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Charles W. Parker, ... , Mary L. Baumann, Mary G. Huber

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Research Article

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Alterations in Cyclic AMP Metabolism in Human Bronchial Asthma

II. LEUKOCYTE AND LYMPHOCYTE RESPONSES TO PROSTAGLANDINS

CHARLES W. PARKER, MARY L. BAUMANN, and MARY G. HUBER

*From the Washington University School of Medicine, Department of Medicine,
St. Louis, Missouri 63110*

ABSTRACT In an effort to clarify the basis for the reduced cyclic AMP response to catecholamines in leukocytes and lymphocytes from asthmatic donors the response of these cells to prostaglandins has been examined. Cells with an impaired beta adrenergic response had an essentially unaltered response to prostaglandin E_1 (PGE_1) indicating the presence of selective beta adrenergic blockade. In contrast to what was observed with cells from asthmatic individuals, in normal control leukocytes with reduced catecholamine responsiveness PGE_1 responses were usually reduced as well, suggesting a different mechanism. The excellent cyclic AMP response to PGE_1 in cells from asthmatic donors would suggest that the defect in catecholamine responsiveness is at the level of the beta adrenergic receptor although a contributory role of altered substrate concentrations or increased phosphodiesterase activity is not formally excluded.

INTRODUCTION

In previous investigations evidence has been presented to indicate that leukocytes (and lymphocytes) from individuals with severe, chronic bronchial asthma have a reduced cyclic AMP response to beta adrenergic agents (1, 2) as well as a decreased cyclic AMP concentration in the absence of hormonal stimulation. While the decreased catecholamine response is consistent with beta adrenergic blockade the decreased cyclic AMP values in unstimulated cells would raise the possibility of an over-

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all, nonselective reduction in adenylate cyclase activity or an increased rate of cyclic AMP degradation. If the reduction in responsiveness is due to beta adrenergic blockade, prostaglandins and other agents which activate adenylate cyclase through nonadrenergic receptors (3) would be expected to produce a normal cyclic AMP response and might be useful therapeutically in individuals with epinephrine resistant asthma. In the present study, the cyclic AMP response of leukocytes and lymphocytes from asthmatic individuals to prostaglandins has been examined in detail and compared with that of cells from normal controls.

METHODS

Prostaglandins were generously provided by Dr. John Pike of the Upjohn Co. Procedures for the preparation of prostaglandin solutions and isolation of leukocytes and lymphocytes were described previously (1, 4). The composition of Gey's solution is given in reference 5. Criteria for the diagnosis of bronchial asthma are discussed in detail in the preceding paper (1). The majority of the patients studied had severe, chronic asthma. Purified cells, $4-8 \times 10^6$ leukocytes/ml or $2-4 \times 10^6$ lymphocytes/ml, were suspended in 0.5 ml Gey's solution and incubated at 37° with 0.05 ml buffer, catecholamine, or PGE_1 solution for various time periods. The cells were centrifuged at 2,500 rpm, the pellets frozen in ethanol-dry ice and their cyclic AMP content determined by radioimmunoassay (1).

RESULTS

Response of leukocytes from asthmatic donors to PGE_1 . The cyclic AMP responses of mixed leukocytes from asthmatic and normal control donors to $30 \mu M$ PGE_1 are shown in Table I. On the basis of the absolute increase in cyclic AMP concentrations (above levels in cells incubated in buffer alone) there was a modest but significant decrease in the PGE_1 response in cells from asthmatic individuals. However, when cyclic AMP stimula-

TABLE I
Effect of PGE₁ on Cyclic AMP Concentrations
in Mixed Leukocytes

	No. sub-jects	No. deter-min-ations	Cyclic AMP		Ratio PGE ₁ : unstimu-lated
			Unstimu-lated	PGE ₁ , 30 μM	
<i>pmol/10⁷ cells*</i>					
Asthma	20	27‡	5.8(±0.5)	39.1(±1.5)	6.7
Normal controls	16	21	8.0(±0.7)	53 (±1.2)	6.6

Mixed leukocytes were incubated in Gey's solution with and without 30 μM PGE₁ for 30 min at 37°. For details of methods see reference 1. The data are from leukocyte preparations containing 25–40% lymphocytes. The normal controls are matched with the asthmatic donors with respect to age, race, and sex. Donors receiving oral contraceptive therapy are excluded.

* ±SEM.

‡ 21 with active asthma; 6 inactive.

tion ratios are compared (PGE₁: buffer control cells) the response in cells from asthmatic individuals was not altered. Thus the leukocyte PGE₁ response was essentially unimpaired in individuals with bronchial asthma, confirming the results of previous studies (2, 6) despite the decreased cyclic AMP values in unstimulated cells.

Responses of normal control lymphocytes and polymorphonuclear leukocytes to PGE₁. We have previously demonstrated that human lymphocytes purified by isopycnic centrifugation in a Ficoll-Hypaque gradient have a much greater cyclic AMP response to isoproterenol (comparing equal numbers of cells) than purified polymorphonuclear leukocytes obtained by a similar purification procedure (1). When lymphocytes were further purified by passage through a nylon fiber column the isoproterenol response/10⁷ cells was decreased, presumably because cells with marked isoproterenol responsiveness selectively adhere to the nylon (1, 7). However, the nylon purified cells still had a much greater catecholamine response than purified polymorphonuclear leukocytes. Studies of PGE₁ responsiveness in the three purified cell populations gave similar results with the order of responsiveness being Ficoll-Hypaque purified lymphocytes > nylon fiber purified lymphocytes >> Ficoll-Hypaque purified polymorphonuclear leukocytes. A representative experiment in which PGE₁ dose response curves of Ficoll-Hypaque purified polymorphonuclear leukocytes and nylon fiber purified lymphocytes from the same individual are compared is shown in Fig. 1. While the threshold PGE₁ concentrations required to produce a response were similar, the magnitude of the response was much greater in the purified lymphocyte preparation. Thus it is unlikely that the relatively good PGE₁ response in leukocytes from asthmatic donors is due to polymorphonuclear leukocytes in the cell mixture.

Bourne, Lehrer, Cline, and Melmon (8) have estimated that lymphocytes contribute only about 25% of the

cyclic AMP response to PGE₁ in mixed leukocyte preparations. However, they used different media and incubation times and cyclic AMP was measured by the ³H adenine-labeled precursor method as compared with our own direct measurements of cyclic AMP in a competitive binding assay. Actually there may be less discrepancy between their results and our own than would appear since they did not examine purified granulocytes and in their calculations it was assumed that glass wool-purified lymphocytes would have the same adenylate cyclase activity as unfractionated lymphocytes.

The time course of PGE₁ stimulation of lymphocyte cyclic AMP concentrations was studied (four experiments). Representative data obtained at 30 μM PGE₁ are given in Fig. 2. The response was maximal at 2 and 10 min, almost maximal at 30 min and markedly reduced at 60 and 120 min. The decrease in cyclic AMP concentration after 30 min was not due to degradation of PGE₁ since supernatants from cells incubated with PGE₁ for 120 min markedly stimulated cyclic AMP accumulation in fresh lymphocytes. The fall was largely prevented by 4 mM theophylline, raising the possibility that it may be due to induction of increased phosphodiesterase activity as suggested in other tissues by Maganiello and Vaughan (9). By contrast the isoproterenol response was not maximal until after 10 min and the response was well sustained at 60 and 120 min, even in the absence of a

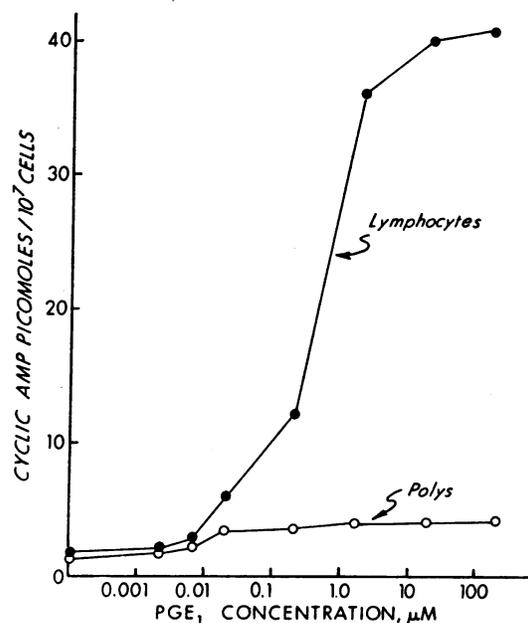


FIGURE 1 Comparative PGE₁ dose response curves of nylon fiber purified lymphocytes and Ficoll-Hypaque purified polymorphonuclear leukocytes from the same individual. 2×10^6 cells were incubated at 37° for 10 min in 0.5 ml Gey's solution.

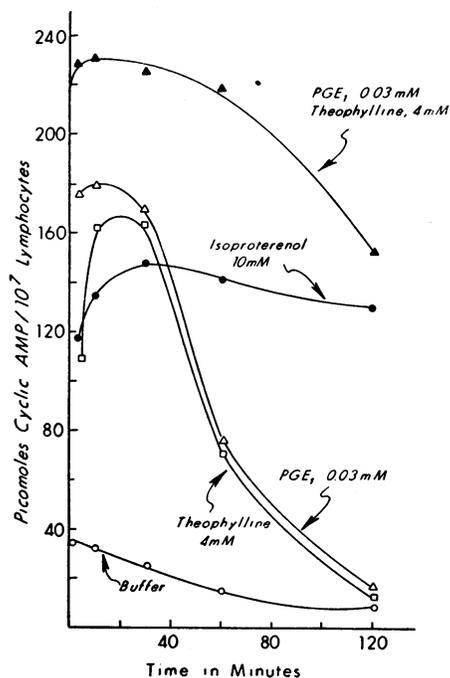


FIGURE 2 Time course of PGE₁ and isoproterenol stimulation in Ficoll-Hypaque purified lymphocytes from a normal control subject. 1.5 million lymphocytes were incubated at 37° in 0.5 ml Gey's solution for 2, 10, 30, 60, and 120 min: ▲—▲, 0.03 mM PGE₁, 4 mM theophylline; □—□, 4 mM theophylline; ●—●, 10 mM isoproterenol; △—△, 0.03 mM PGE₁; ○—○, buffer control.

phosphodiesterase inhibitor. Based on these studies a 30 min stimulation period was selected as providing a maximal or near maximal response to both 10 mM isoproterenol and 30 μM PGE₁ in purified lymphocytes.

Responses of lymphocytes from asthmatic donors to PGE₁. The assumption that the PGE₁ response in leukocytes from asthmatic individuals is largely localized to lymphocytic cells is supported by the results of studies with purified lymphocytes. As shown in Fig. 3, the asthma lymphocyte cyclic AMP response to 30 μM PGE₁ was nearly that of normal control cells and substantially greater than the 10 mM isoproterenol response. The possibility was considered that the more marked PGE₁ response might be due to 30 μM PGE₁ being a more effective adenylate cyclase stimulant than 10 mM isoproterenol (as indicated by a PGE₁:isoproterenol ratio of 1.3 in normal lymphocytes, legend to Fig. 3). The same comparison was therefore made at lower PGE₁ concentrations. At 3 μM PGE₁, which produces essentially the same response as 10 mM isoproterenol in normal lymphocytes (PGE₁:isoproterenol ratio of 1.1), asthma lymphocytes again had a much greater response to PGE₁ than isoproterenol.

As shown in Fig. 3, there was significant overlap between the isoproterenol responses in normal control and asthma cells. When results in selected individuals with chronic, severe asthma were analyzed greater changes in isoproterenol responsiveness were obtained. Fig. 4 compares PGE₁ and isoproterenol responses in the severe asthma group with results in cells from matched (age, race, and sex) normal controls. In this set of normal control leukocytes 30 μM PGE₁ again produced a slightly greater increase in cyclic AMP than 10 mM isoproterenol (a PGE₁:isoproterenol ratio of 1.5) whereas at 10 mM isoproterenol and 3 μM PGE₁ the responses were very similar (a PGE₁:isoproterenol ratio of 1.0). The respective ratios for leukocytes from the asthmatic patients were 4.5 and 3.6, respectively. Similar results were obtained in studies with purified lymphocytes (Fig. 4). The results include cells from one individual with asthma with a poor cyclic AMP response to PGE₁. If these cells are omitted from the pooled data the PGE₁:isoproterenol ratios in lymphocytes from asthmatic donors are even higher. These observations indicate that in cells from donors with unusually severe asthma the discrepancy between isoproterenol and PGE₁ responsive-

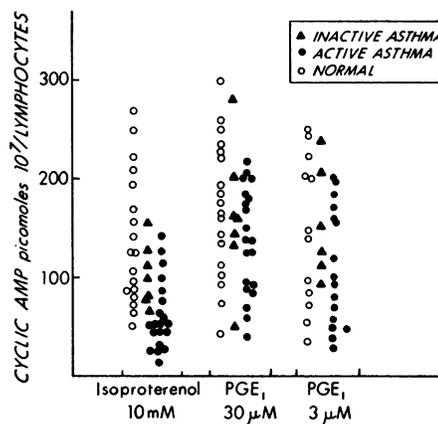


FIGURE 3 Responses of Ficoll-Hypaque purified lymphocytes to isoproterenol and PGE₁. Cells are from adults with clinically active bronchial asthma or matched (age, race, and sex) normal controls. The isoproterenol data are also given in Fig. 5 of reference 1. 1.5×10^6 cells were incubated in 0.5 ml Gey's solution at 37° for 30 min in the presence of 30 μM PGE₁, 3 μM PGE₁, 10 mM isoproterenol or buffer. Buffer control values (\pm SEM) for cells from asthmatic and normal control donors were 18(\pm 2) and 26(\pm 3), respectively. The respective values for 10 mM isoproterenol were 57(\pm 9) and 140(\pm 15); for 30 μM PGE₁, 137(\pm 15) and 170(\pm 18); for 3 μM PGE₁ 113(\pm 15) and 148(\pm 20). The 30 μM PGE₁:10 mM isoproterenol ratios (with buffer control cell values subtracted) for asthma and normal control cells are 3.1 and 1.3, respectively. The corresponding ratios for 3 μM PGE₁:10 mM isoproterenol are 2.4 and 1.1, respectively.

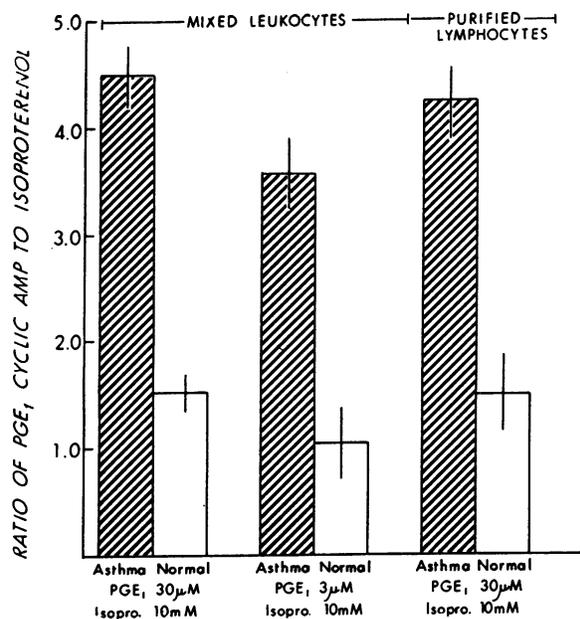


FIGURE 4 Comparative stimulation of leukocyte (or lymphocyte) cyclic AMP accumulation by PGE₁ and isoproterenol in cells from patients with severe asthma and normal controls (expressed as the PGE₁:isoproterenol ratio, e.g. the absolute increase in cyclic AMP above the buffer control with PGE₁ divided by the absolute increase above the control with isoproterenol). 4×10^6 leukocytes or 1.5×10^6 lymphocytes were incubated in 0.5 ml Gey's solution for 30 min at 37° in the presence of 30 µM PGE₁, 10 mM isoproterenol or buffer. The number of subjects (and experiments) in each group are: asthma leukocytes, 12; asthma lymphocytes, 10; normal control leukocytes, 12 (matched with asthma leukocyte donors); normal control lymphocytes, 10 (matched with asthma lymphocyte donors). Leukocyte preparations from asthmatic donors contained an average of 29% lymphocytes as compared with 31% in normal control cells. Buffer control values (in picomoles cyclic AMP/10⁷ cells, \pm SEM) were as follows: asthma leukocytes 4.9 (\pm 0.6); asthma lymphocytes 14.5 (\pm 0.7); normal control leukocytes 7.9 (\pm 0.7); normal control lymphocytes 24.9 (\pm 1.9).

ness is accentuated, as might occur in association with more marked beta adrenergic blockade.

The PGE₁ response in control cells that respond poorly to isoproterenol. Normal control cells stimulated with 10 mM isoproterenol occasionally gave cyclic AMP values in the range of cells from asthmatic donors. It was of interest to examine the PGE₁ response in these cells to see if they exhibited the same alteration in PGE₁:isoproterenol ratio that is found in association with bronchial asthma (Table II). In five experiments in normal control leukocytes with a reduced catecholamine response the PGE₁:isoproterenol ratio (at 30 µM and 10 mM concentrations, respectively) was below 1.5 in two, between 1.5 and 2.0 in two, and 2.5 in one. The corresponding

ratio in leukocytes from donors with severe asthma was above 4.0 (see also, Fig. 4). Thus whatever the basis for the alteration in the isoproterenol response in normal control cells there is ordinarily a parallel reduction in both the PGE₁ and isoproterenol response, differing qualitatively from what is observed with cells from asthmatic donors.

Effect of prostaglandins on the isoproterenol response. The effect of various concentrations of PGE₁ on the catecholamine response in lymphocytes from asthmatic and normal control subjects was investigated. In experiments in which cells were preincubated with PGE₁ for 25 min and then stimulated with 1 µM epinephrine for 5 min the only major changes which occurred could be explained on the basis of simple summation of prostaglandin and catecholamine effects (Fig. 5). Similar results were obtained when 10 mM isoproterenol, 10 µM epinephrine, or 1 µM epinephrine was incubated together with PGE₁ for 10 or 30 min.

DISCUSSION

In the present study the cyclic AMP response of leukocytes and lymphocytes from asthmatic donors to beta adrenergic agents has been compared with the PGE₁ response. It has been possible to show that asthma cells that respond poorly to isoproterenol usually have an essentially unaltered response to PGE₁ over a broad range of PGE₁ concentrations. The better PGE₁ response is not due to its being a more effective adenylate cyclase stimulator than isoproterenol. At 3 µM PGE₁, which produces about the same cyclic AMP response as 10 mM isoproterenol in normal control cells, PGE₁ continued to stimulate asthma cells to a much greater extent than

TABLE II
PGE₁ Responsiveness in Normal Control Leukocytes that Respond Poorly to Isoproterenol

Cell preparation	Cyclic AMP			Ratio PGE ₁ :isoproterenol*
	Control	Isoproterenol, 10 mM	PGE ₁ , 30 µM	
	<i>pmol/10⁷ cells</i>			
1. B. S., leukocytes	5.0	14.0	15.5	1.2
2. E. J., leukocytes	6.0	12.0	14.0	1.3
3. M. B., leukocytes	4.0	11.0	17.0	1.8
4. T. K., leukocytes	8.0	15.0	25.5	2.5
5. M. H., leukocytes	4.0	10.0	15.0	1.8
6. Asthma leukocytes†	4.9	13.1	42.0	4.5
7. Control leukocytes‡	7.9	35.0	48.0	1.5

Leukocytes were incubated in Gey's solution for 30 min at 37°. Data are from leukocyte preparations containing 25–40% lymphocytes.

* Expressed as the absolute increase in cyclic AMP concentrations above the buffer control with PGE₁ divided by the increase above the control with isoproterenol.

† Pooled data obtained with cells from 12 individuals with severe asthma and 12 normal controls. The same data are utilized in Fig. 3.

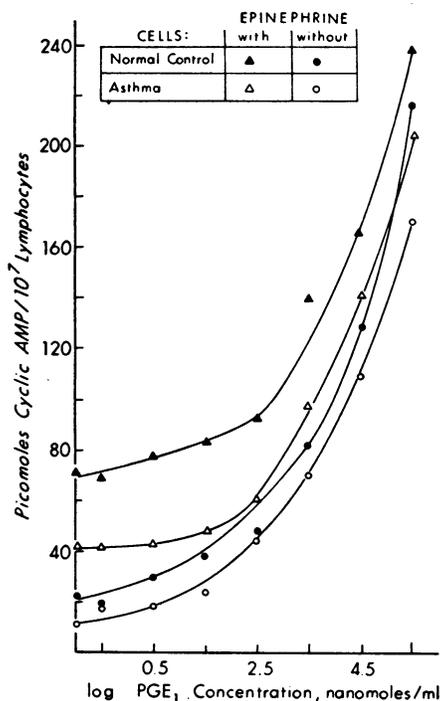


FIGURE 5 Effect of PGE₁ and PGE₂ in combination with 1 μM epinephrine, 0.5 mM theophylline on cyclic AMP accumulation in Ficoll-Hypaque purified lymphocytes. Pooled data from three normal and three asthmatic donors: 1.5 million lymphocytes were incubated at 37° in 0.5 ml Gey's solution for 25 min; 0.05 ml buffer or 0.05 ml of 10 μM epinephrine-0.5 mM theophylline solution was added and cells incubated for an additional 5 min at 37°. ▲—▲, normal control cells with epinephrine; ●—●, normal control cells without epinephrine; △—△, asthma cells with epinephrine; ○—○, asthma cells without epinephrine. Cyclic AMP values for normal control and asthma cells with 0.5 mM theophylline alone were 49 and 22 pmol/10⁷ cells, respectively.

isoproterenol. The increased PGE₁:isoproterenol ratio in association with severe bronchial asthma differed from what was observed with preparations of normal control cells with decreased responsiveness to isoproterenol. Each of five such preparations also had a reduced response to PGE₁, suggesting that when catecholamine responsiveness is diminished in normal control cells the metabolic alteration differs from that in asthma cells.

In considering the mechanism of reduced beta adrenergic responsiveness in leukocytes and lymphocytes from asthmatic donors the possibility was entertained that prostaglandins might exert a catecholamine sensitizing effect, with reduced isoproterenol responsiveness in asthma cells being explained on the basis of systemic prostaglandin deficiency. However, there was no evidence that cells from asthmatic individuals were usually responsive to low concentrations of prostaglandins or that

such concentrations markedly altered catecholamine responsiveness. Any changes that were observed appeared to be explicable on the basis of additive effects of prostaglandins and adrenergic agents without evidence of clear-cut synergism.

The fact that PGE₁ responsiveness is essentially normal in leukocytes and lymphocytes from asthmatic donors must be considered in any theory attempting to explain why adrenergic responsiveness is decreased in these cells. PGE₁ and isoproterenol interact with cell membranes at different receptor sites, as evidenced by the ability of specific blocking agents to inhibit one response and not the other (4, 10). In broken lymphocyte preparations both agents stimulate adenylate cyclase (4) which is presumably the mechanism by which they increase cyclic AMP in intact cells. In view of the unimpaired PGE₁ response it is tempting to assume that the altered response to catecholamines is due to blockade at the level of the beta adrenergic receptor. However, recent studies in this laboratory provide strong presumptive evidence that PGE₁ and isoproterenol act on adenylate cyclase molecules in different regions of the lymphocyte (11). The isoproterenol responsive cyclase appears to be primarily in the nucleus whereas the PGE₁ responsive cyclase is in the cytoplasm. Since isoproterenol and PGE₁ act in different subcellular compartments they do not draw on the same ATP pool and the cyclic AMP they produce may not be equally susceptible to hydrolysis by cyclic AMP phosphodiesterase. Thus, alterations in nuclear ATP concentrations or phosphodiesterase activity may conceivably explain the reduced isoproterenol response (12). It seems more likely that the alteration is localized to the beta adrenergic receptor itself, involving either reduced catecholamine binding, or a decreased ability of membrane-bound catecholamine to influence adenylate cyclase activity. These possibilities are currently being evaluated in isolated lymphocyte nuclei from asthmatic donors. Differences in the primary site of action of isoproterenol and PGE₁ inside the lymphocyte might also explain the more prolonged cyclic AMP response to isoproterenol (Fig. 2) since the nucleus and cytoplasm would presumably contain different phosphodiesterase pools. Another contributory factor might be catecholamine inhibition of phosphodiesterase as recently described by Goren and Rosen (13), although work in progress does not indicate that this is likely to be a major cause.

The basis for the altered catecholamine response in leukocytes from asthmatic patients has been considered in detail in the previous paper in this series (1). Several lines of evidence indicate that bronchodilator therapy per se is not a likely explanation although it is not excluded as a contributing factor in individuals on very aggressive treatment programs. Serial lymphocyte studies

in normal control subjects receiving a commonly used oral bronchodilator agent over a 2 wk period do not reveal changes in catecholamine responsiveness. Moreover, there is a decreased cyclic AMP response to beta adrenergic agents in leukocytes of individuals with recent asthma who have been off of all therapy for at least 2 days or 7 days. This excludes tachyphylaxis as a major factor in the altered response. As a group individuals with asthma that has been inactive over an extended period may have a modest reduction in their catecholamine response although there is extensive overlap with the normal control response. During active asthma a possible role of endogenous catecholamine release in the altered response is not specially excluded. However, the studies of Morris, DeRoche, and Earle indicate that the stress of an acute asthmatic attack is usually not associated with increased catecholamine excretion even though the same individuals mobilize catecholamines in response to a hypoglycemic stimulus (14). Even though the mechanism of the alteration in leukocyte adrenergic responsiveness is presently unclear the fact that PGE₁ responsiveness is retained in these cells could eventually have practical implications. PGE₁ and PGE₂ are known to relax tracheal and bronchial smooth muscle in vitro in lower animals and man (6, 15, 16) and they also produce bronchodilation in human subjects with asthma (17). While their effectiveness as aerosol medications in epinephrine refractory asthma is not yet known, since they do not work through beta adrenergic receptors, it seems quite possible that they would be useful in this situation. The major limiting factor at present is the nonspecific bronchial irritation produced by existing PGE-containing aerosol preparations. If this difficulty can be overcome it seems likely that local prostaglandin therapy will be of practical value in the treatment of bronchial asthma.

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