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EFFECT OF INHIBITORS OF TRANSMETHYLATION ON HISTAMINE RELEASE FROM HUMAN BASOPHILS

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Abstract—Methylation reactions mediated by *S*-adenosyl-L-methionine (AdoMet) play an important role in a number of biological reactions including bacterial and human monocyte chemotaxis. This study evaluated the role of methylation reactions in histamine release from human basophils. Methylation was blocked by several methods. Erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), an inhibitor of adenosine deaminase (EC 3.5.4.4), blocked the IgE-mediated histamine release with an IC_{50} (concentration of drug required to produce 50 per cent inhibition) of 0.33 mM. Preincubation of leukocytes with adenosine caused some inhibition of IgE-mediated histamine release. This inhibition by adenosine was potentiated by the addition of 1×10^{-5} M EHNA which alone did not affect histamine release. Further addition of L-homocysteine thiolactone at 1×10^{-4} M potentiated the inhibitory effect of EHNA plus adenosine. The effect of L-homocysteine thiolactone was dose dependent. 3-Deazaadenosine (DZA), an inhibitor of *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) inhibited IgE-initiated histamine release from human basophils with an IC_{50} of ~1 mM. This inhibition was potentiated by L-homocysteine thiolactone. The inhibition by DZA was not potentiated by two different phosphodiesterase inhibitors suggesting that the action of DZA was not through changes in intracellular levels of cyclic AMP. DZA inhibited during the Ca^{2+}-independent activation step of IgE-mediated basophil histamine release. In contrast, when histamine release was induced by the calcium ionophore A23187, fMet-Leu-Phe or zymosan-activated serum, there was either no inhibition or enhancement of histamine release by these inhibitors of transmethylation. These results suggest that AdoMet-mediated methylation plays an important role in IgE-initiated histamine secretion from human basophils and that release induced by A23187, fMet-Leu-Phe, or C5A, bypasses this reaction step.

Methylation reactions mediated by *S*-adenosyl-L-methionine (AdoMet)* play an important role in a number of biological reactions [1]. *S*-Adenosyl-L-homocysteine (AdoHcy) is produced during reactions in which the methyl group of *S*-adenosyl-L-methionine is transferred to a number of acceptor molecules, e.g. DNA, RNA, lipids, proteins or carbohydrates (Fig. 1). The specific methylation reactions are sensitive to inhibition by AdoHcy. Normal methylation, however, occurs because AdoHcy is rapidly removed as it is formed. Removal is through the actions of *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1). The equilibrium constant for this reaction greatly favors AdoHcy synthesis from adenosine and L-homocysteine: under physiological conditions, these products are rapidly metabolized further by other enzymes; if added in excess to a system, however, they result in increasing levels of AdoHcy and in inhibition of transmethylation reactions. The AdoHcy pathway can also be influenced by two inhibitors. Adenosine deaminase (EC 3.5.4.4) is inhibited by the specific inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA). The addition of EHNA together with adenosine and L-homocysteine has been shown to result in accumulation of intracellular *S*-adenosyl-L-homocysteine in a number of systems [2–4]. The second inhibitor is 3-deazaadenosine (DZA), a structural analogue of

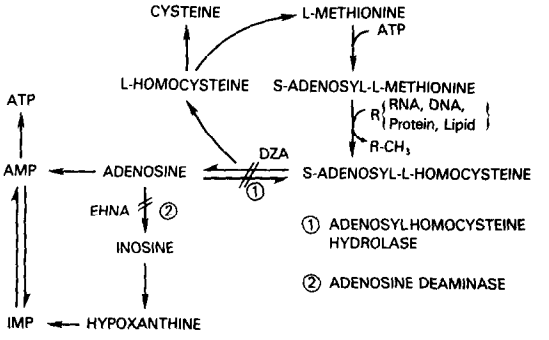

Fig. 1. Pathway of *S*-adenosyl-L-homocysteine metabolism. The sites of action of the two inhibitors, EHNA and DZA, are indicated. The *S*-adenosyl-L-homocysteine hydrolase reaction is reversible—equilibrium constant favors synthesis.

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* Abbreviations: AdoHcy: *S*-adenosyl-L-homocysteine; AdoMet: *S*-adenosyl-L-methionine; cAMP: cyclic AMP; DZA: 3-deazaadenosine; EHNA: erythro-9-(2-hydroxy-3-nonyl) adenine; Hcy: L-homocysteine thiolactone; IBMX: 3-isobutyl-1-methylxanthine; IC_{50}: concentration of drug required to produce 50 per cent inhibition; Pipes: 1, 4-piperazine bis-(ethanesulfonic acid); and RO 20-1724: 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone.

adenosine; DZA is both a substrate and inhibitor of AdoHcy hydrolase [5, 6].

Methylation reactions play a role in both microbial [7] and human monocyte chemotaxis [4]. In bacterial systems, the interaction of the chemotactic stimulus with its specific receptor results in methylation of a certain membrane protein [8]. Mutants that lack the methyl transferase catalyzing the transfer of methyl groups from AdoMet to the membrane protein are defective in chemotaxis [9]. Other mutants lack the specific membrane protein that is methylated, or they are deficient in methionine or AdoMet [7].

A role for methylation reactions in the eukaryotic chemotactic response to the tripeptide fMet-Leu-Phe by rabbit neutrophils has been demonstrated [10]. Treatment of human monocytes with EHNA plus exogenous adenosine and L-homocysteine thiolactone increased intracellular AdoHcy levels, depressed monocyte protein carboxymethylation, and inhibited chemotaxis [4]. Inhibition of methylation reactions, however, does not depress human monocyte phagocytosis, but it inhibits mouse macrophage phagocytic function [11]. Lymphocyte-mediated cytolysis is inhibited by DZA, suggesting that methylation reactions are also important in this reaction [12].

The release of histamine from washed human leukocytes is a useful *in vitro* model for allergic reactions. The IgE-mediated release is initiated by the interaction of antigen or anti-IgE with IgE bound to the basophil membrane [13]. Histamine is released from the basophil, the only cell that contains histamine in human blood [14]. This release of histamine is a secretory phenomenon. The present experiments suggest that AdoMet-mediated methylation plays an important role in IgE-mediated histamine secretion from human basophils. In contrast, release by the calcium ionophore A23187, fMet-Leu-Phe, or C5a bypasses this methylation step.

METHODS

Materials. Pipes, L-homocysteine thiolactone (Hcy), 3-isobutyl-1-methylxanthine and adenosine were obtained from the Sigma Chemical Co., St. Louis, MO. Zymosan was purchased from Schwarz/Mann, Orangeburg, NY. Human serum albumin was obtained as a 25% salt-poor solution from Cutter Laboratories, Berkeley, CA. RO 20-1724 was provided by Hoffaman-LaRoche, Inc., Nutley, NJ. EHNA was from Dr. H. J. Schaeffer, Burroughs Wellcome Co., Research Triangle Park, NC., and DZA was synthesized by Dr. J. A. Montgomery of the Southern Research Institute, Birmingham, AL. Purified ragweed antigen E was provided by the Research Resources Branch, NIAID, NIH, and anti-IgE (Fc specific) by Dr. K. Ishizaka, Good Samaritan Hospital, Baltimore, MD. FMet-Leu-Phe was obtained from Peninsula Laboratories, San Carlos, CA. The calcium ionophore A23187 was donated by Eli Lilly & Co., Indianapolis, IN. These two compounds were dissolved in dimethylsulfoxide (Fisher Scientific Co., Fair Lawn, NJ) at 5×10^{-2} M and 0.5 mg/ml, respectively, and stored at −20°.

Solutions. For histamine-release studies the medium contained 119 mM NaCl, 5 mM KCl, 25 mM Pipes, 40 mM NaOH, 2 mM Ca^{2+}, 0.5 mM Mg^{2+} and 0.03% human serum albumin.

Leukocyte preparations. Washed leukocytes were prepared from venous blood from normal, or ragweed or grass allergic, donors as described previously [15].

Generation of activated serum. Zymosan-activated serum was used as a source of C5a as described previously [16].

IgE-mediated histamine release reactions. To study the effect of inhibitors, washed cells were preincubated with various concentrations of the compound for 60 min at 37°. The histamine-release reaction was initiated by the addition of an optimal concentration of either antigen or anti-IgE and allowed to proceed for 45 min. The reaction was stopped by centrifugation at 700 *g* at 4° for 15 min. The concentration of antigen or anti-IgE was chosen to be optimal by prior titration of the individual's cells. In all experiments, reagent blanks contained cells, the compound being tested, and medium. Experimental tubes were in duplicate or triplicate, with less than 5 per cent difference between duplicates. All experiments were performed at least twice (see also legends). Spontaneous release in the absence of antigen or anti-IgE was consistently less than 5 per cent. For two-stage reactions, leukocytes were washed in medium free of Ca^{2+} and Mg^{2+}, resuspended in the same medium containing 0.04 mM EDTA, prewarmed to 37°, and preincubated with compounds for 90 min and, then, with an optimal concentration of antigen or anti-IgE for 2 min. Ice-cold buffer was added and the tubes were centrifuged for 1 min at 1200 *g* to stop the reaction. Cells were washed at 2° with medium and then resuspended in regular medium containing Ca^{2+} and Mg^{2+}. Reaction was continued at 37° with antigen, anti-IgE, or medium for 45 min. Control leukocytes were incubated without antigen in the first stage for 2 min at 37°.

Histamine release reactions induced by fMet-Leu-Phe, ionophore A23187, *or zymosan-activated serum.* The incubation conditions for fMet-Leu-Phe- or ionophore A23187-induced histamine release were identical to those for the IgE-mediated reaction. For zymosan-activated serum-induced release, however, cells were preincubated with inhibitors for only 20 min at 25° and, after the addition of activated serum, reaction was continued for 30 min at 25°. As a control, the same leukocytes were tested for IgE-mediated histamine release.

Histamine assay. Histamine was assayed by the automated fluorometric technique [17]. Calculations were described previously [15].

Statistical analysis. Student's *t*-tests for paired samples or *t*-tests for two means were used to analyze the histamine release data. Statistical significance is indicated in the figures by the use of asterisks: (*) $P < 0.05$; (**) $P < 0.01$; and (***) $P < 0.001$ (two-tailed). The N indicates the number of individual experiments used in the calculations; results are expressed as means ± S.E.M.

RESULTS

Effects of adenosine, EHNA and L-homocysteine thiolactone on IgE-mediated histamine release.

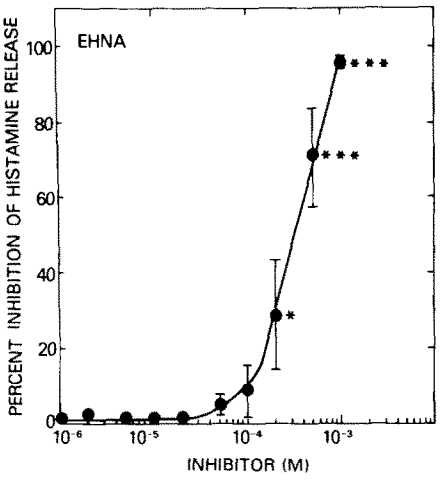

Fig. 2. Inhibition by EHNA of IgE-mediated histamine release from human basophils. Washed leukocytes from normal or allergic individuals were preincubated with the various concentrations of EHNA for 60 min at 37°. Ragweed antigen E or anti-IgE was added, and the incubation continued for another 45 min. Each point is the mean ± S.E.M. from four donors. Release in the absence of inhibitor was $\bar{x} = 59$ per cent (range 45–75 per cent). Statistical significance is indicated by the use of asterisks (*) $P < 0.05$; and (***) $P < 0.001$ (two-tailed).

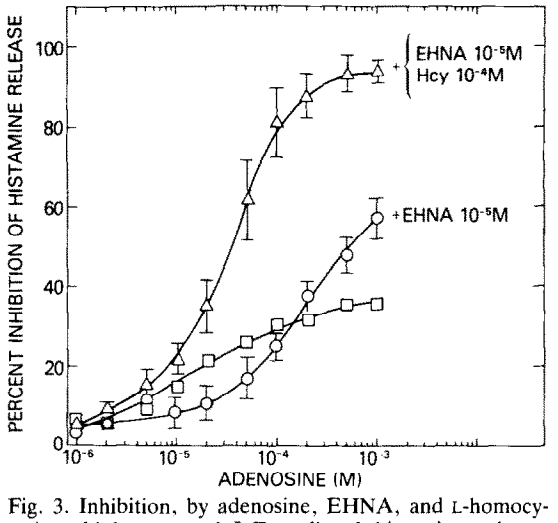

Fig. 3. Inhibition, by adenosine, EHNA, and L-homocysteine thiolactone, of IgE-mediated histamine release. Washed leukocytes were preincubated with the various inhibitors for 60 min at 37°. Histamine release was with antigen or anti-IgE for 45 min. Each point is the mean ± S.E.M. from four donors; the S.E.M. for inhibition with adenosine was less than 3 per cent and is not shown. Release in the absence of inhibitor was $\bar{x} = 57$ per cent (range 45–75 per cent). The inhibition became significant at $P < 0.005$ (Student's *t*-test) with inhibitor concentrations above the following levels: adenosine, 1×10^{-6} M; adenosine (5×10^{-5} M) plus EHNA; adenosine (1×10^{-6} M) and L-homocysteine plus EHNA. The increased inhibition by EHNA above that by adenosine became significant at 5×10^{-4} M adenosine ($P < 0.005$). L-Homocysteine thiolactone potentiation of inhibition caused by EHNA plus adenosine became significant at an adenosine concentration of 2×10^{-5} M ($P < 0.005$). EHNA at 1×10^{-5} M and L-homocysteine thiolactone at 1×10^{-4} M alone had no effect on histamine release.

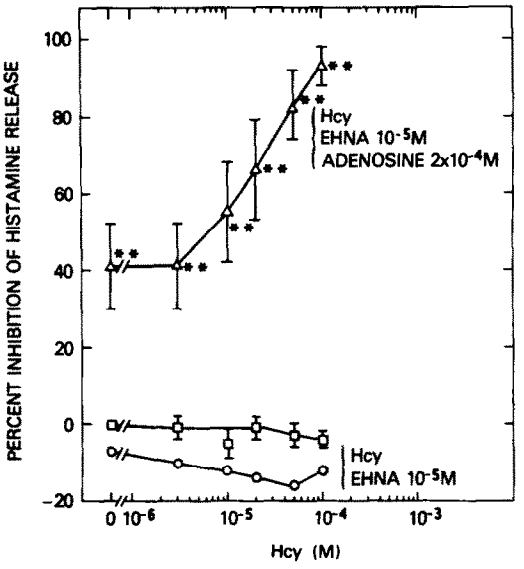

Fig. 4. L-homocysteine thiolactone potentiation of the inhibition induced by EHNA plus adenosine. Leukocytes were preincubated at 37° for 60 min; antigen was then added and the incubation continued for 45 min. Each point is the mean ± S.E.M. from three donors. Release in the absence of any inhibitor was $\bar{x} = 61$ per cent (range 25–82 per cent). The inhibition of histamine release by 1×10^{-5} M EHNA and 2×10^{-4} M adenosine was potentiated by 1×10^{-5} M L-homocysteine thiolactone ($P < 0.001$).

EHNA is an inhibitor of adenosine deaminase, the enzyme that catalyzes the conversion of adenosine to inosine. EHNA inhibited the IgE-mediated histamine release with an IC_{50} of 3.3×10^{-4} M (Fig. 2).

Incubation of leukocytes with adenosine caused some inhibition of histamine release, which reached a maximum of 36 per cent (Fig. 3). The addition of 1×10^{-5} M EHNA to adenosine slightly potentiated this inhibition. Further addition of L-homocysteine

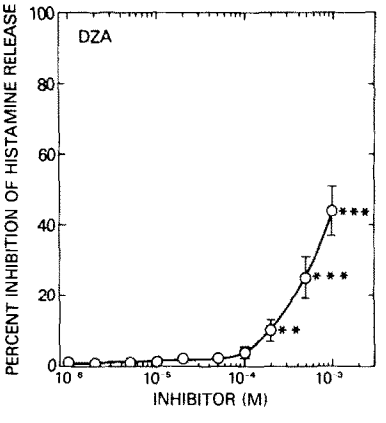

Fig. 5. Inhibition by 3-deazaadenosine of IgE-mediated histamine release from human basophils. The leukocytes were preincubated with various concentrations of 3-deazaadenosine for 60 min at 37°; histamine release was with antigen or anti-IgE for 45 min. Each point is the mean ± S.E.M. from eight experiments. Release in the absence of inhibitor was $\bar{x} = 63$ per cent (range 45–85 per cent). Statistical significance is indicated as follows: (**) $P < 0.01$, and (***) $P < 0.001$ (two-tailed).

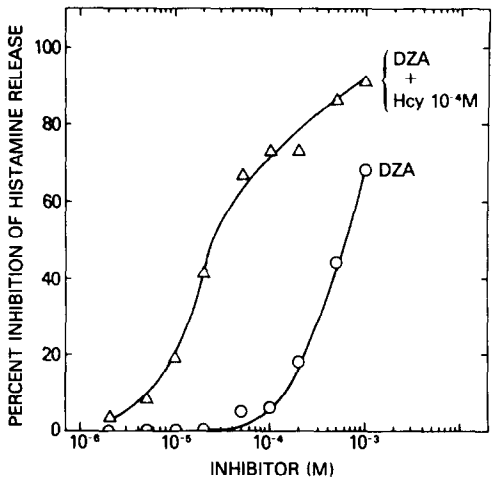

Fig. 6. L-Homocysteine thiolactone potentiation of the 3-deazaadenosine-induced inhibition of histamine release. Leukocytes were preincubated with various concentrations of 3-deazaadenosine and 1×10^{-4} M L-homocysteine thiolactone for 60 min at 37°. Anti-IgE was then added, and the incubation continued for another 45 min. Release in the absence of any inhibitor was 45 per cent. L-Homocysteine thiolactone potentiation of the inhibition of histamine release by 3-deazaadenosine was significant by the paired *t*-test ($P < 0.001$). Similar potentiation was observed in two more experiments.

thiolactone to the adenosine-EHNA further enhanced this inhibition. These concentrations of EHNA and L-homocysteine thiolactone by themselves did not inhibit histamine release. The enhancing effect of L-homocysteine thiolactone was dose-dependent (Fig. 4) and was observed at concentrations greater than 1×10^{-5} M.

The duration of incubation of these inhibitors with the cells prior to activation with anti-IgE or antigen was a critical factor in demonstrating their effects. The maximal effect of adenosine was achieved by a preincubation period of 30 min, whereas the combination of adenosine, EHNA and L-homocysteine required 60 min of preincubation time (data not shown).

Inhibition of basophil histamine release with 3-deazaadenosine. The experiments with EHNA, adenosine and L-homocysteine suggested that inhibitors that elevate the intracellular level of AdoHcy inhibit histamine release by inhibiting methylation. Because cells have adenosine receptors that modulate cyclic AMP (cAMP) [18, 19], however, the observed results could have been due to changes in cAMP levels. Therefore, a series of experiments was performed with 3-deazaadenosine, which is both an inhibitor and substrate for AdoHcy hydrolase. This inhibitor does not elevate cAMP levels [12] and in some systems will decrease intracellular cAMP [20].

Histamine release from leukocytes was inhibited by 3-deazaadenosine, with an IC_{50} of ~1 mM (Fig. 5). The activity of DZA was not affected by the addition of EHNA. This is compatible with the observation that DZA is not a substrate for adenosine deaminase [5]. The effect of DZA was potentiated by the addition of L-homocysteine thiolactone—the IC_{50} for DZA alone was 6×10^{-4} M and shifted to 2.5×10^{-5} M when L-homocysteine thiolactone was also present (Fig. 6). The effect of L-homocysteine thiolactone was concentration dependent; potentiation of inhibition was observed at concentrations of L-homocysteine thiolactone between 5×10^{-5} M and 2×10^{-4} M (data not shown). The action of DZA alone or in combination with L-homocysteine thiolactone required prolonged (up to

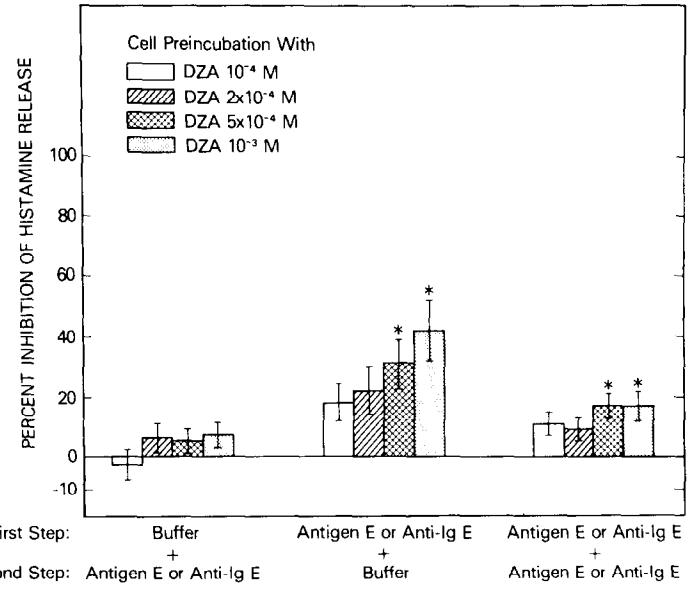

Fig. 7. Effect of 3-deazaadenosine on basophil activation. Aliquots of leukocytes in Ca^{2+}-free medium were incubated for 90 min with 3-deazaadenosine at 0, 1, 2, 5, and 10×10^{-4} M concentrations. The cells were activated with antigen or anti-IgE for 2 min, washed, and suspended in Ca^{2+} medium for 45 min. One group of control cells was not activated in the first step and was challenged only in the second step (first panel): $N = 8$; control release was 67 per cent (range 58–73 per cent). An asterisk indicates statistical significance at $P < 0.05$ (two-tailed).

Table 1. Effects of inhibitors of methylation on ionophore A23187-, fMet-Leu-Phe-, or C5a-induced histamine release from leukocytes

Releasing agent	Control histamine release*	EHNA (10^{-5} M) + Hcy (10^{-4} M) + Adenosine (5×10^{-4} M)		Hcy (10^{-4} M) DZA (5×10^{-4} M)	
		Histamine release	Enhancement (+) Inhibition (−)	Histamine release	Enhancement (+) Inhibition (−)
	% ($\bar{x}$ ± S.E.M.)†				
Ionophore A23187, 0.17 μg/ml‡	73 ± 4	74 ± 4	+7 ± 5	97 ± 2	+27 ± 5§
fMet-Leu-Phe, 1.7×10^{-5} M‡	48 ± 8	57 ± 10	+28 ± 6§	68 ± 10	+33 ± 4§
Anti-IgE or antigen‡	53 ± 11	5 ± 2	−92 ± 3§	16 ± 6	−75 ± 7§
C5a‖	37 ± 3	49 ± 3	+36 ± 9§	53 ± 5	+47 ± 10§
Anti-IgE or antigen‖	39 ± 10	17 ± 9	−60 ± 10§	36 ± 12	−14 ± 7¶

* Total histamine content was 14–33 ng/ml.

† $\bar{x}$ ± S.E.M. for five separate experiments with ionophore and fMet-Leu-Phe and three with C5a. Paired *t*-test was used to compare the results in each experiment.

‡ Leukocytes with inhibitors were preincubated for 60 min at 37°; histamine release step was 40 min at 37°.

§ $P < 0.001$.

‖ Preincubation with inhibitors was at 25° for 20 min; histamine release step with C5a was 30 min at 25° and with IgE-mediated system it was 40 min at 37°. The DZA concentration was 10^{-3} M.

¶ $P < 0.01$.

90 min) preincubation of cells with the inhibitor (data not shown). When cells were incubated with DZA for 60 min, washed, and activated, they released normally; the effect of DZA plus homocysteine, however, was not reversed completely by simply washing the leukocytes.

Further studies were performed to test the possibility that the action of DZA was mediated through the cAMP system. Two different cAMP phosphodiesterase inhibitors, IBMX and RO 20-1724, inhibited histamine release in a dose-dependent fashion. These inhibitors at suboptimal concentrations did not potentiate the inhibition caused by DZA. The lack of a synergistic effect of DZA and the phosphodiesterase inhibitors, together with the data that show that DZA had no effect on intracellular cAMP levels of murine lymphocytes [12], suggests that DZA does not affect the cAMP level of human basophils.

Effects of these inhibitors on the early and late stages of IgE-mediated histamine release. The IgE-mediated histamine release reaction from human basophils can be divided into at least two stages: during the first stage, there is Ca^{2+}-independent activation of the cells, and release occurs during the second Ca^{2+}-dependent step. The effects of the various inhibitors on these two stages were investigated.

Leukocytes were preincubated with DZA for 90 min, activated with antigen E or anti-IgE, washed, and incubated in Ca^{2+}-containing buffer (Fig. 7). There was significant inhibition of histamine release at concentrations of DZA of 0.5 and 1 mM. As observed earlier, washing removed the inhibitory effect of DZA (first panel of Fig. 7). Antigen or anti-IgE was also added in the second stage to cells that had been activated in the first stage in the presence of DZA (third panel of Fig. 7). Under these conditions, there was less inhibition of histamine release.

In other experiments, we observed that adenosine (at 5×10^{-4} M) exerted its action in the first stage of histamine release (data not shown). The inhibition caused by the combination of EHNA, adenosine, and L-homocysteine thiolactone was not reversed by washing the cells; therefore, the experiments could not answer the question whether inhibition occurred only during the first stage.

There was no inhibition by any of these compounds when they were present only during the second stage of histamine release (data not shown).

Effects of these inhibitors on histamine release induced by the calcium ionophore A23187, *fMet-Leu-Phe, or zymosan-activated serum.* The calcium ionophore A23187, formyl methionine-containing peptides, and C5a each release histamine from human basophils by mechanisms that differ from that causing IgE-mediated release [16, 21–26]. The preincubation of washed leukocytes with adenosine and EHNA plus L-homocysteine thiolactone, or with DZA plus L-homocysteine thiolactone, did not cause inhibition of A23187-, fMet-Leu-Phe- or C5a-induced histamine release (Table 1). With both fMet-Leu-Phe- and C5a-induced histamine release there was significant enhancement of histamine release by both combinations of inhibitors, whereas the A23187-induced release was enhanced only by DZA plus L-homocysteine thiolactone. There was inhibition of IgE-mediated histamine release from the same cells tested in the same experiments. The enhancement was dependent on the concentration of inhibitors (data not shown).

DISCUSSION

AdoMet participates in a number of biological reactions. These reactions are sensitive to inhibition by AdoHcy. Under physiological conditions, however, normal methylation occurs because AdoHcy is hydrolyzed to form adenosine and L-homocysteine through the action of AdoHcy hydrolase. This reaction, catalyzed by AdoHcy hydrolase, is reversible, with the equilibrium constant greatly favoring AdoHcy synthesis. The accumulation of AdoHcy in the cell due to inhibitors results in inhibition of

methylation reactions. In the present experiment, two different approaches were used to inhibit intracellular methylation. The first approach was to add EHNA, an inhibitor of adenosine deaminase (Fig. 2). EHNA alone caused inhibition of histamine release. The addition of suboptimal concentrations of EHNA, which alone were not inhibitory, potentiated the action of adenosine, and this inhibition was more pronounced in the presence of L-homocysteine thiolactone (Fig. 3). From the pathways involved, it can be seen that EHNA in combination with adenosine and L-homocysteine thiolactone could inhibit AdoHcy hydrolase and cause the accumulation of AdoHcy within the cells. Such accumulation of AdoHcy under these conditions has been observed in several systems [2–4]. This combination of adenosine and EHNA plus L-homocysteine thiolactone inhibits *in vivo* DNA methylation in cultured S49 mouse lymphoma cells [2] and protein carboxymethylation of human monocytes [4].

The second approach was to use DZA to cause accumulation of intracellular AdoHcy. DZA is a substrate and inhibitor of AdoHcy hydrolase. In the present experiment, it inhibited histamine release (with an IC_{50} of ~1 mM). This inhibition was potentiated by the addition of L-homocysteine thiolactone. DZA has been found to cause accumulation of intracellular AdoHcy in a number of systems [5, 11, 12, 20]. Protein carboxymethylation, lipid methylation, and guanidoacetate methylation have been reported to be inhibited by DZA [20, 27–29]. The inhibitory effect of DZA was not enhanced by the addition of suboptimal concentrations of two different phosphodiesterase inhibitors; this is strong evidence that DZA does not elevate intracellular cAMP levels. Similar results of a lack of an effect of DZA on murine lymphocyte cAMP levels have been reported [12].

In contrast to IgE-mediated reactions, histamine release induced by ionophore A23187, fMet-Leu-Phe, or C5a was not inhibited but was enhanced significantly by the preincubation of the cells with the combination of adenosine, EHNA, and L-homocysteine thiolactone or DZA with L-homocysteine thiolactone. The mechanism for this enhancment is not clear. The results, however, indicate that histamine release induced by these stimuli does not require a methylation step.

Adenosine inhibits IgE-mediated histamine release from human basophils due to the elevation of cAMP levels following its interaction with a specific membrane receptor [19]. Therefore, the inhibition induced by adenosine could be due to changes in intracellular cAMP levels. Adenosine alone, at all concentrations tested, only partially inhibited histamine release. The potentiation of this adenosine inhibition by EHNA plus L-homocysteine thiolactone suggests that the combination of these compounds was blocking methylation. The accumulation of intracellular adenosine and L-homocysteine would inhibit the hydrolysis of AdoHcy and result in decreased methylation reactions. Further evidence that the combination of adenosine, EHNA, and L-homocysteine thiolactone did not act via the cAMP system is our data that these compounds did not inhibit fMet-Leu-Phe- or C5a-induced histamine release, two activators which are inhibited by increased cAMP levels [22, 25].

The two-stage experiments demonstrate that DZA was effective during the Ca^{2+}-independent first stage of histamine release. DZA did not inhibit when added to activated cells during the second Ca^{2+}-requiring stage. These results, however, do not completely exclude the possibility that methylation is also required during the late stages of histamine release. DZA requires prolonged incubation to produce its effect; therefore, DZA might not have had enough time to act during the histamine release step. The action of EHNA–adenosine–L-homocysteine thiolactone was not reversed by washing; therefore, the experiments could not indicate whether the inhibition was during early or late steps in the histamine release process.

The data presented here suggest that methylation reactions are essential for the IgE-mediated triggering of histamine release from human basophils. The evidence also indicates that the methylation-dependent step occurs during the early non-calcium requiring step(s) of the release process. The rapid rate at which the histamine release reaction occurs suggests that DNA methylation is not involved. Previous experiments have also shown that inhibition of protein synthesis does not affect basophil histamine release. It is possible, however, that methylation of either membrane phospholipids or of preformed proteins plays a crucial role in histamine release. For example, the chromaffin granules have the highest concentration of substrate for protein carboxymethylase in the adrenal medulla [30]. It has been speculated that methylation of the granule membrane results in a change in the charge that allows membrane fusion and exocytosis [30]. Whether methylation opens Ca^{2+} channels, or allows assembly of microtubules or membrane fusion, is open to speculation.

Phospholipid methylation may play an important role in membrane function. Two methyltransferases have been described, asymmetrically distributed in the erythrocyte membrane [31, 32]. One enzyme located on the cytoplasmic surface converts phosphatidylethanolamine to phosphatidyl-*N*-monoethylethanolamine. The second enzyme is on the external surface and transfers two additional methyl groups from *S*-adenosyl-L-methionine to form phosphatidylcholine. Methylation of phosphatidylethanolamine increases erythrocyte membrane fluidity [33] and unmasks cryptic β-adrenergic receptors [34]. In rat reticulocyte ghosts, the β-agonist L-isoproterenol stimulates phospholipid methylation and synthesis of both phosphatidyl-*N*-monomethylethanolamine and phosphatidylcholine [35].

The present results suggest that AdoMet-mediated methylation plays an important role in IgE-mediated histamine release from human basophils. While the present experiments were in progress, we learned of experiments which suggest that membrane phospholipid methylation may play a role in histamine release from rat peritoneal mast cells [36]. There is early membrane phospholipid methylation when rat mast cells are stimulated with concanavalin A; these methylated phospholipids rapidly begin to disappear with increased formation of lysophospha-

tidylcholine. Therefore, methylation of specific phospholipids probably is an essential early step in IgE-mediated cell activation and histamine release.

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