We have examined the effect of activated neutrophils on the release of prostacyclin (PGI2) from cultured endothelial cells by radioimmunoassay and thin layer chromatography of its stable metabolite, 6-keto-prostaglandin F1 alpha (6-keto-PGF1 alpha). Phorbol myristate acetate-activated neutrophils induced a time- and dose-dependent release of 6-keto-PGF1 alpha from human and bovine endothelial cell monolayers, whereas phorbol myristate acetate alone and neutrophils alone did not. Pretreatment of the endothelial cells with aspirin prevented neutrophil-mediated 6-keto-PGF1 alpha release, indicating that it did not depend upon neutrophil-generated endoperoxides. Phorbol myristate acetate-activated neutrophils from a patient with chronic granulomatous disease failed to induce endothelial 6-keto-PGF1 alpha release. Addition of catalase but not of superoxide dismutase significantly reduced human and bovine endothelial 6-keto-PGF1 alpha release by phorbol myristate acetate-activated neutrophils. Catalase-inhibitable endothelial 6-keto-PGF1 alpha release was also observed after the addition of the hydrogen peroxide-generating system, glucose-glucose oxidase, to bovine and human endothelial cell monolayers. Bovine endothelial 6-keto-PGF1 alpha release induced by exogenously generated hydrogen peroxide was attenuated by the phospholipase inhibitor mepacrine, suggesting that hydrogen peroxide may act by triggering endothelial membrane phospholipase activation. The release of 6-keto-PGF1 alpha by enzymatically or neutrophil-generated hydrogen peroxide was not associated with endothelial cell lysis as assessed by 51Cr release. We conclude that exogenously generated hydrogen peroxide or a hydrogen peroxide-derived product mediates rapid nonlytic release of PGI2 […]
Role of Hydrogen Peroxide in the Neutrophil-mediated Release of Prostacyclin from Cultured Endothelial Cells

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Abstract. We have examined the effect of activated neutrophils on the release of prostacyclin (PGI₂) from cultured endothelial cells by radioimmunoassay and thin layer chromatography of its stable metabolite, 6-keto-prostaglandin F₁₆ (6-keto-PGF₁₆). Phorbol myristate acetate-activated neutrophils induced a time- and dose-dependent release of 6-keto-PGF₁₆ from human and bovine endothelial cell monolayers, whereas phorbol myristate acetate alone and neutrophils alone did not. Pretreatment of the endothelial cells with aspirin prevented neutrophil-mediated 6-keto-PGF₁₆ release, indicating that it did not depend upon neutrophil-generated endoperoxides. Phorbol myristate acetate-activated neutrophils from a patient with chronic granulomatous disease failed to induce endothelial 6-keto-PGF₁₆ release. Addition of catalase but not of superoxide dismutase significantly reduced human and bovine endothelial 6-keto-PGF₁₆ release by phorbol myristate acetate-activated neutrophils. Catalase-inhibitable endothelial 6-keto-PGF₁₆ release was also observed after the addition of the hydrogen peroxide-generating system, glucose-glucose oxidase, to bovine and human endothelial cell monolayers. Bovine endothelial 6-keto-PGF₁₆ release induced by exogenously generated hydrogen peroxide was attenuated by the phospholipase inhibitor mepacrine, suggesting that hydrogen peroxide may act by triggering endothelial membrane phospholipase activation. The release of 6-keto-PGF₁₆ by enzymatically or neutrophil-generated hydrogen peroxide was not associated with endothelial cell lysis as assessed by ⁵¹Cr release. We conclude that exogenously generated hydrogen peroxide or a hydrogen peroxide-derived product mediates rapid nonlytic release of PGI₂ from cultured endothelial cells.

Introduction

Since augmented neutrophil adherence to vascular endothelium is an early response to inflammation (1), neutrophils have been implicated in the pathogenesis of the altered vascular permeability associated with acute inflammation (2). The observation that neutrophil depletion attenuates permeability edema formation in several experimental models supports this hypothesis (3–5). Activated neutrophils have been shown to produce endothelial injury in vitro through the generation of toxic oxygen products (6, 7) and the release of granule proteases (8). Since prostacyclin (PGI₂) has been demonstrated to have proinflammatory effects and to enhance edema formation (2, 9–16), we have examined the effect of activated neutrophils on endothelial PGI₂ release by the use of purified human peripheral blood neutrophils, cultured bovine and human endothelial cells (ECs), and radioimmunoassay (RIA) and thin-layer chromatography (TLC) of 6-keto-prostaglandin F₁₆ (6-keto-PGF₁₆), the stable breakdown product of PGI₂.

Methods

ECs. Bovine aortic and pulmonary artery and human umbilical vein ECs were prepared by collageanse treatment of vessels as previously described (17, 18) and maintained in 10% newborn calf serum (NBCS) (Gibco Laboratories, Gibco Div., Grand Island, NY) in Waymouth's medium (Gibco Laboratories, Gibco Div.). Bovine ECs were used in the 6th through the 15th passage. Individual experiments were performed with bovine ECs of the same strain and passage number. Human umbilical vein ECs were used in the first passage.

Preparation of neutrophils. Purified human peripheral blood neutrophils were prepared from heparinized blood from normal healthy donors by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ)

1. Abbreviations used in this paper: EC, endothelial cell; NBCS, newborn calf serum; PGE₁, PGE₂, PGF₁₆, PGF₂₆, prostaglandins E₁, E₂, F₁₆, F₂₆; PGI₂, prostacyclin; PMA, phorbol myristate acetate; RIA, radioimmunoassay; SBTI, soybean trypsin inhibitor; SOD, superoxide dismutase; TLC, thin-layer chromatography.

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gradient centrifugation and dextran sedimentation with hypotonic lysis of contaminating erythrocytes (19). Peripheral blood neutrophils were similarly prepared from blood obtained from a patient with chronic granulomatous disease and a patient with myeloperoxidase deficiency (courtesy of Dr. Henry Rosen, University of Washington, Seattle, WA). Neutrophils were suspended in Neuman-Tytell medium (Gibco Laboratories, Gibco Div.) at a concentration of 10^7 cells/ml.

RIA of EC 6-keto-PGF1α release. ECs were plated at confluent density (10^5 cells/cm²) in 10% NBCS in Waymouth's medium in 16-mm or 23-mm wells (Cluster 3524 and 3512; Costar, Cambridge, MA) and allowed to adhere overnight. Before the experiment, monolayers were washed twice with serum-free Neuman-Tytell medium. The medium was then gently decanted and control or test agents or neutrophils in serum-free Neuman-Tytell medium were added. The monolayers were incubated at 37°C in 5% CO₂ and aliquots of cell-free supernatant medium were removed at intervals for determination of 6-keto-PGF1α. Cell counts were performed on replicate wells by electronic counter (Particle Data, Inc., Elmhurst, IL).

The measurement of unextracted, cell-free supernatant medium from the endothelial cultures for 6-keto-PGF1α was performed in some samples by the determination of competitive inhibition of 3H-6-keto-PGF1α to anti-6-keto-PGF1α binding using the labeled tracers, standards, and antiserum from New England Nuclear (Boston, MA) (New England Nuclear Technical Bulletin NEK 008) (20). The standard curve range was 10 to 500 pg added per 100 μl, with a normalized percent inhibition at 50% of 100–130 pg. Cross-reactivity of the 6-keto-PGF1α antibody at a normalized percent inhibition of 50% was PGI2, 7.8%; prostaglandin E1 (PGE1), 3%; prostaglandin F2α (PGE2α), 2.7%; prostaglandin E2 (PGE2), 2%; prostaglandin A1, <0.3%; prostaglandin A2, <0.1%; thromboxane B2, <0.1%; and 11,14-dihydro-15-keto-PGF2α, <0.02%. (New England Nuclear Technical Bulletin NEK-008). In other samples, 6-keto-PGF1α levels were determined by RIA with 3H-6-keto-PGF1α (Amersham Corp., Arlington Heights, IL), authentic 6-keto-PGF1α standard kindly provided by Douglas McCarter and Dr. John Pike (Upjohn Co., Kalamazoo, MI), and antiserum generously supplied by Dr. William B. Campbell, University of Texas Health Science Center at Dallas. This anti-6-keto-PGF1α antibody cross-reacted less than 0.9% with PGE1, PGE2, PGF2α, PGI2, prostaglandin D2, prostaglandin A2, and thromboxane B2, 3.5% with 6,15-diketo-PGF1α, and 70% with 6-keto-PGF1α. The level of sensitivity was 10 pg/300 μl. Assays were run in duplicate and at two dilutions when necessary. Results were confirmed in selected samples by independent RIA of 6-keto-PGF1α kindly performed by Dr. William B. Campbell (21). Results obtained in the RIA are expressed as picograms of 6-keto-PGF1α released per 10⁶ endothelial cells. For statistical calculations, samples with undetectable levels of 6-keto-PGF1α were assumed to be equal to the lower limit of detection in the assay.

TLC of bovine EC 6-keto-PGF1α release. In some experiments, EC prostaglandin release was also assessed by TLC (22). EC lipids were labeled by the incubating of the cells for 6 h with 1.0 μCi [3H]arachidonic acid (87.4 Ci/mmol, New England Nuclear) in Neuman-Tytell media. The cell monolayers were then washed three times with 10 mM Hepes buffer (Gibco Laboratories, Gibco Div.) containing 0.15 M NaCl, 0.005 M KCl, 0.0018 M CaCl2, 0.001 M MgCl2, glucose 1 g/liter, and fatty acid-free bovine serum albumin (Sigma Chemical Co., St. Louis, MO) to remove the unincorporated [3H]arachidonic acid. The monolayers were then incubated with control or test medium or agents. Cell-free supernatant medium was collected from each well after 30–60 min of incubation. The monolayers were then washed once with the Hepes buffer solution and the wash was pooled with the original supernatant medium.

The supernatant medium was acidified to pH 3.5 with glacial acetic acid and extracted twice with five times volume of ethyl acetate/cyclohexane (1:1). The organic layer was evaporated to dryness with nitrogen. Unlabeled 6-keto-PGF1α, and PGE2 (Sigma Chemical Co.) were added before extraction to localize these prostaglandins during chromatography. A small aliquot was also removed before and after extraction to correct for recovery. The dried residue was resuspended in 100 μl of ethyl acetate and applied to a 20 × 20 cm silica gel 60 TLC plate (Merck and Co., Darmstadt, Germany). The plates were developed twice in a water-saturated mixture of ethyl acetate/glacial acetic acid/isooctane (110:20:50), and dried in air, and the spots were located with iodine vapor. Areas corresponding to 6-keto-PGF1α, PGE2, and arachidonic acid were then removed by scraping, extracted with chloroform/methanol (9:1), and dried under nitrogen, and scintillate was added for counting.

31Cr-release assay. ECs were plated on Microtest III plates (Falcon Labware, Div. Becton-Dickinson and Co., Oxnard, CA) and labeled with sodium (31Cr) chromate (New England Nuclear) as previously described (8). After an overnight incubation, cells were washed three times with 1% NBCS in phosphate buffered saline (Gibco Laboratories, Gibco Div.) and then incubated with either test or control medium or cells. 50–100 μl of cell-free supernatant medium were removed at 4–6 h for determination of specific 31Cr release by the formula (A – B)/(C – B) × 100%. A represents the mean test 31Cr counts per minute released, B represents the mean spontaneous 31Cr counts per minute released, and C represents the mean maximum 31Cr counts per minute released. Maximum 31Cr release was determined by incubation in 1% Triton X-100 (New England Nuclear). Spontaneous 31Cr release was determined in control monolayers incubated in Neuman-Tytell medium only and was 5–10% of maximum 31Cr release after 4 h of incubation. Statistical significance was determined by comparison of mean test and mean spontaneous 31Cr-counts per minute released by two-tailed, unpaired t statistic.

Reagents. Acetylsalicylic acid, A23187, phorbol myristate acetate (PMA), soybean trypsin inhibitor (SBTI), mepracine (quinacrine dihydrochloride), fatty acid-free albumin, catalase (bovine liver, 11,800 U/mg), superoxide dismutase (SOD) (2,700 U/mg), and glucose oxidase (grade VII) were obtained from Sigma Chemical Co. Sodium arachidonate was obtained from Bio/Data Corp. (Horsham, PA).

Results

Neutrophil-mediated bovine endothelial 6-keto-PGF1α release. Incubation of bovine EC monolayers with PMA alone or neutrophils alone did not induce significant release of 6-keto-PGF1α. When neutrophils were activated by PMA, however, a marked increase in 6-keto-PGF1α release was noted. Because baseline (medium alone) and stimulated (arachidonic acid) release of 6-keto-PGF1α from bovine EC monolayers varied considerably among strains and passage number in the same strain, results from individual experiments were expressed as the fold-increase in 6-keto-PGF1α release (Table I). The increase in 6-keto-PGF1α was due to release from ECs and not from neutrophils, since 6-keto-PGF1α could not be detected when neutrophils were activated by PMA in the absence of ECs (<100 pg/10⁷ neutrophils in nine experiments).

Time course and dose response of neutrophil-mediated bovine EC 6-keto-PGF1α release. Significant 6-keto-PGF1α release was observed after a 15-min incubation of bovine EC monolayers...
Control medium, PMA (10 ng/ml), neutrophils (neutrophil-to-endothelial ratio of 10 to 1), neutrophils and PMA, or arachidonic acid (50 µg/ml) were added to bovine aortic or pulmonary artery EC monolayers. Aliquots of cell-free supernatant medium were removed after 30 min of incubation for determination of 6-keto-PGF_1α release by RIA. Values represent the mean±1 SE of (n) separate experiments. In each individual experiment the fold-increase in 6-keto-PGF_1α release was determined from the mean of 2–4 replicate wells as (test − control)/control, where control represents mean 6-keto-PGF_1α (picograms per 10⁶ cells) released with medium alone and test represents mean 6-keto-PGF_1α (picograms per 10⁶ cells) released after incubation with test agent or cell (i.e., PMA, neutrophils, PMA and neutrophils, or arachidonic acid). The P value was determined by comparison of the mean value of 6-keto-PGF_1α released in control and test in each experiment by paired t statistic.

Table I. Effect of PMA-activated Neutrophils on Bovine EC 6-keto-PGF_1α Release

<table>
<thead>
<tr>
<th>Addition to endothelial monolayer</th>
<th>Fold-increase in 6-keto-PGF_1α release</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA (4)</td>
<td>1.1±0.7 (P &gt; 0.05)</td>
</tr>
<tr>
<td>Neutrophils (4)</td>
<td>1.1±0.5 (P &gt; 0.05)</td>
</tr>
<tr>
<td>Neutrophils and PMA (18)</td>
<td>12.2±1.5 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Arachidonic acid (6)</td>
<td>35.1±3.8 (P &lt; 0.001)</td>
</tr>
</tbody>
</table>

Effect of inhibitors on neutrophil-mediated bovine endothelial 6-keto-PGF_1α release. The effect of inhibitors on bovine endothelial 6-keto-PGF_1α release induced by PMA-activated neutrophils is summarized in Table II. Aspirin pretreatment of the bovine EC monolayers prevented neutrophil-activated 6-keto-PGF_1α release. SBTI, a potent inhibitor of neutrophil serine proteases, had no significant inhibitory effect. Superoxide dismutase also failed to prevent endothelial 6-keto-PGF_1α release. Catalase significantly reduced bovine endothelial 6-keto-PGF_1α release induced by PMA-activated neutrophils, although the degree of inhibition varied somewhat between experiments (range, 30–95%). Catalase did not inhibit endothelial 6-keto-PGF_1α release induced by arachidonic acid or calcium ionophore.

Effect of PMA-activated chronic granulomatous disease neutrophils on bovine EC 6-keto-PGF_1α release. The inhibition of endothelial 6-keto-PGF_1α release after the addition of catalase to PMA-activated neutrophils suggested that hydrogen peroxide or a hydrogen peroxide-derived product was a critical mediator. To assess further the role of hydrogen peroxide, we examined the effect of PMA-activated neutrophils from a patient with chronic granulomatous disease and a patient with myeloperoxidase deficiency on endothelial 6-keto-PGF_1α release. Chronic granulomatous disease neutrophils, which do not generate hydrogen peroxide, failed to induce 6-keto-PGF_1α release when activated by PMA (Table III). Note that PMA-activated myeloperoxidase-deficient neutrophils, which produce hydrogen peroxide but not hypochlorous acid, induced 6-keto-PGF_1α release.

![Figure 1](image-url)  
Figure 1. Time- and dose-response of neutrophil-mediated bovine EC 6-keto-PGF_1α release. PMA (10 ng/ml) and neutrophils (PMN) were added to bovine aortic EC monolayers. In A, the neutrophil-to-endothelial ratio was 50 to 1. In B, the incubation was 30 min. Values represent mean±1 SE of three replicate wells.

Table II. Effect of Inhibitors on Neutrophil-mediated Bovine EC 6-keto-PGF_1α Release

<table>
<thead>
<tr>
<th>Addition to endothelial monolayer</th>
<th>Inhibitor</th>
<th>% Inhibition of endothelial 6-keto-PGF_1α release</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA + neutrophils</td>
<td>Aspirin pretreatment (2)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>SBTI (2)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SOD (6)</td>
<td>6±2 (P &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>Catalase (11)</td>
<td>52±12 (P &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>Catalase boiled (2)</td>
<td>0</td>
</tr>
<tr>
<td>A23187</td>
<td>Catalase (1)</td>
<td>0</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>Catalase (1)</td>
<td>0</td>
</tr>
</tbody>
</table>
Release similar to that induced by normal neutrophils (720±100 pg/10^6 cells with normal PMA-activated neutrophils; 780±33 pg/10^6 cells with myeloperoxidase-deficient PMA-activated neutrophils, mean±1 SE of triplicate wells).

**Table III. Role of Oxygen Radicals in Bovine EC 6-keto-PGF<sub>1α</sub> Release Induced by PMA-activated Neutrophils**

<table>
<thead>
<tr>
<th>Addition to endothelial monolayer</th>
<th>6-keto-PGF&lt;sub&gt;1α&lt;/sub&gt;</th>
<th>pg/10&lt;sup&gt;6&lt;/sup&gt; EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal neutrophils + PMA (3)</td>
<td>247</td>
<td>523±142</td>
</tr>
<tr>
<td>CGD neutrophils + PMA (3)</td>
<td>&lt;30</td>
<td>2,452±140</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal neutrophils + PMA (2)</td>
<td>227</td>
<td>404±95</td>
</tr>
<tr>
<td>CGD neutrophils + PMA (2)</td>
<td>&lt;30</td>
<td>380±48</td>
</tr>
</tbody>
</table>

In two separate experiments bovine aortic EC monolayers were incubated with normal or chronic granulomatous disease neutrophils (neutrophil-to-endothelial ratio of 10 to 1) and PMA (10 ng/ml). After 60 min of incubation, an aliquot of cell-free supernatant medium was removed for determination of 6-keto-PGF<sub>1α</sub> by RIA. Values represent means of (n) replicate wells.

**Effect of glucose-glucose oxidase on bovine endothelial 6-keto-PGF<sub>1α</sub> release.** After the observations that endothelial 6-keto-PGF<sub>1α</sub> release required a normal neutrophil oxidative burst and was catalase inhabitable, we examined the effect of a cell-free hydrogen peroxide-generating system, glucose-glucose oxidase, on bovine endothelial 6-keto-PGF<sub>1α</sub> release. The addition of glucose-glucose oxidase to bovine EC monolayers produced significant catalase-inhibitable 6-keto-PGF<sub>1α</sub> release (Table IV A). The boiling of glucose oxidase before its addition to the monolayer prevented 6-keto-PGF<sub>1α</sub> release, and the boiling of catalase abolished its inhibitory effect, indicating that for both, enzymatic activity was required (Table IV A). Bovine EC 6-keto-PGF<sub>1α</sub> release induced by glucose-glucose oxidase was inhibited by the phospholipase inhibitor mepacrine (23) (Table IV B).

**Effect of hydrogen peroxide on human EC 6-keto-PGF<sub>1α</sub> release.** Because the bovine EC strains employed in these studies were multiply passaged, we were concerned that the effect of hydrogen peroxide on PG12 release might be due to some alteration in arachidonic acid metabolism that occurred with prolonged passage. It is known, for example, that the amount of 6-keto-PGF<sub>1α</sub> released from bovine aortic and pulmonary artery ECs after stimulation with ionophore or arachidonic acid decreases markedly with passage and that there is a shift from production of PG12 to production of other prostanooids (24, 25). For this reason, we also examined the effect of hydrogen peroxide on first passage human umbilical vein ECs.

Both PMA-activated neutrophils and glucose-glucose oxidase induced dose-dependent release of 6-keto-PGF<sub>1α</sub> from human umbilical vein ECs (Fig. 2). The amount of 6-keto-PGF<sub>1α</sub> released after incubation of the first passage human umbilical vein ECs with PMA-activated neutrophils or the hydrogen peroxide-generating system were considerably greater than that observed.
with the bovine cells. However, the levels of 6-keto-PGF\textsubscript{1\alpha} released by A23187 or arachidonic acid were likewise five- to tenfold greater in human cells than in bovine cells (data not shown). As with bovine ECs, the release of 6-keto-PGF\textsubscript{1\alpha} from the human ECs incubated with PMA-activated neutrophils (Fig. 2A) and with glucose-glucose oxidase (data not shown) was inhibited by catalase.

**TLC of hydrogen peroxide-mediated EC 6-keto-PGF\textsubscript{1\alpha} release.** The release of 6-keto-PGF\textsubscript{1\alpha} from ECs induced by neutrophil-derived and enzymatically generated hydrogen peroxide was confirmed by TLC. Table V A demonstrates the catalase-inhibitable release of labeled 6-keto-PGF\textsubscript{1\alpha}, PGE\textsubscript{2}, and arachidonic acid from prelabeled human EC monolayers incubated with PMA-activated neutrophils. Table V B demonstrates the catalase-inhibitable release of these labeled products from prelabeled human EC monolayers incubated with glucose-glucose oxidase.

**Relation of hydrogen peroxide-mediated endothelial 6-keto-PGF\textsubscript{1\alpha} release to cell lysis.** The rapid release of 6-keto-PGF\textsubscript{1\alpha} from bovine EC monolayers during incubation with PMA-activated neutrophils was not correlated with EC lysis, as determined by \(^{31}\text{Cr} \) release. Specific endothelial \(^{31}\text{Cr} \) release was not observed even after a 4-h incubation of \(^{31}\text{Cr} \)-labeled EC monolayers with PMA-activated neutrophils (Table VI). PMA-activated neutrophils also failed to induce significant specific \(^{31}\text{Cr} \) release from human EC monolayers at 4 h (data not shown).

**Table V. TLC of H\textsubscript{2}O\textsubscript{2}-mediated Human EC 6-Keto-PGF\textsubscript{1\alpha} Release**

<table>
<thead>
<tr>
<th>Addition to endothelial monolayers</th>
<th>(^{31}\text{Cr} )-counts per minute released</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-keto-PGF\textsubscript{1\alpha}</td>
<td>PGE\textsubscript{2}</td>
</tr>
<tr>
<td>A Control medium</td>
<td>38±4</td>
</tr>
<tr>
<td>PMA + neutrophils</td>
<td>267±85</td>
</tr>
<tr>
<td>PMA + neutrophils + catalase</td>
<td>91±29</td>
</tr>
<tr>
<td>B Control medium</td>
<td>93±9</td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>292±69</td>
</tr>
<tr>
<td>Glucose oxidase + catalase</td>
<td>102±28</td>
</tr>
</tbody>
</table>

In A, human umbilical vein EC monolayers were labeled with \(^{1}\text{H} \)-arachidonic acid, washed, and then incubated for 60 min with control medium, or PMA (10 ng/ml) and neutrophils (neutrophil-to-endothelial cell ratio of 10 to 1) with and without catalase (3000 U/ml). In B, control medium or glucose oxidase (50 mU/ml) with and without catalase (3000 U/ml) were incubated for 30 min with \(^{1}\text{H} \)-arachidonic acid-labeled human umbilical vein EC monolayers. After incubation the cell-free supernatant medium was removed for determination of labeled 6-keto-PGF\textsubscript{1\alpha}, PGE\textsubscript{2}, and arachidonic acid release by TLC. Values represent mean±1 SD of duplicate wells.

**Table VI. Effect of PMA-activated Neutrophils on Bovine EC Lysis**

<table>
<thead>
<tr>
<th>Addition to endothelial monolayers</th>
<th>(^{31}\text{Cr} ) release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm released</td>
</tr>
<tr>
<td>Control medium (8)</td>
<td>234±15</td>
</tr>
<tr>
<td>Neutrophils (8)</td>
<td>195±8</td>
</tr>
<tr>
<td>PMA (8)</td>
<td>236±20</td>
</tr>
<tr>
<td>PMA and neutrophils (8)</td>
<td>249±18</td>
</tr>
<tr>
<td>Triton X-100 (4)</td>
<td>2,202±11</td>
</tr>
</tbody>
</table>

Bovine aortic EC monolayers were labeled overnight with \(^{31}\text{Cr} \), washed, and then incubated with control medium, neutrophils (neutrophil to endothelial ratio of 10 to 1), PMA (10 ng/ml), neutrophils and PMA, or Triton X-100 (0.1%). After 4 h an aliquot of cell-free supernatant medium was removed for determination of \(^{31}\text{Cr} \) release. Values represent mean±1 SE of (n) replicates.

**Discussion**

The acute inflammatory response is characterized by neutrophil adherence to endothelium and neutrophil accumulation at the inflammatory site (1). The vascular response to neutrophil infiltration includes an increase in local blood flow and altered vascular permeability, resulting in edema formation. Numerous studies have implicated prostaglandins, particularly PGE\textsubscript{2} and PG\textsubscript{I2}, as critical mediators of the hyperemia and increased permeability that accompany the neutrophil infiltration (2, 9–16). Because the vascular endothelium releases PGI\textsubscript{2} and PGE\textsubscript{2} when stimulated (26), ECs are a potential source for the local production of these prostaglandins during the acute inflammatory response.

Our studies demonstrate that PMA-activated human peripheral blood neutrophils induce a time- and dose-dependent release of PGI\textsubscript{2} from cultured ECs. Since PMA does not induce the release of arachidonic acid metabolites from human neutrophils (27), and aspirin pretreatment of the endothelium prevented 6-keto-PGF\textsubscript{1\alpha} release, it is unlikely that endothelial PGI\textsubscript{2} release occurs as a result of the provision of a substrate for endothelial cyclooxygenase by neutrophil-generated endoperoxides, as has been described for platelet-endothelial interactions (28). The failure of PMA to induce arachidonic acid release in human neutrophils also suggests that neutrophil lipooxygenase products are not involved in EC PGI\textsubscript{2} release by neutrophils stimulated with PMA. Since leukotrienes C\textsubscript{4} and D\textsubscript{4} have been demonstrated to induce endothelial PGI\textsubscript{2} synthesis (29), it is possible that neutrophil-activating agents that result in leukotriene production could provoke endothelial PGI\textsubscript{2} release through generation of leukotriene C\textsubscript{4} or D\textsubscript{4}.

Because neutral proteases such as trypsin and thrombin have been demonstrated to stimulate endothelial PGI\textsubscript{2} release (30), neutrophil-derived neutral proteases such as elastase and cathepsin G might be expected to mediate endothelial 6-keto-
PGF$_{1a}$ release. Several factors, however, suggest that neutrophil-derived neutral proteases are not involved in the 6-keto-PGF$_{1a}$ release induced by PMA-activated neutrophils. First, at the concentration of PMA (10 ng/ml) and time course (15–30 min) used in these studies, there is minimal release of the neutrophil azurophilic granules that contain the neutral proteases (31). Second, SBTI, a potent serine protease inhibitor, did not prevent 6-keto-PGF$_{1a}$ release induced by PMA-activated neutrophils. Finally, purified elastase and cathepsin G do not directly affect baseline endothelial PGI$_{1a}$ release (32).

The failure of PMA-activated neutrophils from a patient with chronic granulomatous disease to induce endothelial PGI$_{2}$ release indicates that an oxygen radical is involved. Since catalase, but not superoxide dismutase, significantly reduces 6-keto-PGF$_{1a}$ release from ECs incubated with PMA-activated neutrophils, it appears that hydrogen peroxide or a hydrogen peroxide-derived product is the critical neutrophil-generated mediator. This is supported by the observation that the hydrogen peroxide-generating system, glucose-glucose oxidase, can also induce catalase-inhibitable PGI$_{2}$ release from cultured ECs over the same time course. Catalase did not always completely inhibit neutrophil-mediated PGI$_{2}$ release, whereas it totally prevented glucose-glucose oxidase-mediated release; this may be due to exclusion of this enzyme at sites where neutrophils and ECs adhere closely.

Our observation that peroxide-stimulated prostaglandin generation by ECs is not associated with cellular lysis has also been recently reported by Ager and Gordon (33). These investigators found that porcine aortic endothelium synthesized PGI$_{2}$ and PGE$_{2}$ in response to enzymatically generated hydrogen peroxide at a concentration significantly lower than that which was lethal to the cells.

The results obtained by TLC confirm those obtained by RIA. The TLC results demonstrate that enzymatically or neutrophil-generated hydrogen peroxide induces the release of labeled arachidonic acid, 6-keto-PGF$_{1a}$, and PGE$_{2}$ from prelabeled endothelial cells. Hydrogen peroxide has been observed to induce PGE$_{2}$ release from fibroblasts, and an organic peroxide has been reported to induce PGI$_{2}$ release from ECs (34, 35). The inhibition of hydrogen peroxide-induced endothelial PGI$_{2}$ release by the phospholipid inhibitor mepagaine A ($\Delta$) suggests that hydrogen peroxide may act by triggering membrane phospholipase A$_{2}$, thus liberating arachidonic acid as a substrate for cyclooxygenase.

The conversion of the arachidonic acid released by the exogenously generated hydrogen peroxide to PGI$_{2}$ is a cyclooxygenase-catalyzed event, since it is inhibited by aspirin. The reported effects of peroxides on the cyclooxygenase-dependent conversion are variable. Peroxides have been observed to both activate (36, 37) and inactivate cyclooxygenase (38). Since hydroperoxides can inactivate endothelial PGI$_{2}$ synthetase (39), peroxides may also affect the subsequent conversion of the endoperoxides generated by cyclooxygenase. If cyclooxygenase is stimulated while PGI$_{2}$ synthetase is inhibited, enhanced synthesis of PGE$_{2}$ may occur (40).

The effects of exogenously generated peroxides on endothelial arachidonic acid metabolism may be critically dose dependent. Taylor et al. noted that high concentrations of hydroperoxides inhibited 6-keto-PGF$_{1a}$ production in bovine ECs and PGE$_{2}$ in fibroblasts, whereas low concentrations stimulated prostaglandin release (35). Hydrogen peroxide-induced endothelial prostaglandin production may thus be a net result of complex stimulatory and inhibitory effects on the various enzyme systems involved.

The observation that exogenously generated hydrogen peroxide can induce the release of PGI$_{2}$ from cultured ECs is of particular interest in acute inflammation where the endothelium may be exposed to hydrogen peroxide generated by inflammatory cells such as neutrophils and monocytes. The release of a potent vasodilator such as PGI$_{2}$ may augment local edema formation (2, 9–16). Alternatively, the release of PGI$_{2}$ could prevent the adherence and activation of additional inflammatory cells by elevating intracellular cyclic AMP (41–44). The demonstration that a toxic oxygen radical generated by one cell can trigger the release of arachidonic acid metabolites by another cell further illustrates the possibilities for interactions between inflammatory mediators.

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