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Human granulocyte adhesion to glass capillary tubes was tested in the presence of agents that increase intracellular levels of cyclic 3',5'-adenosine monophosphate (cAMP). Adhesion was significantly reduced by  $10^{-3}$ - $10^{-4}$  M dibutyryl cAMP,  $10^{-4}$ - $10^{-6}$  M prostaglandin E<sub>1</sub> (PGE<sub>1</sub>),  $10^{-4}$ - $10^{-6}$  M histamine, or  $10^{-3}$  M theophylline. Adhesion was not suppressed by  $10^{-4}$  M theophylline unless it was combined with PGE<sub>1</sub> or histamine. Eosinophil and basophil adhesion was especially sensitive to suppression by the above agents. These findings suggest that intracellular cAMP may play a role in regulation of adhesiveness of human basophils, eosinophils, and neutrophils.

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# The Effect of 3',5'-Adenosine Monophosphate on Granulocyte Adhesion

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**ABSTRACT** Human granulocyte adhesion to glass capillary tubes was tested in the presence of agents that increase intracellular levels of cyclic 3',5'-adenosine monophosphate (cAMP). Adhesion was significantly reduced by  $10^{-8}$ – $10^{-4}$  M dibutyryl cAMP,  $10^{-4}$ – $10^{-6}$  M prostaglandin  $E_1$  (PGE $_1$ ),  $10^{-4}$ – $10^{-6}$  M histamine, or  $10^{-8}$  M theophylline. Adhesion was not suppressed by  $10^{-4}$  M theophylline unless it was combined with PGE $_1$  or histamine. Eosinophil and basophil adhesion was especially sensitive to suppression by the above agents. These findings suggest that intracellular cAMP may play a role in regulation of adhesiveness of human basophils, eosinophils, and neutrophils.

## INTRODUCTION

Adhesiveness is a poorly understood cell property that may affect granulocyte margination in capillaries, migration in tissues, and phagocytosis of microorganisms. Several observations suggest that intracellular cyclic 3',5'-adenosine monophosphate (cAMP)<sup>1</sup> might affect granulocyte adhesion. (a) Intracellular cAMP appears to play a role in regulation of human platelet adhesiveness (1). Thus, there is a precedent for modification of adhesiveness attributable to intracellular cAMP. (b) Agents altering intracellular cAMP have been shown to modify granulocyte motility (2), lysosomal enzyme release (3), and histamine excretion (4), thus documenting alteration of leukocyte function attributable to cAMP. And (c) suppression of adhesion by increased

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<sup>1</sup>Abbreviations used in this paper: cAMP, cyclic 3',5'-adenosine monophosphate; cGMP, 3',5'-guanosine monophosphate; LRP, leukocyte-rich plasma; PGE $_1$ , prostaglandin  $E_1$ .

cAMP is consistent with the theoretical consideration that increased intracellular cAMP may suppress participation of leukocytes in the inflammatory process (4, 5). The present study evaluates changes in granulocyte adhesiveness observed after incubation of leukocytes with dibutyryl cAMP, prostaglandin  $E_1$  (PGE $_1$ ), histamine, or theophylline.

## METHODS

PGE $_1$  was kindly furnished by Dr. John Pike, Upjohn Co., Kalamazoo, Mich. Other materials were obtained as follows: theophylline, Mann Research Labs, Inc., New York; histamine, dihydrochloride, EDTA, and dibutyryl 3',5'-adenosine monophosphate (dibutyryl cAMP), Sigma Chemical Co., St. Louis, Mo.; heparin, Connaught Laboratories, Westlake, Ontario, Canada; isoton and zap isoton, Coulter Electronics, Inc., Hialeah, Fla. A Coulter Counter was obtained from Coulter Electronics, Inc.

Human peripheral blood leukocytes were obtained from healthy adult males. Leukocyte-rich plasma (LRP), prepared by previously described methods, had normal differential white blood counts and contained  $1 \times 10^7$  leukocytes/ml and 3.3 mg/100 ml of heparin (6). Dibutyryl cAMP, PGE $_1$ , histamine, and theophylline were prepared and diluted in 0.02 M Tris buffer in isotonic saline adjusted to pH 7.5. Additives were mixed with LRP in a ratio of 1:10 and incubated 10 min at 37°C before performance of adhesion studies. Controls were treated identically but received buffered isotonic saline.

Cell adhesion was tested in glass capillary tubes (6). Mixtures of LRP and additives were placed in capillary tubes, the end was sealed, and tubes were incubated horizontally at 37°C for 10 min before centrifugation at 11,500 rpm for 2 min in an Adams Microhematocrit centrifuge (Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, N. J.). The distal 5 mm of the sealed end of each tube was discarded. Liquid content of each capillary was expelled and discarded and capillary tubes were immersed in 8 ml of isoton containing 10 mM EDTA. Adherent cells were dislodged with injection of 12 ml of isoton-EDTA solution through each tube. Incubation and wash solutions were pooled and mixed with 6 drops of zap isoton. Cells were counted with a model F Coulter Counter. Background counts of tubes incubated with cell-free plasma were subtracted from control and test counts. Adhesion of cells in five tubes was tested for each variable or control. Studies

were performed three times and expressed as mean $\pm$ SEM percent of control adhesion.

Basophil and eosinophil adhesion was tested with LRP similar to that above. This method used incubation, centrifugation, and removal of the liquid content of capillary tubes as described above. Basophils were stained for 5 s with Cooper's stain (7), and tubes were emptied and dried in a slanting position. Eosinophils were stained by Discombe's stain. Basophils or eosinophils adhering to capillary tubes were counted microscopically at 1,250 $\times$  or 500 $\times$ , respectively. Five tubes were examined for each variable. Studies were performed three times and expressed as mean  $\pm$ SEM of the percent of cells present in controls. Specimens were coded so that results were recorded before identification of variables tested. The possibility that change in leukocyte adhesion was due to increased cell aggregation was excluded by demonstration that granulocyte aggregation was reduced by incubation with  $1 \times 10^{-8}$  M PGE<sub>1</sub> (data not shown).

## RESULTS

Changes in leukocyte adhesion after incubation with dibutyryl cAMP, PGE<sub>1</sub>, histamine, or theophylline are shown in Table I. There was an inverse relationship between concentration of dibutyryl cAMP and cell adhesion. Eosinophil and basophil adhesion were especially sensitive to suppression by dibutyryl cAMP. PGE<sub>1</sub> and histamine suppressed adhesion at concentrations of  $10^{-4}$ – $10^{-6}$  M. Both agents produced greater effects on eosinophil and basophil adhesion. Histamine had a slightly

greater suppressive effect on basophils, whereas PGE<sub>1</sub> had a slightly greater effect on eosinophils.

Cell adhesion was suppressed by  $1 \times 10^{-4}$  M theophylline but not by  $10^{-4}$  M theophylline (Table II). Suppressive effects of PGE<sub>1</sub> and histamine were enhanced by  $10^{-4}$  M theophylline and were most clearly shown with eosinophils and basophils.

"Leukocyte" adhesion quantitated by the present methods is best understood in relation to the type of cells adhering to glass despite centrifugation. Three-fourths or more of cells adhering in capillary tubes are neutrophils (6). Thus, changes in "leukocyte adhesion" quantitated with the Coulter Counter are primarily due to changes in neutrophil adhesion.

## DISCUSSION

The importance of leukocyte adhesiveness in the pathophysiology of infection is supported by several considerations. Ingestion of bacteria or incubation with endotoxin enhances neutrophil phagocytic capacity (8, 9). Similarly, ingestion of bacteria or incubation with endotoxin or immune precipitates enhances neutrophil adhesiveness (10, 11). These findings suggest that increased phagocytic capacity of neutrophils after phagocytosis may be attributable to increased leukocyte adhesiveness. Similarly, the enhanced phagocytic capacity

TABLE I  
Effect of Dibutyryl cAMP, PGE<sub>1</sub>, Histamine, and Theophylline on Leukocyte Adhesion

Compound	Concentration	Leukocyte adhesion*	Eosinophil adhesion	Basophil adhesion
	<i>M</i>		Percent of control† (Mean $\pm$ SEM)	
Dibutyryl cAMP	$1 \times 10^{-3}$	60 $\pm$ 4	9 $\pm$ 1	19 $\pm$ 2
	$1 \times 10^{-4}$	79 $\pm$ 3	22 $\pm$ 5	17 $\pm$ 6
	$1 \times 10^{-5}$	97 $\pm$ 2	60 $\pm$ 6	98 $\pm$ 4
	$1 \times 10^{-6}$	98 $\pm$ 2	77 $\pm$ 4	—
PGE <sub>1</sub>	$1 \times 10^{-4}$	42 $\pm$ 5	5 $\pm$ 1	19 $\pm$ 3
	$1 \times 10^{-5}$	50 $\pm$ 6	9 $\pm$ 1	19 $\pm$ 5
	$1 \times 10^{-6}$	77 $\pm$ 5	43 $\pm$ 6	38 $\pm$ 4
Histamine	$1 \times 10^{-4}$	38 $\pm$ 3	13 $\pm$ 3	4 $\pm$ 1
	$1 \times 10^{-5}$	34 $\pm$ 5	16 $\pm$ 4	4 $\pm$ 1
	$1 \times 10^{-6}$	78 $\pm$ 2	76 $\pm$ 7	29 $\pm$ 2
Theophylline	$5 \times 10^{-3}$	26 $\pm$ 2	1 $\pm$ 0.3	<1
	$1 \times 10^{-3}$	63 $\pm$ 7	18 $\pm$ 5	32 $\pm$ 9
	$1 \times 10^{-4}$	91 $\pm$ 3	82 $\pm$ 2	116 $\pm$ 3

\* Adhering cells are 75–80% neutrophils, 3–6% eosinophils, 1–3% basophils, and 12–15% monocytes.

† Percent of control adhesion =  $100 \times (\text{no. of cells adhering in test}) / (\text{no. of cells adhering in control})$ . Mean number of cells adhering per tube for control studies done with dibutyryl cAMP, PGE<sub>1</sub>, histamine, and theophylline were as follows: leukocyte adhesion = 7,947, 5,768, 6,830, and 8,040, respectively; eosinophil adhesion = 1,265, 920, 815, and 765, respectively; basophil adhesion = 425, 270, 391, and 551, respectively.

TABLE II  
Effect of Drug Combinations on Leukocyte Adhesion

Compound	Concentration	Leukocyte adhesion*	Eosinophil adhesion	Basophil adhesion
	<i>M</i>		Percent of control‡ (Mean ± SEM)	
Theophylline	1 × 10 <sup>-4</sup>	99 ± 3	95 ± 7	102 ± 7
PGE <sub>1</sub>	1 × 10 <sup>-6</sup>	60 ± 2	43 ± 4	57 ± 6
PGE <sub>1</sub>	1 × 10 <sup>-6</sup>	48 ± 3	10 ± 1	14 ± 2
+ theophylline	1 × 10 <sup>-4</sup>			
Histamine	1 × 10 <sup>-6</sup>	65 ± 3	78 ± 8	20 ± 3
Histamine	1 × 10 <sup>-6</sup>	43 ± 4	12 ± 4	4 ± 1
+ theophylline	1 × 10 <sup>-4</sup>			

\* Adhering cells are 75–80% neutrophils, 3–6% eosinophils, 1–3% basophils, and 12–15% monocytes.

‡ Percent of control adhesion = 100 × (no. of cells adhering in test)/(no. of cells adhering in control). Mean number of cells adhering per tube for controls of leukocyte adhesion, eosinophil adhesion, or basophil adhesion were 6,677, 765, and 394, respectively.

of leukocytes during “surface phagocytosis” on filter paper may reflect the necessity of leukocytes to adhere well to surfaces in order to move and ingest organisms efficiently (12).

Leukocyte margination is one of the first demonstrable changes in the inflammatory process but is greatly suppressed by corticosteroids (13). After demonstration that corticosteroids suppressed leukocyte adhesion to glass (personal observation) and that hyperosmolality, which suppressed leukocyte delivery to renal medullary tissues, also suppressed the leukocyte adhesiveness per se (14), it appeared likely that the property of granulocyte adhesiveness might significantly affect delivery and local function of leukocytes in the infectious or immunologically mediated inflammatory process.

Eosinophil and basophil adhesiveness was especially sensitive to suppression and suggests that intracellular cAMP may play a significant role in bioregulation of those cells *in vivo*.

Platelet adhesiveness is suppressed by cAMP and may be regulated in part by intracellular cAMP (1). Comparable suppression of adhesiveness of basophils, eosinophils, and neutrophils by cAMP is demonstrated in the present study. This is a new finding and suggests that regulation of cell adhesiveness by cAMP may represent a bioregulatory mechanism common to a number of cell types.

Increased intracellular cAMP inhibits a number of other granulocyte functions such as secretion of histamine by basophils (4), release of lysosomal enzymes (3), and leukocyte motility (2). The concentrations of agents used to produce such effects were comparable

to those used in the present study. The effect of cyclic 3',5'-guanosine monophosphate (cGMP) on release of lysosomal enzymes appears to be opposite to that of cAMP (3). The effect of cGMP on adhesion is untested.

Histamine is thought to increase intracellular cAMP, which in turn is thought to affect both absorption and excretion of histamine by basophils (4, 15). Since histamine suppressed granulocyte adhesiveness, it is possible that intracellular cAMP of basophils or mast cells may affect histamine release and, therefore, secondarily affect granulocytes.

It has been postulated that increased intracellular cAMP might exert a suppressive effect on the inflammatory process (4, 5). The present studies support that hypothesis and suggest that increased intracellular cAMP may suppress the inflammatory response by suppressing leukocyte adhesiveness and thereby modifying delivery of leukocytes to sites of infection. This suggestion is speculative and should be substantiated by studies performed *in vivo*. Clarification of this point may provide new insight into mechanisms regulating leukocyte adhesiveness and participation in the inflammatory process.

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