Histamine Release Induced by Human Leukocyte Lysates

REABSORPTION OF PREVIOUSLY RELEASED HISTAMINE AFTER EXPOSURE TO CYCLIC AMP-ACTIVE AGENTS

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ABSTRACT The role of cyclic AMP in histamine release induced by human leukocyte lysates was investigated. Leukocytes were incubated with leukocyte lysates prepared by ultrasonic disruption, and histamine was determined fluorimetrically. Several cyclic AMP-active agents had a marked inhibitory effect on histamine release. Theophylline and isoproterenol produced 50% inhibition at concentrations of less than 10^{-6} M. Prostaglandin E_1 and dibutyryl cyclic AMP inhibited release by 50% at 7×10^{-8} M and 6×10^{-5} M concentrations, respectively. Histamine, which has recently been shown to increase leukocyte cyclic AMP, had a pronounced inhibitory effect on lysate-induced histamine release, producing 50% inhibition at a concentration of only 2.5×10^{-12} M.

Leukocytes, incubated with leukocyte lysates, were sampled at various times and assayed for free histamine released into the incubation mixture supernates, and for bound histamine associated with the leukocyte buttons after centrifugation. Theophylline, prostaglandin E_1 and dibutyryl cyclic AMP not only blocked histamine release, but also caused a progressive decrease in free histamine when added at any time up to 30 min after initiation of the release reaction. As the free histamine decreased after addition of the inhibitors, there was a corresponding increase in the bound histamine, suggesting that previously released histamine was reabsorbed by the leukocytes after exposure to cyclic AMP-active agents. Continued incubation of leukocytes in their own histamine after completion of the release reaction also resulted in reabsorption of the previously released histamine. Previous studies have indicated that cyclic AMP inhibits leukocyte histamine release. The results of the present studies suggest that cyclic AMP modulates histamine release induced by human leukocyte lysates by stimulating reabsorption of histamine from the extracellular environment. These studies also suggest that previously released extracellular histamine may stimulate its own reabsorption by increasing the intracellular level of cyclic AMP.

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INTRODUCTION

Histamine plays a central role in the development of the vasodilation and increased vascular permeability which characterize the acute inflammatory response. The mechanisms of histamine release and its control are, therefore, important aspects of research in acute inflammation. Previous studies have shown that ultrasonic lysates of human leukocytes release histamine from intact human leukocytes in vitro (1). This system provides a model for studies of endogenous mechanisms of histamine release in human inflammation.

Several studies have indicated that cyclic AMP (adenosine 3′,5′-monophosphate) is important in the control of histamine release. Thus, allergic histamine release from leukocytes (2–4), immunological histamine release from human lung (5, 6), and anaphylactic histamine release (7) are inhibited by increased intracellular levels of cyclic AMP. In the present studies, the role of cyclic AMP in leukocyte histamine release induced by human leukocyte lysates was investigated. The results suggest that cyclic AMP not only prevents lysate-induced histamine release but also stimulates reabsorption of previously released histamine.

METHODS

Preparation of leukocytes and leukocyte lysates. The leukocytes used in this study were prepared from the peripheral blood of normal human donors by dextran sedimentation, as previously described (1). Leukocytes were

washed and suspended at a concentration of 1×10^6/ml in an isotonic salts solution containing human serum albumin (tris A) (8). Lysates of these suspensions were prepared by ultrasonic disruption, as previously described (1). For histamine release assays, the leukocytes were washed in tris A and resuspended at a concentration of 1×10^7/ml in tris ACM (tris A supplemented with Ca^{++} and Mg^{++}) (8).

Test agents. Stock solutions of theophylline, isoproterenol (Schwarz/Mann Div. of Becton, Dickinson & Co., Orangeburg, N. Y.), dibutyryl cyclic AMP (N^6,O^2-dibutyryl adenosine 3',5'-cyclic monophosphoric acid (Sigma Chemical Co., St. Louis, Mo.), histamine (Fisher Scientific Co., Fair Lawn, N. J.), and PGE_1 (prostaglandin E_1) (kindly furnished by Dr. John Pike, Upjohn Co., Kalamazoo, Mich.) were prepared in tris ACM and stored at −70°C. The stock solutions were diluted in tris ACM to the desired concentrations just before use.

Histamine release assay. 10 million leukocytes (final concentration 2.5×10^6/ml) were incubated for 30 min at 38°C in 3.5 ml tris ACM containing 0–10^{-2} M concentrations of various agents to be tested. After equilibration, 0.5 ml of leukocyte lysate in tris ACM, containing an appropriate concentration of test agent, was added to the leukocyte suspensions and incubated for 60 min at 38°C. The final concentration of lysate was adjusted to 0.25–1.0 μg protein/ml which provided optimal histamine release. The histamine released into the incubation mixture supernates was assayed fluorimetrically (8), and histamine values were determined from a histamine dihydrochloride standard curve. All values are expressed in terms of histamine dihydrochloride or the percent of the total available histamine released into the incubation mixture supernates.

Control incubations not containing test agent were included in each experiment, and percent inhibition values were calculated based on these controls. Assays of standard histamine solutions containing the maximum concentrations of the inhibitors used in these studies demonstrated that these agents did not interfere with the histamine assay. Spontaneous histamine release in the absence of lysate was monitored in each experiment, and it was always less than 5% of the total available histamine. All experiments were repeated a minimum of five times using the cells of at least two different donors.

In experiments concerning the kinetics of histamine release leukocytes were incubated in the presence of 0.25–1.0 μg lysate protein/ml which provided optimal histamine release. Samples were withdrawn at intervals, and the leukocytes were removed by centrifugation. The resulting supernates were assayed for histamine release. In some experiments, the cell-associated histamine was also measured for each sample after lysis of the sedimented leukocytes in perchloric acid (8).

RESULTS

Effect of theophylline and isoproterenol. Leukocytes were incubated for 30 min in the presence of varied concentrations of isoproterenol or theophylline ranging from 0 to 10^{-3} M. Subsequent challenge with leukocyte lysate revealed the marked inhibitory effect of these agents on histamine release (Table I). Both agents produced 90–100% inhibition at 10^{-3} M concentrations, and the 50% inhibitory concentrations were less than 10^{-5} M. Therefore, catecholamines and methylxanthines are potent inhibitors of histamine release induced by human leukocyte lysates.

TABLE I

Effects of Catecholamines and Methylxyanthines on Histamine Release

Concentration	Percent Inhibition*	
	Isoproterenol	Theophylline
M		
1×10^{-3}	98.0±2.0	88.0±6.0
5×10^{-4}	94.0±3.5	80.0±5.5
1×10^{-4}	82.0±6.0	77.0±1.0
5×10^{-5}	76.0±4.0	69.0±5.0
1×10^{-5}	61.0±2.0	58.0±2.0

* Values expressed as mean ±standard error of the mean for five experiments. Total histamine concentration 0.122±0.005 μg/ml. Average histamine release in the absence of inhibitors was 40.0±3.5% of the total available histamine.

Effect of PGE_1 and dibutyryl cyclic AMP. We next examined the effect of PGE_1 on leukocyte lysate-induced histamine release. Leukocytes were incubated for 30 min in the presence of 10^{-5} M to 10^{-3} M concentrations of PGE_1 and then exposed to leukocyte lysate. PGE_1 was found to be another potent inhibitor of histamine release (Fig. 1) with a 50% inhibitory concentration of approximately 7×10^{-8} M.

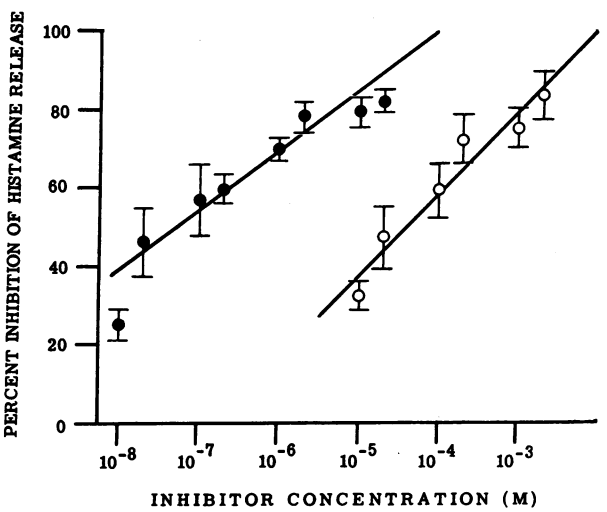

FIGURE 1 The effect of prostaglandin E_1 and dibutyryl cyclic AMP on histamine release induced by human leukocyte lysates. Leukocytes were challenged with leukocyte lysate in the presence or absence of PGE_1 (●–●) or dibutyryl cyclic AMP (○–○), and the percent inhibition of histamine release was determined. Values expressed as mean ±standard error of the mean for five experiments. Total histamine concentration 0.100±0.007 μg/ml. Average histamine release in the absence of inhibitors was 50.0±3.0% of the total available histamine.

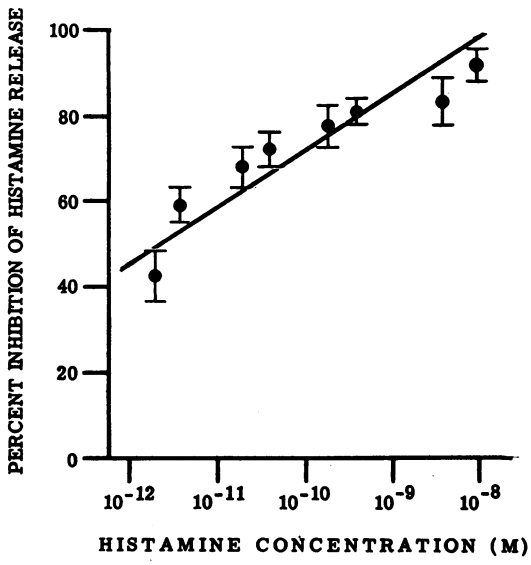

FIGURE 2 Effect of exogenous histamine on leukocyte histamine release induced by human leukocyte lysates. Leukocytes were challenged with leukocyte lysate in the presence or absence of histamine, and the percent inhibition of histamine release was determined. Values expressed as mean ±standard error of the mean for five experiments. Total histamine concentration 0.091±0.007 μg/ml. Average histamine release in the absence of exogenous histamine was 39.0 ±1.5% of the total available histamine.

Since PGE_1, isoproterenol and theophylline are known to increase leukocyte cyclic AMP (9), the effect of dibutyryl cyclic AMP, a lipid soluble form of the active nucleotide, was examined. Leukocytes exposed to this agent produced markedly reduced histamine release upon subsequent challenge with lysate (Fig. 1). The highest concentration tested, 3×10^{-3} M, produced 83% inhibition of histamine release, and a concentration of approximately 6×10^{-5} M dibutyryl cyclic AMP was required for 50% inhibition of release.

Effect of exogenous histamine. Histamine itself has recently been found to increase cyclic AMP in human leukocytes (2). Therefore, we examined the effects of various concentrations of exogenous histamine on the release of endogenous histamine in our system. Leukocytes were incubated in the presence of 0 to 10^{-3} M histamine. After 30 min, the leukocytes were exposed to lysate to determine histamine release. Exogenous histamine was a very strong inhibitor of leukocyte histamine release (Fig. 2). A concentration of 10^{-3} M histamine produced 92% inhibition of histamine release, and 50% inhibition required only about 2.5×10^{-12} M histamine.

Effect of cyclic AMP-active agents on the kinetics of histamine release. In order to assess the timing of the inhibitory effect of cyclic AMP-active agents on lysate-induced histamine release, leukocytes were incubated with lysate, and theophylline was added to a final concentration of 10^{-3} M at 5, 10, and 30 min after the initiation of histamine release. Samples were withdrawn at intervals up to 60 min, and histamine release was determined. Theophylline inhibited histamine release when added to the incubation mixtures at any time up to 30 min (Fig. 3). Interestingly, theophylline not only halted additional histamine release, but also induced a progressive decrease in the free histamine already released (Fig. 3).

This phenomenon was further investigated to determine the fate of the free histamine after addition of theophylline. Time course experiments were performed as above, and theophylline was added 30 min after the leukocyte lysate. Samples were withdrawn at 10 min intervals and assayed for free histamine released into the incubation mixture supernatants. The leukocytes from each sample were treated with perchloric acid (8), and the cell-associated histamine was determined. Histamine release increased progressively, and the cell-associated histamine decreased correspondingly with time until theophylline was added (Fig. 4). In the control not exposed to theophylline, free histamine continued to increase while the cell-associated histamine decreased. In the incubations containing theophylline, free histamine decreased progressively with time (Fig.

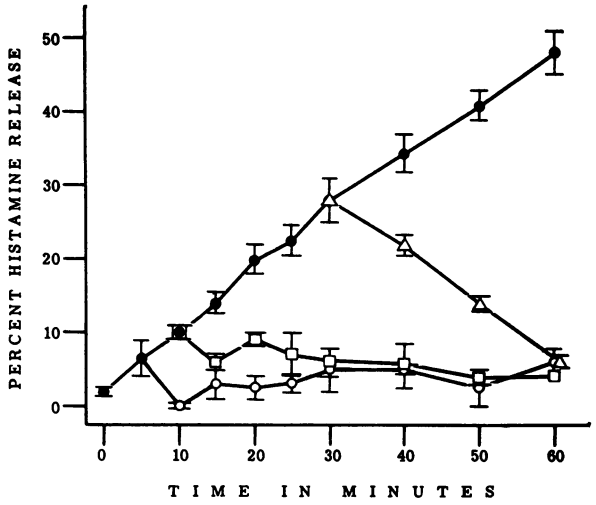

FIGURE 3 Time course of theophylline inhibition of histamine release induced by human leukocyte lysates. Leukocytes were incubated with leukocyte lysate, and the percent histamine release was determined on samples withdrawn at various times (●–●). Theophylline (10^{-3} M final concentration) was added to aliquots of the incubation mixture at 5 (○–○), 10 (□–□), and 30 (△–△) min after the addition of lysate. Values expressed as mean ±standard error of the mean for 12 experiments. Total histamine concentration 0.114±0.004 μg/ml.

4), as noted in the previous experiment. However, the cell-associated histamine increased progressively after the addition of theophylline. The total amount of histamine in the incubation mixtures remained essentially constant in the presence or in the absence of theophylline. This suggests that theophylline not only inhibits release, but it also causes the cells to reabsorb previously released histamine. This experiment was repeated twelve times using the cells of three different donors with the same results although the absolute amounts of leukocyte histamine and the level of histamine release varied from donor to donor.

The effects of other cyclic AMP-active agents on histamine flux in this system were also examined. The addition of 10^{-3} M dibutyryl cyclic AMP or 10^{-6} M PGE_1 after 30 min of incubation with lysate resulted in reabsorption of free histamine nearly identical to that obtained with theophylline.

Since histamine itself is a cyclic AMP-active agent, experiments were done to determine if histamine might stimulate its own reabsorption. Kinetic studies were performed as above except that no inhibitors were added, and samples were withdrawn at 10-min intervals for 2 h. Samples were assayed for both free and cell-associated histamine. In these experiments, histamine was released until a maximum of approximately 0.03 μg/ml (1.6×10^{-7} M) was reached after 50–70 min of incubation (Fig. 5). This rise in free histamine was associated with a concomitant decrease in the cell-associated histamine. Subsequently, the free histamine decreased progressively with time. The cell-associated histamine increased correspondingly during this time to near the original level at the end of 120 min. This experiment was repeated six times using the cells from five different donors with the same result although the absolute amounts of leukocyte histamine and the level of histamine release varied from donor to donor.

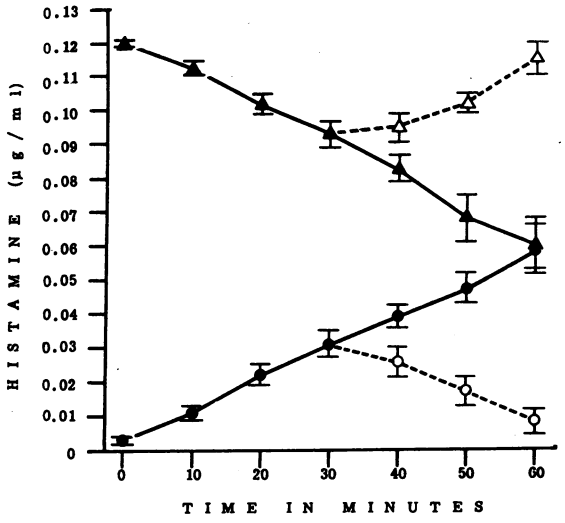

FIGURE 4 Fate of leukocyte histamine after inhibition by theophylline of leukocyte lysate-induced histamine release. Leukocytes were incubated with leukocyte lysate in duplicate suspensions, and samples were withdrawn at 10-min intervals for histamine assays. Samples were assayed for free histamine, released into the supernates, and for histamine associated with the leukocyte buttons after centrifugation. After 30 min of incubation, theophylline (10^{-3} M) was added to one leukocyte suspension. ●—●, free histamine, no theophylline; ○- - -○, free histamine, 10^{-3} M theophylline; ▲—▲, cell-associated histamine, no theophylline; △- - -△, cell-associated histamine, 10^{-3} M theophylline. Values expressed as mean ±standard error of the mean for four experiments using cells from donor R. S.

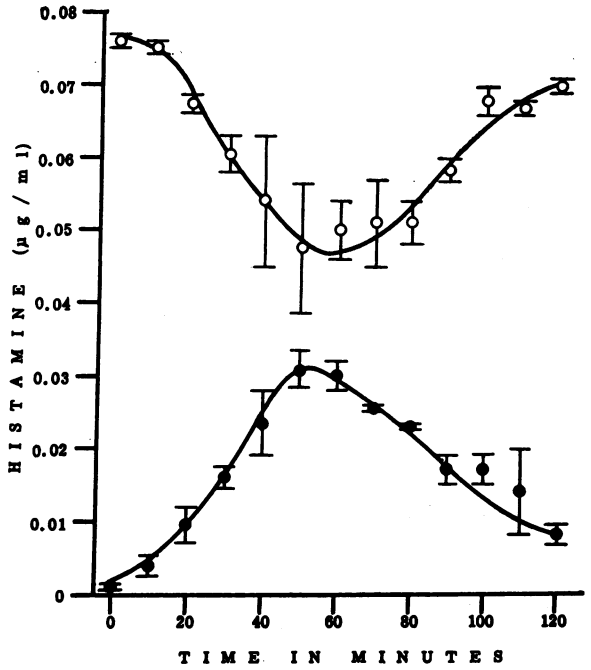

FIGURE 5 Fate of leukocyte histamine after leukocyte lysate-induced histamine release. Leukocytes were incubated with leukocyte lysate, and samples were withdrawn at 10-min intervals for 2 h. Samples were assayed for free histamine released into the supernates, and for bound histamine associated with the leukocyte buttons after centrifugation. ●—●, free histamine; ○—○, cell-associated histamine. Values expressed as mean ±standard error of the mean for six experiments.

DISCUSSION

Our previous studies (1) demonstrated that lysates of human leukocytes release histamine from intact human leukocytes in vitro. We also demonstrated that leukocyte lysates induce histamine release in vivo, using the intact dog as a model (10). More recently, we have obtained evidence that lysate-induced histamine release

involves a specific, complement-independent, noncytotoxic reaction which proceeds optimally under physiological conditions. This system requires oxidative metabolism and is inhibited by mono- and disaccharides.[1] The present study demonstrates that histamine release induced by human leukocyte lysates is inhibited by agents which increase the intracellular levels of cyclic AMP in human leukocytes.

Catecholamines and prostaglandins increase cyclic AMP levels in human leukocytes by stimulation of adenyl cyclase (9). Methylxyanthines augment leukocyte cyclic AMP by inhibition of phosphodiesterase (9). Therefore, the inhibitory effects of isoproterenol, theophylline, and PGE_1 on histamine release induced by human leukocyte lysates strongly suggest that increased intracellular levels of cyclic AMP inhibit histamine release in this system. This role of cyclic AMP is further substantiated by the inhibitory effect of dibutyryl cyclic AMP, a lipid soluble analogue of the endogenous nucleotide.

Cyclic AMP plays a regulatory role in several other histamine releasing systems. Assem and Schild (7) demonstrated that catecholamines and methylxyanthines inhibit anaphylactic histamine release, and Orange, Austen, and Austen (5) presented similar findings for immunological release of histamine from sensitized human lung. Using allergic histamine release from human leukocytes, Lichtenstein and Margolis (11) and Lichtenstein and De Bernardo (3) demonstrated that the cyclic AMP-active agents, PGE_1, isoproterenol, theophylline, and dibutyryl cyclic AMP all inhibit the release reaction. These findings have led to the proposal that cyclic AMP may act as a "second messenger" in histamine release of immunological origin (2, 6). The present studies suggest that cyclic AMP also modulates histamine release induced by human leukocyte lysates.

Our studies suggest a possible mechanism for the regulatory role of cyclic AMP in this system. Theophylline, PGE_1, and dibutyryl cyclic AMP inhibited additional histamine release when added to incubation mixtures at various times, and the extracellular histamine present at the time of addition of these agents decreased progressively with time thereafter (Fig. 3). Kinetic studies of free and cell-associated histamine demonstrated that the leukocyte histamine increased as the extracellular histamine decreased following the addition of cyclic AMP active agents (Fig. 4). These results suggested that previously released histamine was reabsorbed by the leukocytes. The possibility of net synthesis of leukocyte histamine with concomitant degradation of extracellular histamine cannot be excluded. However, this possibility seems unlikely since the total amount of histamine remained nearly constant, and this would require net synthesis exactly equal to the net degradation of histamine in the incubation mixtures. Thus, previously released histamine appears to be reabsorbed after exposure of leukocyte suspensions to agents which increase intracellular levels of cyclic AMP.

Although the basophil leukocytes present in the incubation mixtures are most likely involved in the release of histamine, the elements responsible for histamine reabsorption are not clearly defined by these studies. Histamine may be both released and reabsorbed by basophils, or it may be reabsorbed by other elements, such as eosinophils or platelets, which were present in the incubation mixtures.

This release and reabsorption of histamine in response to different stimuli suggests that control of histamine release may result from regulation of the direction of flow of the amine across the leukocyte membrane. The inducers of histamine release in leukocyte lysates may produce membrane changes which favor the movement of histamine out of the cells, resulting in extracellular accumulation. On the other hand, increased intracellular levels of cyclic AMP may favor the intracellular accumulation of histamine. Thus, the effect of cyclic AMP on the leukocyte lysate system may reflect a change in histamine flux across the leukocyte membrane rather than simply inhibition of histamine release.

Studies of the physiological characteristics and the effects of metabolic inhibitors on lysate-induced histamine release suggest that this system is unlike any previously described.[2] Accordingly, the reversibility of amine flux in response to cyclic AMP in this system must be interpreted cautiously as applied to other types of histamine release. Cyclic AMP-active agents inhibit allergic histamine release only when present during the stage of antigen activation, and reabsorption of histamine has not been demonstrated (3). Thus, reversible amine flux across the leukocyte membrane may not occur in this system. Anaphylactic histamine release is also inhibited by cyclic AMP-active agents, but the effect of these agents after the release reaction is in progress has not been studied (5, 7). Therefore, the reversibility of amine flux in these systems remains to be investigated.

Recently, histamine itself has been shown to increase leukocyte cyclic AMP and to inhibit allergic histamine release (2, 4). In the present studies, exogenous histamine was a potent inhibitor of histamine release induced by leukocyte lysates. However, it should be noted that maximum inhibition was obtained with only 1×10^{-8} M histamine in this system whereas allergic his-

[1] To be published.

[2] To be published.

tamine release required 2×10^{-6} M concentrations (4). This disparity is difficult to explain, but it may represent a unique characteristic of leukocyte lysate-induced histamine release. Such a finding is not without precedent for other cyclic AMP-active agents since Assem and Schild (7) found that anaphylactic histamine release from human lung was maximally inhibited by catecholamine concentrations which had little or no effect on allergic histamine release in the studies of Bourne, Lichtenstein, and Melmon (4). Although no firm conclusions can be drawn, these studies suggest that histamine and cyclic AMP may be linked in a negative feedback system for control of histamine release in inflammation.

The kinetic studies of leukocyte lysate-induced histamine release over a 2-h period revealed that histamine was released during the first hour and reabsorbed during the second hour (Fig. 5). It should be noted that reabsorption of histamine did not begin until the extracellular concentration reached approximately 1×10^{-7} M. In the experiments cited earlier (Fig. 2), histamine release was maximally inhibited by 10-fold lower concentrations of exogenous histamine. This apparent discrepancy may be explained by the fact that the leukocytes were incubated with histamine for 30 min before exposure to lysate in the experiments in Fig. 2 while extracellular histamine was not present until after the addition of lysate in the present experiments. In addition, exogenous histamine dihydrochloride may not influence leukocyte function in exactly the same way as the endogenous amine.

One possible explanation of the histamine release and reabsorption in the 2-h kinetic studies could be that the lysate histamine-releasing factor is inactivated during incubation at 38°C. The histamine-releasing factor is heat labile, and a second dose of leukocyte lysate in such an experiment after 60 min stabilized the level of extracellular histamine during the second hour (unpublished observations). Therefore, one interpretation of this experiment could be that the histamine-releasing factor is inactivated after 60 min, and the cells resynthesize some component which reverses histamine flux during the second 60 min of incubation.

Although such an interpretation cannot be excluded, the data more strongly suggest a mechanism involving cyclic AMP. Histamine is a cyclic AMP-active agent, and, as noted above, agents which increase leukocyte cyclic AMP induced reabsorption of previously released histamine even if added at a time when histamine was being actively released (Fig. 4). A similar mechanism may be involved in the kinetic studies of histamine release over a 2 h period (Fig. 5). As extracellular histamine accumulates during the release phase, it approaches levels ($> 1 \times 10^{-7}$ M) within 50–60 min which are sufficient to stimulate an increase in leukocyte AMP and maximally inhibit histamine release. Such an increase in cyclic AMP could lead to a reversal of histamine flux during the second phase of the reaction.

On a purely speculative basis, histamine flux in this system might be viewed as an equilibrium which is balanced by the interaction of leukocyte lysate and cyclic AMP. Thus, early in the release reaction (Fig. 5) the lysate activity exceeds cyclic AMP activity and histamine is released. Later in the reaction, cyclic AMP activity (stimulated by histamine) may exceed the lysate activity, and histamine is reabsorbed. If the cyclic AMP level is increased early in the reaction, e.g., theophylline added at 30 min (Fig. 4), histamine reabsorption begins earlier. On the other hand, if additional lysate is added at 60 min, the lysate activity is increased, and histamine reabsorption does not occur.

The implications of such a system functioning in vivo are clear. Thus, as extracellular histamine increases at a site of inflammation in vivo, it may increase intracellular levels of cyclic AMP and reverse the direction of histamine flux. Such a negative feedback control mechanism would allow precise control of the histamine levels in inflammatory foci.

The role of histamine in the development of the vasodilation and increased vascular permeability which characterize the acute inflammatory response has been well established (12). Therefore, the mechanisms of histamine release in inflammation have received considerable attention. Histamine release induced by human leukocyte lysates provides a unique system for studies of acute inflammation in man. Other human systems have yielded important information about histamine release in allergic reactions (13) and in anaphylaxis (6), but these systems require exogenous antigens acting on specifically sensitized cells to elicit histamine release. Leukocyte lysate-induced histamine release is a completely endogenous system which may participate in inflammatory states of diverse origin. In this system, the agents of histamine release can be derived from circulating leukocytes and platelets, and they may release histamine from stores in circulating basophiles or tissue mast cells. Since leukocytes and platelets are early inhabitants of most inflammatory foci, the leukocyte lysate histamine release system may play a role in generation of the acute inflammatory response in man. The control of histamine release and the reabsorption of extracellular histamine in response to cyclic AMP-active agents in this system could provide precise control of that part of the inflammatory response due to histamine.

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