Regulation of Endothelial Cell Cyclic Nucleotide Metabolism by Prostacyclin

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ABSTRACT An analysis of prostaglandin-stimulated adenosine 3′,5′-cyclic monophosphate (cyclic AMP) accumulation in cultured human umbilical vein endothelial cells showed prostacyclin (PGI_2) to be the most potent agonist followed by prostaglandin (PG)H_2, which was more potent than PGE_2, while PGD_2 was essentially inactive.

The endothelial cells studied apparently have a high rate of cyclic AMP phosphodiesterase activity because significant PGI_2-mediated increases in cyclic AMP could only be shown in the presence of the phosphodiesterase inhibitor isobutylmethylxanthine (MIX).

Endoperoxide PGH_2-stimulation of cyclic AMP accumulation was inhibited 75–80% by the prostacyclin synthetase inhibitors 12-hydroperoxyeicosatetraenoic acid or 9,11-azoprosta-5,13-dienoic acid. These data indicate that the PGH_2-stimulation is due primarily to conversion to PGI_2.

The beta-adrenergic agonist L-isoproterenol stimulated cyclic AMP accumulation in the endothelial cells. This accumulation was completely blocked by propranolol. However, stimulation of cyclic AMP accumulation by the beta-adrenergic agent did not equal that induced by PGI_2. Furthermore, the PGI_2 response could not be blocked by propranolol.

Thrombin-stimulated PGI_2 biosynthesis was attenuated by PGE_1 or isoproterenol in the presence of MIX. MIX alone was less effective than a combination of PGE_1 or isoproterenol plus MIX.

These data suggest two potential effects of PGI_2 biosynthesis by endothelial cells: first, the PGI_2 can elevate cyclic AMP in platelets, and second, endothelial cell cyclic AMP can be elevated as well, so that subsequent PGI_2 synthesis will be attenuated.

INTRODUCTION

Prostacyclin [(5Z)-9-deoxy-6,9-α-epoxy-Δ^5-prostaglandin $F_{1\alpha}$] (PGI_2)[1] is a labile prostaglandin that is a potent vasodilator and the most powerful inhibitor of human platelet aggregation yet described (1–4). Vascular tissue and especially vascular endothelium are rich sources of PGI_2 synthetase activity (5–7), and the relationship(s) between the synthesis of the antiaggregatory PGI_2 by vascular elements and the synthesis of the proaggregatory molecule thromboxane A_2 by platelets have been widely discussed (3, 4, 8, 9).

PGI_2 is a potent stimulator of platelet adenylate cyclase (3, 4), and elevates cyclic AMP levels in cultured human foreskin fibroblasts (10), human fat cell ghosts (11), and several clonal cell lines of central nervous system origin (12). PGI_2 also elevates cyclic AMP levels in arterial rings (13), but no experiments on the effect of PGI_2 on cyclic AMP levels in endothelial cells have been reported to date.

In the present study we show that PGI_2 is a potent stimulator of cultured human umbilical vein endothelial cell cyclic AMP levels, and suggest that endogenously produced PGI_2 may regulate subsequent PGI_2 biosynthesis by stimulating adenylate cyclase.

METHODS

Materials. Prostaglandins (PG) I_2, E_1, E_2, D_2, and the prostaglandin analog 9,11 azoprosta-5,13-dienoic acid (azo analog I) were obtained from The Upjohn Company, Kalamazoo, Mich. PGH_2 and 12-hydroperoxyeicosatetraenoic acid (12-HPETE) were biosynthesized according to Gorman et al. (14) and Nugteren et al. (15). [α-^{32}P] ATP (10–30 Ci/mmol) was purchased from ICN Pharmaceuticals, Irvine, Calif., and [^{3}H]cyclic AMP was obtained from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y. [^{3}H] 6-Keto-$PGF_{1\alpha}$ was purchased from New England Nuclear, Boston, Mass. Dowex AG 50W-4X was purchased from Bio-Rad Laboratories, Richmond, Calif. Type I collagenase was purchased from Worthington Biochemical Corp., Freehold, N. J. [^{125}I]-2′*O*-Succinyl cyclic AMP tyrosine methyl ester and cyclic AMP antiserum were purchased from Collaborative Research Inc., Waltham, Mass. Isobutylmethylxanthine (MIX) was purchased from Aldrich Chemical Co., Milwaukee, Wis.

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[1] *Abbreviations used in this paper*: azo analog I, 9,11-azoprosta-5, 13-dienoic acid; 12-HPETE, 12-hydroperoxyeicosatetraenoic acid; MEM, minimal essential medium; MIX, isobutylmethylxanthine; PG, prostaglandin; PGI_2, prostacyclin.

L-Isoproterenol hydrochloride, D,L-propranolol hydrochloride, thrombin (3,000 U/ml) and ristocetin were purchased from Sigma Chemical Co., St. Louis, Mo. Human Factor VIII antiserum was purchased from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif., and the fluorescein conjugated goat anti-rabbit antiserum was obtained from N. L. Cappel Laboratories Inc., Cochranville, Pa. Plastic tissue culture flasks and plates were from Falcon Plastics, Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif. and Costar, Data Packaging, Cambridge, Mass.

Medium 199 with Earle's salts and L-glutamine as well as Hepes buffer were purchased from Microbiological Associates, Walkersville, Md.

The medium 199 was supplemented with sterile filtered human serum to a volume of 20% serum, Hepes buffer (15 mM, pH 7.5), L-glutamine (2 mM), sodium penicillin G (100 U/ml, The Upjohn Co.) and streptomycin sulfate (100 μg/ml, Eli Lilly Co., Indianapolis, Ind.). Sterile saline and distilled water were purchased from Abbott Diagnostics, Chicago, Ill.

Radioimmunoassay of cyclic AMP. Cyclic AMP was measured in confluent endothelial cell monolayers ~0.8×10^6 cells/35-mm well. Each well was washed once with 2.0 ml of warmed minimal essential medium (MEM) containing 25 mM Hepes buffer (no serum or phenol indicator). The cells were allowed to equilibrate with 1.0 ml of MEM-Hepes containing 1.0 mM MIX for 10 min before the addition of agonists. The reactions were terminated by the addition of 0.5 ml of 10% trichloroacetic acid, and the aqueous samples extracted three times with 10 vol of diethylether. Cyclic AMP was measured by radioimmunoassay according to Steiner et al. (16) with the incorporation of the acetylation modification of Harper and Brooker (17). All samples were tested at two dilutions. Data are presented as the mean±SEM of triplicate determinations.

Radioimmunoassay of 6-keto $PGF_{1\alpha}$. Thombin-stimulated PGI_2 biosynthesis was studied by measuring 6-keto-$PGF_{1\alpha}$ by radioimmunoassay according to Dray et al. (18). The antiserum was a gift from Dr. Fernando Dray, The Pasteur Institute, Paris, France. All samples were assayed at two dilutions. Data are presented as mean±SEM of triplicate determinations.

Endothelial cell monolayers were washed once with 2.0 ml of MEM-Hepes buffer and allowed to equilibrate with an additional 1.0 ml of MEM-Hepes for 10 min at 37°C. After the equilibration period, the cells were challenged with 2.0 U of thrombin, and allowed to incubate for an additional 10 min at 37°C. The media was then immediately withdrawn and frozen in liquid N_2, and aliquots of the samples were analyzed for 6-keto-$PGF_{1\alpha}$.

In experiments where PGE_1 was used to stimulate cyclic AMP levels, all of the samples and standards were assayed before and after alkali treatment. This was done to dehydrate PGE_1 to PGA_1 and eliminate PGE_1 crossreaction with the 6-keto-$PGF_{1\alpha}$ antiserum. Base treatment was done by adjusting the samples to pH 10.0 with $NaHCO_3$, followed by boiling to dryness. The dried samples were reconstituted with H_2O and the pH readjusted to 7.4 for subsequent radioimmunoassay. Authentic 6-keto-$PGF_{1\alpha}$ was degraded <5% by this procedure, whereas PGE_1 was essentially completely dehydrated to PGA_1.

Preparation of particulate fraction for adenylate cyclase assay. To eliminate PGI_2 biosynthesis during processing, particulate fractions were prepared from endothelial cells pretreated for 1 h with 1 mM acetylsalicyclic acid. Previous experience has shown that this treatment inhibits at least 95% of the thrombin-stimulated PGI_2 biosynthesis. Cells were harvested with a rubber spatula, and the pooled cells centrifuged at 4°C for 15 min at 2,000 × *g*. The cell pellet was resuspended in cold 50 mM Tris-HCl, 0.15M NaCl buffer, pH 7.50, and frozen and thawed three times in liquid N_2, followed by 10 strokes of a Dounce homogenizer (Kontes Co., Vineland, N. J.) (glass/glass). The homogenate was then centrifuged at 10,000 × *g* for 15 min at 4°C. The resulting pellet was then resuspended in 1 mM $KHCO_3$ and used directly in the adenylate cyclase assay.

Adenylate cyclase activity was measured according to Rodbell (19) with modifications as reported by Salomon et al. (20), from the rate of formation of cyclic AMP from [α-^{32}P]ATP. All incubations contained 10 mM theophylline. Data are presented as picomoles cyclic AMP per 10 min per milligram protein determined according to Lowry et al. (21).

Culture and characterization of endothelial cells. Endothelial cells were derived from human umbilical cord veins as described by Jaffe et al. (22). Cords were stored in sterile containers with 20 ml of cord buffer (137 mM NaCl, 4 mM KCl, 10 mM Hepes, pH 7.5, and 11 mM glucose). A disposable syringe was attached to a blunt needle and the vein was flushed with 50 ml of cord buffer to remove any blood. The vein was then perfused with 10 ml of 0.1% collagenase in cord buffer, and incubated at 37°C in a bath of cord buffer for 10 min. The collagenase-cell mixture was flushed from the vein with 30 ml of buffer into a plastic centrifuge tube that contained 10 ml of medium 199 and centrifuged at 200 *g* for 5 min. The cell pellet was resuspended in 10 ml of fresh medium 199 and added to a T-75 flask. Cells were exposed to an atmosphere of 95% air – 5% CO_2 and were fed twice a week until subculturing. Confluency was usually reached in 4–5 d.

For subculturing the medium was removed and the cells rinsed once with cord buffer. A 5-min incubation with a 1:1 mixture of 0.02% EDTA: 0.2% collagenase followed by centrifugation was used to harvest the cells. Cells were grown in either T-150 culture flasks or distributed into 35-mm wells.

Endothelial cells were characterized by (*a*) the presence of Weibel-Palade bodies as observed by electron microscopy (23), (*b*) the ability of the medium from cultured cells to support ristocetin-induced agglutination of washed human platelets (24), and (*c*) by immunofluorescence studies of Factor VIII antigen using a rabbit antibody to human Factor VIII, and a fluorescein-conjugated goat anti-rabbit gamma globulin (25).

RESULTS

Incubation of intact endothelial cell monolayers with 2.8 μM PGI_2 resulted in a marked increase in cyclic AMP within 1 min. The response was maximal at 10 min, and then gradually declined over the next 50 min (Fig. 1). Most of the cyclic AMP remained intracellular for at least 60 min (Fig. 1). We included 1 mM MIX, the cyclic AMP phosphodiesterase inhibitor, in all of our experiments because without MIX there was only a small increase in cyclic AMP in response to PGI_2 (Fig. 1).

Dose-response analysis indicated that PGI_2 was a more potent stimulator of endothelial cell cyclic AMP levels than either PGH_2 or PGE_2, whereas PGD_2 was essentially inactive (Fig. 2). As little as 28 nM PGI_2 significantly elevated cyclic AMP levels above basal levels, and 2.8 μM PGI_2 gave maximal stimulation (Fig. 2). This dose-response profile was similar to data previously reported in cultured human foreskin fibroblasts (10).

Most of the stimulation of cyclic AMP levels in response to PGH_2 was shown to be due to conversion of PGH_2 to PGI_2 by the endothelial cell monolayers. Incu-

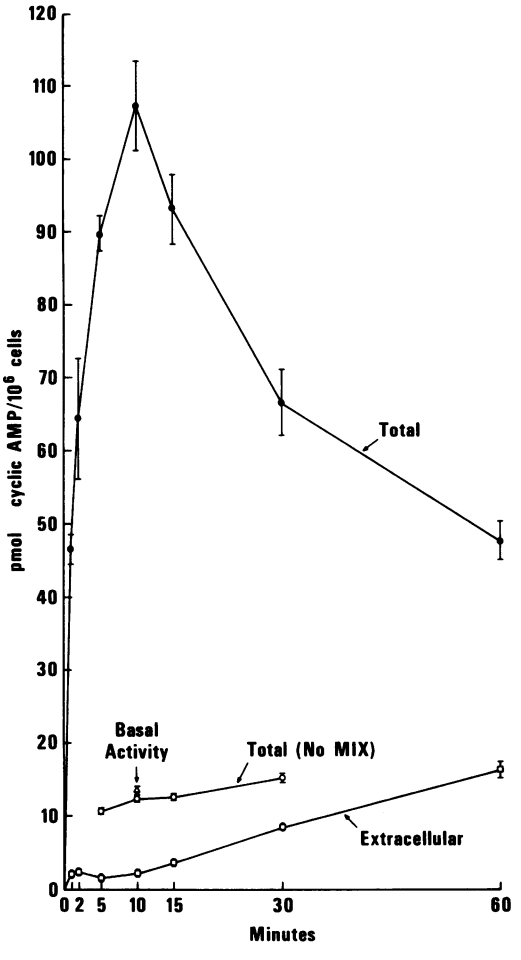

FIGURE 1 Time-course of total and extracellular cyclic AMP levels in human endothelial cells. Cells were stimulated with 2.8 μM PGI_2 from 1 to 60 min and both total (●) and extracellular (□) cyclic AMP levels measured in the presence of 1 mM MIX. Other wells of endothelial cells were challenged with the same concentration of PGI_2 but without MIX (○) present. Data are presented as mean±SEM pmol cyclic AMP/10^6 cells of triplicate samples.

bation of endothelial cell monolayers with the PGI_2 synthetase inhibitors 12-HPETE (28 μM), or azo analog I(2.8 μM) inhibited PGH_2-stimulated cyclic AMP levels 75 and 80%, respectively when base-line activity was subtracted (Fig. 3). Neither inhibitor significantly changed the basal level of cyclic AMP (Fig. 3).

As shown previously (26), endothelial cells also have a β-adrenergic receptor that is coupled to the adenylate cyclase. Our data shows that the maximal β-adrenergic stimulation of cyclic AMP accumulation by L-isoproterenol is considerably less than the stimulation observed with 2.8 μM PGI_2 (Fig. 4). The isoproterenol-stimulation was completely blocked by 10 μM propranolol, but propranolol did not inhibit the PGI_2-stimulation of cyclic AMP levels (Fig. 4).

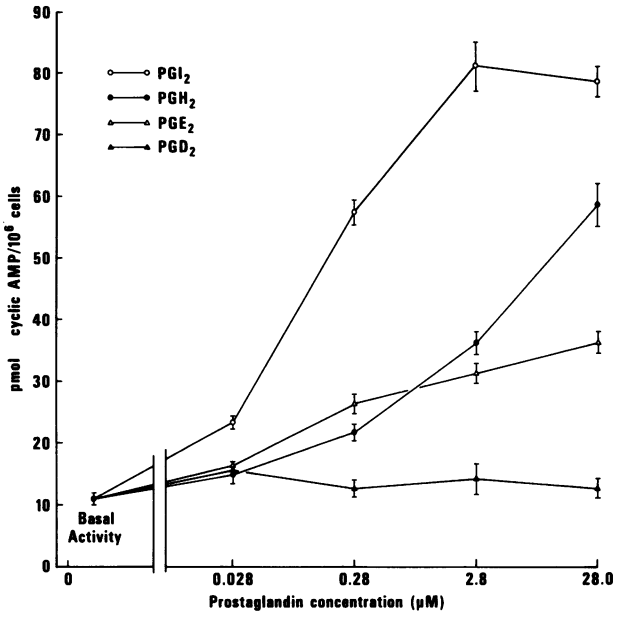

FIGURE 2 Dose-response relationship for prostaglandin stimulation of cyclic AMP. Endothelial cells were incubated for 10 min with 1 mM MIX before challenging with from 28 nM to 28.0 μM PGI_2, PGH_2, PGE_2, or PGD_2. All prostaglandins were incubated with the cells for 10 min at 37°C. Data are presented as mean±SEM pmol cyclic AMP/10^6 cells of triplicate samples.

In contrast, the α-adrenergic agonist phenylephrine did not stimulate or inhibit endothelial cell adenylate cyclase (unpublished experiments).

In addition to measuring cyclic AMP in intact endo-

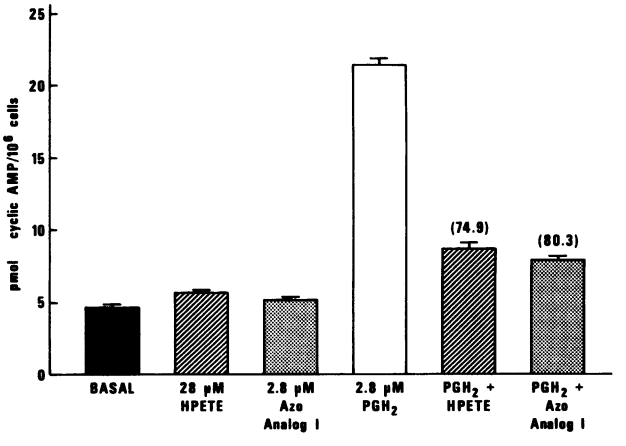

FIGURE 3 Inhibition of PGH_2 stimulation of cyclic AMP accumulation by 12-HPETE or azo analog I. Confluent human endothelial cells were preincubated for 10 min at 37°C with 1 mM MIX and with either 28 μM 12-HPETE or 2.8 μM azo analog I before exposure to 2.8 μM PGH_2. Numbers in brackets above bar graphs represent percent inhibition of PGH_2-stimulated cyclic AMP accumulation when basal levels of cyclic AMP are subtracted. Data reported as mean±SEM pmol cyclic AMP/10^6 cells of triplicate samples.

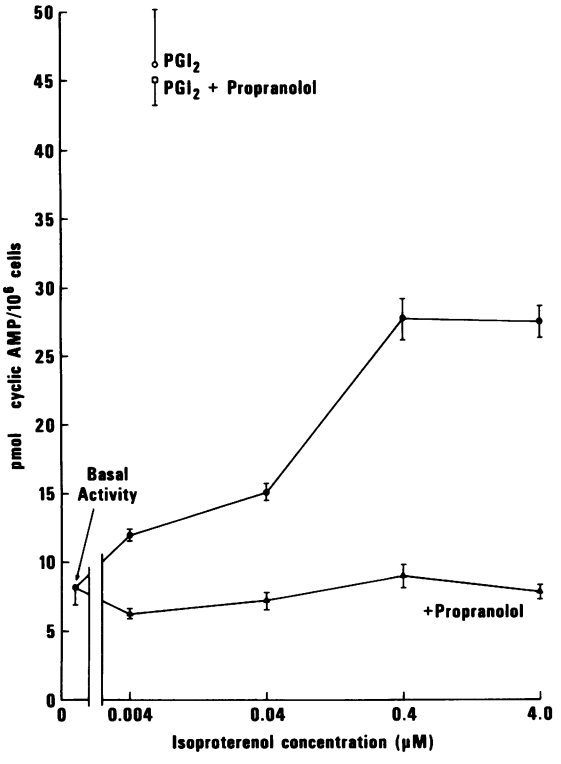

FIGURE 4 Isoproterenol dose-response curve. Endothelial cells were incubated for 10 min at 37°C with from 4 nM to 4 μM isoproterenol in the presence of 1 mM MIX. Another group of cells were preincubated for 10 min with MIX plus 33.8 μM propranolol before challenging with several concentrations of isoproterenol. Stimulation of cyclic AMP levels by 2.8 μM PGI_2 was also done with and without propranolol. Data are presented as mean±SEM pmol cyclic AMP/10^6 cells of triplicate samples.

thelial cell monolayers, we also measured adenylate cyclase activity in homogenates prepared from endothelial cells. PGI_2 and PGE_1 gave modest increases in enzyme activity and isoproterenol an even smaller stimulation (Table I). Both PGD_2 and adenosine were inactive as stimulators of adenylate cyclase (Table I).

Weksler et al. (27) have shown that thrombin stimulates endothelial cell PGI_2 biosynthesis. We have found that thrombin-stimulated PGI_2 biosynthesis (as measured by 6-keto-$PGF_{1\alpha}$ levels) was attenuated by both PGE_1 and L-isoproterenol when the phosphodiesterase was inhibited (Table II). Neither PGE_1 nor L-isoproterenol inhibited PGI_2 biosynthesis in the absence of phosphodiesterase inhibition (Table II). The data presented were obtained before and after base treatment (Methods), because PGE_1 was used to elevate cyclic AMP levels, and PGE_1 cross-reacts with the 6-keto-$PGF_{1\alpha}$ antibody. Authentic PGI_2 could not be used because this would have generated too much exogenous 6-keto-$PGF_{1\alpha}$, and hampered the subsequent measurement of endogenously synthesized PGI_2. Base

TABLE I
Endothelial Cell Adenylate Cyclase Activity

Additions	Adenylate cyclase activity
	pmol cyclic AMP/mg protein
None-Basal	633.6±44
28 μM PGI_2	976.5±74
28 μM PGE_1	957.9±66
28 μM PGD_2	624.6±32
10 μM Isoproterenol	721.1±38
10 μM Adenosine	605.4±42

A particulate preparation of homogenized endothelial cells (82 μg protein) was incubated for 10 min at 30°C with either 28 μM PGI_2, PGE_1, or PGD_2, or with 10 μM isoproterenol or adenosine. Assay conditions and purification of cyclic AMP are described in the Methods. Data are representative of three confirmatory experiments and are presented as the mean ±SEM of triplicate samples. Membranes prepared in this manner converted 15.4% of exogenously added [1-^{14}C]PGH_2 to 6-keto-$PGF_{1\alpha}$ and the synthesis of 6-keto-$PGF_{1\alpha}$ was blocked by 2.8 μM azo analog I. This rate of conversion is analogous to previously published data in endothelial cells (7).

treatment of samples hydrolyzes PGE_1 to PGA_1, and PGA_1 does not interfere with the measurement of 6-keto-$PGF_{1\alpha}$ (18). Authentic 6-keto-$PGF_{1\alpha}$ is not degraded by base treatment (Table II). Of particular interest was the observation that 1.0 mM MIX alone could also reduce thrombin-stimulated PGI_2 biosynthesis (Table II). A combination of PGE_1 and 1.0 mM MIX inhibited PGI_2 biosynthesis 70.0% whereas 1.0 mM MIX inhibited synthesis 38% (Table II).

TABLE II
Inhibition of PGI_2 Biosynthesis by PGE_1 and L-*Isoproterenol*

Treatment	Before base treatment	After base treatment
	ng 6-Keto-$PGF_{1\alpha}$/10^6 Cells	
None-basal	1.8±0.3	1.7±0.2
2 U Thrombin	18.2±0.7	18.8±1.0
0.28 μM PGE_1	22.4±2.0	1.1±0.1
Thrombin + PGE_1	51.4±2.7	21.2±0.5
Thrombin + PGE_1 + 1.0 mM MIX	31.2±0.7	5.6±0.3
4.0 μM Isoproterenol	1.5±0.3	1.4±0.1
Thrombin + isoproterenol	18.9±1.5	18.1±1.3
Thrombin + iso + 1.0 mM MIX	6.1±0.2	6.2±0.4
Thrombin + 1.0 mM MIX	12.4±0.4	11.6±0.6

Confluent monolayers of endothelial cells in 35-mm wells were washed once with 2.0 ml of warm MEM containing 25 mM Hepes buffer. The cells were allowed to equilibrate with 1.0 ml of MEM-Hepes with or without PGE_1, isoproterenol or 1.0 mM MIX for 10 min at 37°C. Thrombin was added at the end of the 10-min equilibration period and allowed to incubate for an additional 10 min with the cells. Data are reported as mean±SEM of triplicate samples.

TABLE III
Elevation of Endothelial Cell Cyclic AMP Levels by PGI_2 and Thrombin

Additions	Cyclic AMP
	pmol/10^6 cells
None-basal	1.9±0.2
0.1 mM MIX	4.6±0.1
1.0 mM MIX	8.7±0.6
0.28 μM PGI_2	3.4±0.1
2 U Thrombin	2.6±0.2
0.1 mM MIX + PGI_2	15.2±0.4
1.0 mM MIX + PGI_2	64.4±2.5
0.1 mM MIX + thrombin	8.4±1.2
1.0 mM MIX + thrombin	22.8±0.6

Confluent monolayers of endothelial cells in 35-mm wells were washed once with 2.0 ml of warm MEM containing 25 mM Hepes buffer. The cells were then allowed to equilibrate with 1.0 ml of MEM-Hepes buffer with or without 0.1 or 1.0 mM MIX for 10 min at 37°C. After the 10-min equilibration period the cells were exposed to either 0.28 μM PGI_2 or 2 U of thrombin for an additional 10 min at 37°C. Data are presented as mean±SEM of triplicate samples.

It should be emphasized that marked elevations in cyclic AMP and inhibition of thrombin-stimulated PGI_2 biosynthesis were observed only when the phosphodiesterase was inhibited.

To assess the possible correlation between cyclic AMP elevation and the inhibition of PGI_2 biosynthesis, we measured cyclic AMP levels in endothelial cells exposed to either PGI_2 or thrombin with or without 0.1 or 1.0 mM MIX (Table III). Treatment of the monolayers with 0.1 mM or 1.0 mM MIX increased cyclic AMP levels two- and four-fold, respectively (Table III). PGI_2 or thrombin gave small increases in cyclic AMP without MIX, but 0.1 mM MIX potentiated the PGI_2 response to an eight-fold increase, and the thrombin response to a fourfold increase. In the presence of 1.0 mM MIX PGI_2 increased cyclic AMP levels 33-fold, and thrombin 12-fold (Table III).

DISCUSSION

The observation that endothelial cells can synthesize PGI_2 from either endogenous or exogenous endoperoxide PGH_2 or arachidonic acid (6, 7) has generated a great deal of research concerning endothelial cell-platelet interactions. The thrust of the work has been to assess the influence of PGI_2 produced by the endothelium on platelet aggregation and/or adhesion (5–10), but the possible effects of endogenously produced PGI_2 on endothelial cell physiology per se have not as yet been explored.

Since PGI_2 is a potent stimulator of adenylate cyclase in a variety of cell types (10–12) we felt it was important to assess the influence of PGI_2 on endothelial cell adenylate cyclase as well. PGI_2 proved to be a potent stimulator of endothelial cell cyclic AMP accumulation when the cyclic AMP phosphodiesterase was inhibited. This dependence on phosphodiesterase inhibition contrasts sharply with the observed potency in other cultured cell lines (10, 12), but we have previously observed an analogous high level of cyclic AMP phosphodiesterase activity in isolated arterial rings (13). The control of endothelial cell sensitivity to PGI_2 may be indirectly regulated through the phosphodiesterase, and any agent that suppresses phosphodiesterase activity would enhance the PGI_2 response.

In addition to PGI_2, exogenously added PGH_2 was also readily converted to PGI_2 by the endothelial cells resulting in an increase in endothelial cyclic AMP levels. The PGI_2 dependence of the PGH_2 response was evident by the ability of the prostacyclin synthetase inhibitors 12-HPETE or azo analog I to inhibit most of the PGH_2 response. Complete inhibition of the PGH_2 response is probably not attainable because considerable amounts of PGE_2 are synthesized from PGH_2 when the PGI_2 synthetase is blocked, and PGE_2 itself is a weak stimulator of cyclic AMP accumulation. The inhibition of the PGI_2 synthetase by 12-HPETE is potentially of in vivo significance. Since 12-HPETE is produced by the platelet (28, 29), it is possible that platelets interacting with the endothelium could inhibit PGI_2 biosynthesis by producing 12-HPETE. Interestingly, aspirin treatment of platelets could exacerbate any 12-HPETE inhibition by shunting arachidonic acid toward the platelet lipoxygenase.

Besides prostaglandins, endothelial cell cyclic AMP levels can also be elevated by isoproterenol, but the β-adrenergic stimulation does not approach the magnitude of the PGI_2 response.

The direct assay of adenylate cyclase activity in endothelial cell homogenates was not as successful as measurement of cyclic AMP levels in monolayers. The stimulations by both prostaglandins and isoproterenol were quite small, but correlate qualitatively with the data obtained in intact cells. The problem of loss of hormone responsive adenylate cyclase during cell processing is not a new one, but appears to be particularly troublesome in endothelial cells.

Our direct measurements of 6-keto-$PGF_{1\alpha}$ levels in thrombin-stimulated endothelial cells supports the work of Weksler et al. (27) that showed thrombin stimulation of PGI_2 biosynthesis. In addition, we observed that when the phosphodiesterase was inhibited, agents that elevated cyclic AMP levels also attenuated thrombin-stimulated PGI_2 biosynthesis. Our data showed that the phosphodiesterase inhibitor MIX at 1.0

mM can also inhibit thrombin-stimulated PGI_2 biosynthesis. These data at first were somewhat confusing because 1.0 mM MIX alone did not markedly elevate cyclic AMP levels. However, these data are quite consistent with the other data because a combination of 1.0 mM MIX and thrombin elevated cyclic AMP levels 12-fold due to the PGI_2 biosynthesized in response to thrombin (Tables II and III). Subsequent experiments have shown that either 1 mM aspirin or 10 μM indomethacin completely inhibit thrombin-stimulated cyclic AMP accumulation.

The finding that PGI_2 was a potent stimulator of cyclic AMP accumulation in endothelial cells suggests that once PGI_2 is produced it can have at least two actions. Newly synthesized PGI_2 can interact with platelets, elevate cyclic AMP, and inhibit aggregation. Concurrently, it would be capable of elevating endothelial cell cyclic AMP levels and attenuating further PGI_2 biosynthesis. This type of feedback inhibition would have to be highly sensitive to small increases in cyclic AMP, because without phosphodiesterase inhibition, there was little increase in cyclic AMP. In the human platelet, PGI_2 inhibits platelet aggregation in the nanomolar range by elevating cyclic AMP 10–15 pmol/10^9 platelets above basal levels, but the platelet is capable of making 6,000–7,000 pmol of cyclic AMP in response to 2.8 μM PGI_2 (30). Clearly most of the potential responsiveness to PGI_2 in the platelet is not expressed. An analogous situation may exist in the endothelial cell.

The precise physiological significance of the PGI_2 elevation of endothelial cell cyclic AMP levels and subsequent inhibition of PGI_2 biosynthesis cannot be established by this paper. Previous work has shown that by elevating cyclic AMP, PGI_2 can inhibit arachidonic acid transformation in the human platelet (31). The inhibition by cyclic AMP was at the level of the phospholipase. Our data are consistent with a phospholipase inhibition, but do not rule out inhibition at the level of the cyclooxygenase or the PGI_2 synthetase.

We are presently studying PGI_2 biosynthesis in endothelial cells to establish exactly at what level(s) biosynthesis is interrupted. Regardless of the exact point of control, it is clear that endothelial biosynthesis of PGI_2 is not a passive phenomenon. The cells are responsive to this compound as well.

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