagle's minimum essential medium with Earle's balanced salt solution (MEM) [Grand Island Biological Co. (GIBCO), Grand Island, NY] supplemented with 15% heat-inactivated fetal calf serum (Flow Laboratories, McLean, VA), 100 units/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml gentamycin and 2 mM L-glutamine (GIBCO). Cultures were maintained under 5% CO_2 at 36° in tissue culture flasks (150 cm^2, Costar, Cambridge, MA) and passaged as described previously [1]. Histamine release with these cells was as described in the legend of Table 1.

Histamine release from rat peritoneal mast cells. Washed peritoneal cells were prepared as described previously [7]. The cells at 1.9–5.9 × 10^5/ml were preincubated with the inhibitors for 15–60 min at 37° and with the histamine-releasing agents for another 30 min. The reaction was stopped by centrifugation at 400 *g* for 15 min at 4° and the supernatant fractions were assayed for histamine by the automated fluorometric technique [8]. Phosphatidylserine at a final concentration of 100 μg/ml was included in the reaction mixture when histamine release was induced by anti-IgE or dextran.

Results and discussion

In our studies with rat mast cells, 3-deaza-SIBA inhibited release initiated by all secretagogues tested, such as anti-IgE, ionophore A23187, dextran, polymyxin, compound 48/80 and ATP (Table 1). In contrast, 3-deazaadenosine, which can cause inhibition of methylation reactions by increasing intracellular levels of *S*-adenosylhomocysteine and *S*-3-deazaadenosylhomocysteine [9–11], inhibited IgE-mediated histamine release from rat mast cells, but not the histamine release induced by the other secretagogues.

The discrepancy between the effects of 3-deazaadenosine and 3-deaza-SIBA suggested that 3-deaza-SIBA might have influenced biochemical variables in the cell other than methylation reactions. Recently, it has been suggested that 3-deaza-SIBA inhibits phosphatidylcholine biosynthesis in cultured NG 108-15 neuroblastoma × glioma hybrid cells, without inhibiting phospholipid methylation [13]. We, therefore, investivated the action of 3-deaza-SIBA on the biosynthetic pathway of phosphatidylcholine via choline incorporation, and found that 3-deaza-SIBA inhibited [^{14}C]choline incorporation into the total lipid fractions of RBL-2H3 cells, i.e. cells preincubated with 5 × 10^{-4} M 3-deaza-SIBA for 90 min incorporated 25% less [^{14}C]choline during the next 20 min than the control cells. Moreover, after incubation with 3-deaza-SIBA, the uptake of

Table 1. Effect of 3-deaza-SIBA and 3-deazaadenosine plus L-homocysteine thiolactone on histamine release

Cells	Releasing agents	Concentrations	Control release*	ID_{50}† (M)	
				3-Deaza-SIBA	3-Deazaadenosine‡
I. RBL-2H3	Antigen§	10 μg/ml	60	4.5×10^{-4}	0.11×10^{-4}
	A23187	0.2 μg/ml	58	1.5×10^{-4}	‖
II. Rat mast cells	Anti-IgE¶	100 μg/ml	79	1.5×10^{-4}	1.6×10^{-4}
	Dextran**	5 μg/ml	27	0.3×10^{-4}	††
	A23187‡‡	0.2 μg/ml	38	1.9×10^{-4}	††
	Compound 48/80‡‡	5 μg/ml	50	4.7×10^{-4}	††
	Polymyxin B‡‡	2 μg/ml	31	2.1×10^{-4}	††
	ATP‡‡	5×10^{5} M (added)	29	0.5×10^{-4}	††

* Per cent of total available histamine released in the absence of inhibitors.

† Inhibitors were added in duplicate at concentrations from 1×10^{-6} M to 1×10^{-3} M, and the concentration of the drug that inhibited 50% of the control release was calculated. Each is the mean of duplicate determinations from two separate experiments.

‡ L-Homocysteine thiolactone (10^{-4} M) was added to all 3-deazaadenosine concentrations to generate *S*-3-deazaadenosylhomocysteine [11, 12].

§ RBL-2H3 cells, plated at 1×10^{5} cells per 16 mm diameter well, were sensitized with ovalbumin-specific IgE for 90 min at 36°, washed, and then preincubated with inhibitors in Pipes-buffered saline. Antigen (ovalbumin, 10 μg/ml) was then added and the incubation was continued for another 30 min.

‖ Forty per cent inhibition at 1×10^{-3} M.

¶ Rat peritoneal mast cells were sensitized with ovalbumin-specific IgE antibody for 20 hr, washed, and preincubated at 37° with 3-deaza-SIBA for 30 min or with 3-deazaadenosine plus L-homocysteine thiolactone for 60 min. Anti-IgE was then added at 37° for another 30 min.

** Rat peritoneal mast cells were exposed to inhibitors for 15 min at 37° and then challenged with dextran for 30 min. The reaction was in Krebs–Ringer phosphate buffer (139 mM NaCl, 2.4 mM KCl, 3.3 mM KH_2PO_4, 3.3 mM Na_2HPO_4, 1 mM Ca^{2+}, 0.1% bovine serum albumin, pH 7.0).

†† Less than 10% inhibition at 1×10^{-3} M.

‡‡ Rat peritoneal mast cells were preincubated with inhibitors as in the ¶ footnote. Histamine-releasing agents were then added and the reaction was continued for 30 min.

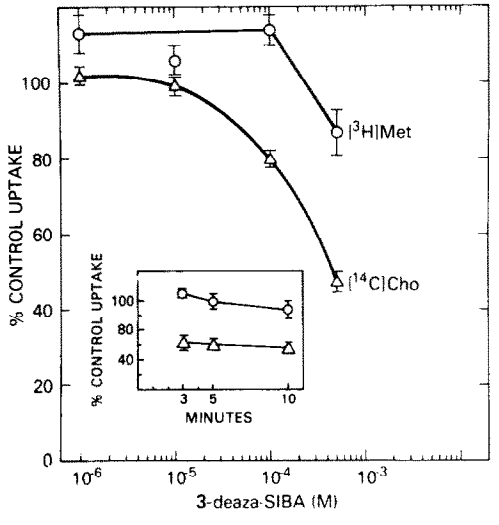


Fig. 1. Effect of 3-deaza-SIBA on the uptake of [^{3}H-methyl]methionine and [^{14}C]choline into RBL-2H3 cells. RBL-2H3 cells were plated at 0.4×10^{6} per 35 mm diameter well in 3 ml of MEM. After 30 hr, the medium was replaced with MEM containing 0.1% bovine serum albumin and ovalbumin-specific IgE antibody for 2 hr. After washing with MEM, cells were first incubated with 3-deaza-SIBA or MEM; 60 min later 84 μmoles per ml L-[methyl-^{3}H]methionine (200 mCi/mmole) and 78 μmoles per ml [1,2-^{14}C]choline (5.4 mCi/mmole) were added. After 10 min, cells were washed twice with MEM and solubilized in 2 ml of 1 N NaOH. Inset shows time course of uptake at 5×10^{-4} M 3-deaza-SIBA concentration. Key: (○——○) [^{3}H-methyl]methionine uptake and (△——△) [^{14}C]choline uptake.

[^{14}C]choline into the cells was inhibited (Fig. 1). At 5×10^{-4} M 3-deaza-SIBA, there was a 50% inhibition of [^{14}C]choline uptake; the uptake of [^{3}H]methionine seemed to be affected to a smaller extent. 3-Deaza-SIBA inhibited histamine release at the same concentrations that inhibited [^{14}C]choline uptake, and it caused a 30–50% inhibition of the incorporation of [^{14}C]choline into phosphatidylcholine and lysophosphatidylcholine in RBL-2H3 cells in the presence or absence of antigen (data not shown). The inhibition of histamine release by 3-deaza-SIBA was observed in reaction mixtures which contained no exogenous choline; therefore, the drug may have acted at sites within the cell on the phosphatidylcholine biosynthetic pathway. It has been found that 3-deaza-SIBA inhibits phosphatidylcholine cytidyltransferase (A. DeBlas, M. Nirenberg, P. K. Chiang and G. L. Cantoni, manuscript in preparation).

These experiments further support the suggestion that alterations in phospholipid metabolism may play an important role in biochemical mechanisms leading to histamine release. Phosphatidylcholine is a major component of membrane phospholipids; it can be synthesized either by the choline incorporation pathway or by three successive *N*-methylations of phosphatidylethanolamine [14, 15]. Increased incorporation of [^{14}C]choline into phosphatidylcholine might occur during histamine release probably due to increased turnover of phosphatidylcholine during the secretory process. Similarly, increased incorporation of $^{32}PO_4$ into phosphatidylcholine, phosphatidylinositol and phosphatidic acid was demonstrated in mast cells activated with various secretagogues [16, 17].

In summary, 3-deaza-SIBA, a postulated analogue of *S*-adenosylhomocysteine, inhibited the IgE-mediated histamine release and blocked both the choline uptake by the rat basophilic leukemia cells and the incorporation of choline into phosphatidylcholine and lysophosphatidylcholine. Unlike inhibitors of methylation which inhibit only dextran- and IgE-mediated reactions, 3-deaza-SIBA also

blocked the histamine release from mast cells induced by other secretagogues, such as compound 48/80, ionophore A23187, polymyxin B and ATP. 3-Deaza-SIBA may perturb membrane functions of basophilic leukemia cells and mast cells by inhibiting the biosynthesis of phosphatidylcholine via choline incorporation, the turnover of which is probably required for cellular degranulation and the release of histamine. Previous studies with 3-deaza-SIBA have been mainly interpreted in terms of its structural similarity to *S*-adenosylhomocysteine and its potential use as an inhibitor of methylation reactions. In light of our present findings, experiments utilizing 3-deaza-SIBA as a biochemical probe have to be carefully interpreted.

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Glutathione *S*-transferase and glutathione reductase activities in hepatic and extrahepatic tissues of female mice as a function of age

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Gluthatione (GSH) is the most abundant low molecular weight thiol-containing compound in living cells, and in the reduced form it functions as an antioxidant in protecting sulfhydryl groups of functional cellular macromolecules [1, 2]. GSH also participates in the detoxification of electrophilic intermediates of drugs and foreign chemicals produced by the mixed-function oxidases of the body [3]. Furthermore, GSH conjugation may provide the main protective mechanism against the formation of the carcinogenic forms of polycyclic aromatic hydrocarbons [4] and other tumorigenic chemicals.

The glutathione *S*-transferases (EC 2.5.1.18) form a group of enzymes which catalyze the conjugation of GSH with a wide vareity of xenobiotics [5, 6]. Several glutathione *S*-transferases (GST) have been identified in the rat liver cytoplasm and microsomal fractions, and these enzymes have been shown to have broad, and overlapping, substrate specificities [7, 8]. GST activities have been measured in other tissues as well as liver, including lung [9–12], intestine [13], and kidney [6, 11, 14].

When GSH serves as a reductant in oxidation–reduction processes, this function results in the formation of glutathione disulfide (GSSG; oxidized glutathione) [1]. The reduction of GSSG is consequently of fundamental importance for the metabolic function of glutathione, and this reaction is catalyzed by the enzyme glutathione reductase (EC 1.6.4.2) with the coenzyme NADPH. Glutathione reductase (GR) is believed to be as ubiquitous as GSH and has been studied in various tissues including liver [15–17], lung [16], kidney [18], and erythrocytes [19].

Stohs *et al.* [20] have shown that decreased glutathione levels occur in blood, liver, kidneys and intestinal mucosa of male CBF-1 mice as a function of age and have suggested that a decrease in cellular GSH with time may contribute