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THE EFFECT OF METHYLTRANSFERASE INHIBITORS ON HISTAMINE RELEASE FROM HUMAN DISPERSED LUNG MAST CELLS ACTIVATED WITH ANTI-HUMAN IgE AND CALCIUM IONOPHORE A23187

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Abstract—Inhibitors of adenosylmethionine (AdoMet)-dependent methyltransferases reduce histamine release from enzymatically dispersed human lung mast cells activated with either anti-human IgE or calcium ionophore A23187. The IC_{25} values for adenosine and 3-deazaadenosine (DZA) inhibiting anti-IgE-induced histamine release were 395 μM and 301 μM respectively. The addition of homocysteine thiolactone (Hcy) potentiated the effects of adenosine and DZA, reducing their IC_{25} values to 32 μM and 10.5 μM respectively. The adenosine deaminase (adenosine aminohydrolase EC 3.5.4.4) inhibitors erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) inhibited anti-IgE-induced histamine release with an IC_{50} of 162 μM. This inhibition was not potentiated by Hcy. The combination of DZA and Hcy effectively inhibited histamine release induced by concentrations of A23187 which released a similar amount of histamine to anti-IgE. However the combination was 17 times less potent against A23187- compared with anti-IgE-induced release.

These observations suggest that AdoMet-dependent methyltransferases play an important role in IgE-dependent histamine release from human lung mast cells but their role in A23187-induced release is less clear.

The IgE-dependent activation of mast cells and basophils initiates a series of membrane and cytoplasmic events which culminate in the non-cytotoxic release of preformed and newly generated mediators of inflammation. Cell activation occurs through antigen cross-linkage of cell-bound IgE with the subsequent bridging of IgE-Fc receptors [1]. There has been much recent interest in the biochemical events which link the IgE-Fc receptor signal to the intracellular mechanism of granule secretion. Activation of purified rat peritoneal mast cells with anti-rat IgE, anti-receptor antibody [2] or the lectin concanavalin A [3], is reported to cause a rapid and transient incorporation of methyl groups from *S*-adenosylmethionine (AdoMet)† into plasma membrane phospholipids through the *N*-methylation of phosphatidylethanolamine. Analysis of similar methylation reactions in microsomes of bovine adrenal medulla [4], rat erythrocyte membranes [5] and rat brain synaptosomes [6] indicates that conversion of phosphatidylethanolamine to its trimethylated derivative phosphatidylcholine occurs in two steps which are catalysed by separate enzymes. Phosphatidylethanolamine becomes methylated to phosphatidyl-*N*-monomethylethanolamine (PME) by a magnesium-dependent methyltransferase (methyltransferase I) which is orientated toward the cytoplasmic surface of the plasma membrane. After reorientating within the cell membrane, PME becomes a substrate for a second methyltransferase (methyltransferase II) which is situated on the outer surface of the membrane and incorporates two further methyl groups into PME to form phosphatidylcholine.

That phospholipid methylation precedes the influx of calcium ions necessary for histamine release from activated rat peritoneal mast cells [2] and leukaemic basophils [7], has led to the suggestion that phospholipid methylation is involved in the calcium gating mechanism. Reorientation of phospholipids in the region of activated IgE-Fc receptors, followed by calcium ion influx activates phospholipase A_2 to liberate free arachidonic acid for oxidative metabolism to newly generated mediators [7].

Methylation reactions are considered to be obligatory for IgE-dependent triggering of histamine secretion from rat mast cells [2] and leukaemic basophils [7] because agents such as 3-deazaadenosine and *S*-isobutyryl-3-deazaadenosine which inhibit methyltransferase activity also inhibit calcium ion influx and histamine secretion over the same concentration range. Furthermore McGivney *et al.* [8] have shown that genetic deletion of methyltransferase I or II from rat basophil leukaemia cells totally

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† *Abbreviations used:* AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine; DZA, 3-deazaadenosine; EHNA, erythro-9-(2-hydroxy-3-nonyl)-adenine; Hcy, L-homocysteine thiolactone; IC_{50} (IC_{25}), concentration of drug calculated to produce 50 (25) per cent inhibition; PME, L-α-phosphatidyl-*N*-monomethylethanolamine; cyclic AMP, 3′,5′-cyclic adenosine monophosphate; HBSS, HEPES buffered salt solution.

impairs their ability to release histamine upon immunological stimulation while their response to calcium ionophore A23187 remains unaffected.

Ishizaka and co-workers [9] have recently reported increased phospholipid methylation following IgE-dependent activation of human lung mast cells. Methylation reaches a maximum later in human cells (around 30 sec) than in rat mast cells (around 10–15 sec) but still precedes both calcium ion influx and histamine secretion. We have investigated the role of methylation reactions in histamine secretion from mast cells dispersed from human lung by examining the effects of methyltransferase inhibitors on cells activated with anti-human IgE or with the calcium ionophore A23187.

MATERIALS AND METHODS

Materials. Erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) was supplied by Burroughs Wellcome Co. (Research Triangle Park, NC, U.S.A.) and 3-deazaadenosine (DZA) by Southern Research Institute (Birmingham, AL). Adenosine, homocysteine thiolactone (Hcy), calcium ionophore A23187, human serum albumin (fraction V), pronase, chymopapain and deoxyribonuclease (bovine pancreas) were all obtained from Sigma (Poole, U.K.). All other reagents were of analytical grade. Heat inactivated goat anti-human IgE serum was prepared as described previously [10]. HEPES-buffered salt solution (HBSS) contained 137 mM NaCl, 5.6 mM glucose, 10 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulphonic acid (HEPES), 0.4 mM NaH_2PO_4, 2.7 mM KCl, 0.5 mM $MgCl_2$, 1.0 mM $CaCl_2$ and 0.03% human serum albumin. The pH was adjusted to 7.4 by addition of aqueous NaOH.

Dispersion of human lung mast cells. Macroscopically normal human lung tissue, obtained within 1 hr of resection from patients undergoing surgery for carcinoma of the bronchus, was dissected free of tumour and major bronchi, chopped finely with scissors and suspended in HBSS solution. After washing, the fragments were incubated with 2 mg/ml pronase and 0.5 mg/ml chymopapain in HBSS for 30 min at 37°. Cells dispersed during this procedure were separated from undigested tissue by sequential sieving through 800 μm and 60 μm nylon gauze. Dispersed cells were washed three times with cold HBSS and to minimise cell aggregation were agitated with 0.02 mg/ml deoxyribonuclease in HBSS for 20 min at 37°. Mast cells were identified microscopically by metachromasia with Kimura's stain and comprised 3–5% of the total nucleated cells.

Drug incubations and assessment of histamine release. Duplicate 900 μl aliquots of dispersed lung cells containing 3 to 6 $\times$ 10^4 mast cells in HBSS were incubated alone or with pharmacological agents at 37° for 1 hr unless stated otherwise. The mast cells were activated for histamine release by addition of 100 μl of anti-human IgE to achieve a final dilution of 1/10, or 100 μl of calcium ionophore A23187 to achieve a final concentration of 0.1–1 μM. After mixing, histamine release was allowed to proceed for 15 min at 37° before being stopped by centrifugation at 250 *g* for 10 min at 4°. The supernatant was removed, acidified with trichloroacetic acid (TCA) to a final concentration of 5% and kept at −20° until assay. Total cellular histamine was measured in parallel tubes by disintegrating cells in 5% TCA. Histamine levels in supernatants and cell pellets were measured by an automated spectrofluorometric method [11]. Histamine release induced by the secretogogues is expressed as percentage of total histamine and corrected for spontaneous release in the absence of secretogogue.

Data analysis. The significance of the differences between release of histamine in the presence of drug to that in its absence was analysed using Student's *t*-test for paired data. Concentration–response curves were compared by covariant analysis and drug potencies expressed as the concentration calculated to inhibit histamine release by either 25 or 50% (IC_{25} or IC_{50}).

RESULTS

3-Deazaadenosine on IgE-dependent histamine release

Preincubation of human lung mast cells for 1 hr with 3-deazaadenosine (DZA) inhibited anti-IgE-induced histamine release in a concentration-dependent manner (Fig. 1). The maximum inhibition achieved was 42.3 ± 6.7% (mean ± S.E.M.) at a DZA concentration of 1000 μM ($P < 0.001$). Preincubation of mast cells for 1 hr with homocysteine thiolactone (Hcy), 100 μM, had a slight (3.6 ± 3.5%), but not significant effect in potentiating histamine release. In contrast, Hcy, 100 μM, potentiated the DZA-induced inhibition of histamine release. At DZA concentrations between 10 and 1000 μM, addition of Hcy caused a parallel displacement of the DZA concentration–reponse

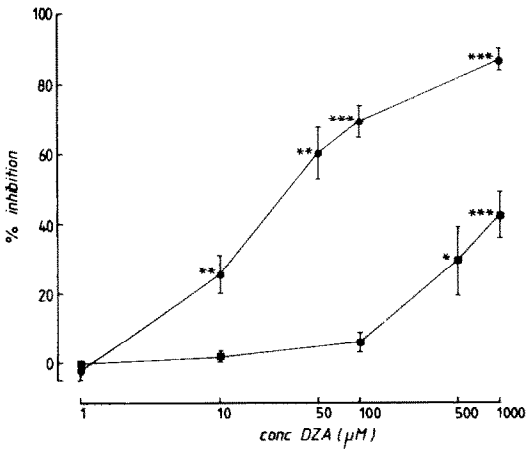

Fig. 1. Inhibition by DZA (■) and DZA in the presence of Hcy, 100 μM, (●) of histamine release induced by anti-human IgE. Net histamine release induced by anti-IgE in the absence of drugs was 30.2 ± 2.4% and spontaneous release was 9.1 ± 0.4%. Each point represents the mean ± S.E.M. of duplicate samples of cells for 5–11 experiments carried out on separate lungs. *, ** and *** represent values significantly different from control, $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

curves to the left (Fig. 1). The geometric mean concentration of DZA required to inhibit IgE-dependent histamine release by 25% (IC_{25}) decreased from 300 μM in the absence of Hcy to 10.5 μM in its presence ($P < 0.001$). The geometric mean IC_{50} for the combination of DZA and Hcy was 35 μM. Inhibition of histamine release by DZA, 1000 μM, was potentiated by Hcy (50–400 μM) in a concentration-dependent manner. The combination of DZA with Hcy, 400 μM, totally inhibited IgE-dependent histamine release (99.3 ± 0.6% inhibition). In contrast, incubation of mast cells with Hcy alone potentiated histamine release by a maximum of 16.3 ± 2.1% ($P < 0.01$) at a concentration of 400 μM.

Inhibition of histamine release by DZA, 100 μM, plus Hcy, 100 μM, increased with prolongation of the preincubation time before challenge, inhibition after 5 and 60 min preincubation being 13.0 ± 2.0% and 77.5 ± 7.5% respectively (Fig. 2).

Adenosine on IgE-dependent histamine release

Preincubation of mast cells for 1 hr with adenosine (1–1000 μM) inhibited histamine release by a maximum of 27.5 ± 8.1% at 1000 μM ($P < 0.05$) (Fig. 3). Homocysteine thiolactone, 100 μM, potentiated the inhibitory effect of adenosine as reflected by a parallel displacement to the left of the linear part of the adenosine concentration–response curve. Thus, Hcy significantly decreased the geometric mean IC_{25} for adenosine from 394 μM in its absence to 32 μM in its presence ($P < 0.01$). As with DZA, the inhibitory effects of the combination of adenosine, 100 μM, plus Hcy, 100 μM, increased with length of preincubation (Fig. 2).

EHNA on IgE-dependent histamine release

Preincubation of mast cells for one hour with EHNA, an inhibitor of adenosine deaminase, caused a concentration-related inhibition of histamine release with a geometric mean IC_{50} of 162 μM (Fig. 4). The presence of Hcy, 100 μM, in 3 experiments had no significant effect on the inhibition of histamine release produced by any concentration of EHNA. At a concentration of 10 μM, EHNA did not enhance adenosine-induced inhibition of histamine secretion (Fig. 3).

DZA on histamine release induced by calcium ionophore A23187

In three experiments A23187 at concentrations of 0.4, 0.6 and 1 μM induced 57, 63 and 62% histamine release respectively. DZA in the presence of Hcy, 100 μM, produced a similar concentration-related inhibition of histamine release at each ionophore concentration. In contrast to its effect on anti-IgE-

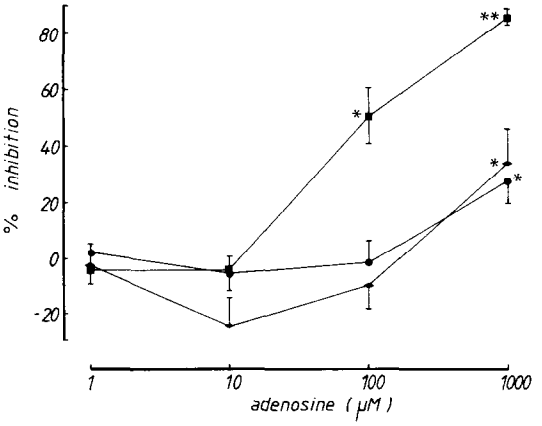

Fig. 3. Inhibition by adenosine (●), adenosine plus EHNA, 10 μM, (◆) and adenosine plus Hcy, 100 μM, (■) of anti-IgE induced histamine release. Net histamine release induced by anti-IgE in the absence of inhibitors was 30.9 ± 3.2% and spontaneous release was 9.5 ± 0.3%. Each point represents the mean ± S.E.M. of duplicate samples of cells from five lungs. The asterisks represent statistical significance as in Fig. 1.

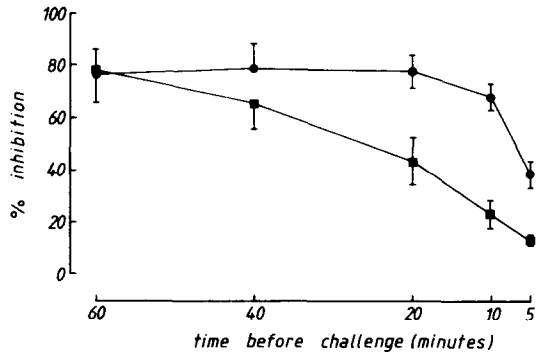

Fig. 2. Effect of preincubation time on inhibition of anti-IgE-induced histamine release by DZA, 100 μM, plus Hcy, 100 μM, (■) or adenosine, 100 μM, plus Hcy, 100 μM, (●). Net histamine release induced by anti-IgE in the absence of inhibitors was 33.7 ± 2.7% and spontaneous release was 12.8 ± 1.2%. Each point represents the mean ± S.E.M. of duplicate samples of cells from three lungs incubated with inhibitors for the stated times before challenge.

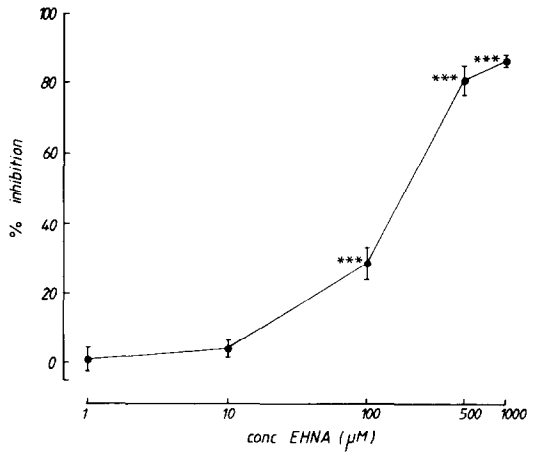

Fig. 4. Inhibition by EHNA of anti-IgE-induced histamine release. Net histamine release induced by anti-IgE in the absence of EHNA was 28.4 ± 2% and spontaneous release was 9.5 ± 0.8%. Each point represents the mean ± S.E.M. of duplicate samples of cells from five lungs. The asterisks represent statistical significance as in Fig. 1.

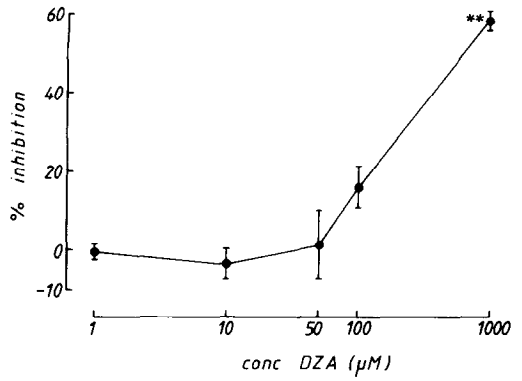

Fig. 5. Inhibition by DZA plus Hcy, 100 μM, of histamine release induced by calcium ionophore A23187, 0.15–0.30 μM. Net histamine release induced by ionophore in the absence of drugs was 23.5 ± 2.9% and spontaneous release was 16.6 ± 1.6%. Homocysteine thiolactone, 100 μM, alone potentiated histamine release by 12.7 ± 4.9%. Each point represents the mean ± S.E.M. of duplicate samples of cells from four lungs. The asterisks represent statistical significance as in Fig. 1.

induced histamine release, this inhibition reached a maximum of only 22.3 ± 2.7% at a DZA concentration of 1000 μM. Because in these experiments A23187-induced histamine secretion was almost twice that observed in experiments with anti-human IgE, further studies were undertaken in which release induced by ionophore was more closely matched to that of anti-IgE, i.e. 30%. In four experiments in which mean A23187-induced histamine release was 23.5 ± 2.9%, DZA in the presence of Hcy, 100 μM, produced a dose-related inhibition of histamine release with a geometric mean IC_{50} of 605 μM (Fig. 5). Although histamine release induced by A23187 and anti-IgE were not significantly different, the combination of DZA and Hcy was 17 times less potent ($P < 0.001$) in inhibiting A23187-induced release.

DISCUSSION

This study demonstrates that adenosine and its 3-deaza-analogue inhibit IgE-dependent histamine release from enzymatically dispersed human lung mast cells in a concentration-dependent fashion. The inhibitory effects of both drugs are potentiated by homocysteine thiolactone at a concentration which alone has no significant effect on histamine release. These pharmacological interactions suggest that methylation reactions play an important role in IgE-dependent histamine release from human lung mast cells.

S-adenosylmethionine-dependent methyltransferases are subject to potent end product inhibition by *S*-adenosylhomocysteine (AdoHcy) [12]. Intracellular concentrations of AdoHcy are maintained at a low level by its metabolism to adenosine and homocysteine catalysed by *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1). This reaction is readily reversible and although the equilibrium favours AdoHcy synthesis [13], the hydrolytic reaction occurs due to subsequent rapid metabolism of adenosine and homocysteine. Adenosine and several of its analogues are inhibitors of AdoHcy hydrolase and, in the presence of increased cellular homocysteine, act as substrates for the reverse condensation reaction which forms the corresponding nucleosidyl-homocysteine derivative [14, 15]. For example, 3-deazaadenosine serves as a substrate, yielding 3-deazaadenosylhomocysteine, in addition to being a competitive inhibitor of AdoHcy hydrolase. This analogue of AdoHcy has been shown to inhibit a variety of methyltransferases both *in vitro* and *in vivo* [16, 17]. Thus, in the presence of DZA or increased cellular levels of adenosine, potent inhibition of mast cell methyltransferase activity would be expected.

In this study homocysteine thiolactone potentiated the inhibitory capacity of both DZA and adenosine on IgE-dependent histamine release. This provides strong evidence that these drugs are acting by inhibiting methyltransferase reactions which are linked to the mast cell activation-secretion mechanism. The IC_{50} value of 35 μM for DZA in the presence of Hcy, 100 μM, in human mast cells is similar to that observed in rat peritoneal mast cells (33 μM) [18], rat basophil leukaemia cells (12 μM) [19], and human basophils (25 μM) [20]. In a single experiment Ishizaka *et al.* [9] have also demonstrated that this drug combination produces concentration-dependent inhibition of histamine release from purified human lung mast cells, although the observed IC_{50} was much higher (200 μM). However, in the same cell preparations the IC_{50} values for DZA inhibition of calcium ion influx, lipid methylation and the early IgE-dependent increase in cellular levels of cyclic AMP were in the range 20–40 μM [9], consistent with our results.

The demonstration that adenosine inhibition of immunological histamine release caused by preincubation of human lung mast cells for 1 hr with 100–1000 μM adenosine was potentiated by homocysteine thiolactone suggests an effect on methylation reactions. In a previous study we demonstrated that preincubation of mechanically dispersed human lung mast cells [21], and human basophil leucocytes [22] for 15 min with lower concentration of adenosine (1–100 μM), inhibited histamine release. This occurred by an adenosine A_2-receptor mediated mechanism which activates adenylate cyclase to increase cellular levels of cyclic AMP [23]. Adenosine may, therefore, have complex effects on the secretory mechanism of human mast cells involving both stimulation of cell surface A_2-purinoceptors and of inhibition of methyltransferase activity. The relative contributions of these actions may depend on adenosine concentration and the duration of preincubation before challenge.

The effects of DZA on human lung mast cells are less complex than those of adenosine. Deazaadenosine does not raise cellular cyclic AMP levels in mouse lymphocytes [24] and its effects in human basophils are not potentiated by phosphodiesterase inhibitors [20]. The observation that, in human lung mast cells, inhibition of IgE-dependent histamine release by adenosine or DZA, in the presence of Hcy, increases with length of preincubation time (Fig. 2) suggests a time-dependent accumulation of an inhibitory product.

Morita *et al.* have shown that EHNA inhibits IgE-dependent histamine release from human basophils [20]. As this compound is a potent inhibitor of adenosine deaminase [25] its action could lead to increased cellular levels of adenosine and inhibition of AdoHcy hydrolysis. However, while we were able clearly to demonstrate that EHNA was an inhibitor of IgE-dependent mediator release from human lung mast cells, its effects were not potentiated by homocysteine thiolactone as had been observed with adenosine. Furthermore, EHNA at a concentration of 10 μM, which in other whole cell systems almost totally inhibits adenosine deaminase [26], failed to potentiate adenosine-induced inhibition of immunological histamine release. These observations suggest that EHNA decreases histamine release from human lung mast cells by mechanisms other than by methyltransferase inhibition.

IgE-dependent activation of rat mast cells [2], rat basophilic leukaemia (RBL) cells [7], and human lung mast cells [9], is associated with increased phospholipid methylation. In contrast, no such increase occurs when RBL-cells are activated with calcium ionophore A23187, a secretogogue which bypasses the IgE-Fc receptor. Furthermore, in rat mast cells the combination of DZA and Hcy, which effectively inhibits IgE-dependent histamine release, has little effect on histamine release induced by A23187, compound 48/80 or polymyxin B [19]. These findings suggest that phospholipid methylation is pertinent only to the activation–secretion mechanism initiated through the IgE-Fc receptor. In the present study, we have shown that DZA, in the presence of Hcy, inhibits histamine secretion induced by optimum concentrations of A23187 but is substantially less effective than in IgE-dependent release. Pearce and coworkers have demonstrated that drugs such as β-adrenergic agonists, methylxanthines and sodium cromoglycate are of similar efficacy in inhibiting histamine release induced by either an IgE-dependent mechanism or by a calcium ionophore provided that the amount of histamine release was similar [27, 28]. Although reduction of the A23187 stimulus markedly enhanced the efficacy of DZA plus Hcy, with approximately matched histamine releases the drug combination was some 17 times less effective against A23187-induced release compared with immunological release. Phosphatidylethanolamine methylation is particularly sensitive to inhibition by DZA [29, 30], but the higher concentrations of drug required to inhibit A23187-induced histamine release may affect other methylation reactions involved in ionophore-induced histamine release. For example, protein carboxymethylation is involved in exocytosis from rat chromaffin cells [31, 32] and pancreatic acinar cells [33].

In conclusion, we have demonstrated that DZA and adenosine inhibit IgE-dependent histamine release from human lung mast cells. As the inhibition is potentiated by homocysteine thiolactone we suggest the mechanism involves inhibition of methyltransferase activity. Although the findings with immunological stimulation would support the observations of Ishizaka *et al.* [9] that phospholipid methylation is an early obligatory event in mast cell IgE-dependent activation-secretion coupling, the observations with A23187 indicate that the drugs used in this study may also inhibit methylation reactions other than phospholipid methylation. Therefore, further studies are necessary to define the precise mechanisms of action of methyltransferase inhibitors in mast cell mediator secretion.

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