Induction of endothelial cell cytoplasmic lipid bodies during hypoxia

LISA M. SCARFO,1 PETER F. WELLER,2 AND HARRISON W. FARBER1

1Pulmonary Center, Boston University School of Medicine, Boston, 02118; and
2Charles A. Dana Research Institute, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215

Received 5 May 2000; accepted in final form 7 August 2000

Scarfo, Lisa M., Peter F. Weller, and Harrison W. Farber. Induction of endothelial cell cytoplasmic lipid bodies during hypoxia. Am J Physiol Heart Circ Physiol 280: H294–H301, 2001.—Lipid bodies (LBs), lipid-rich cytoplasmic inclusions found in many cell types, seem to act as nonmembrane sites of eicosanoid formation. Because alterations in eicosanoid products have been demonstrated in endothelial cells (ECs) during hypoxia, we investigated induction of LBs in systemic and pulmonary ECs exposed to acute and/or chronic hypoxia. LBs in ECs were O2-concentration dependent, increasing approximately fivefold during acute exposure to 0% O2 in both cell types. During chronic exposure to 3% O2, LBs were induced only in systemic ECs. LBs were not induced by other cellular stresses (heat shock or glucose deprivation). Subsequent studies suggested that protein kinase C-dependent and tyrosine kinase-dependent pathways are important in LB induction during hypoxia. PGH synthase was demonstrated in LBs in every case in which they were induced. These are the initial studies to demonstrate induction of LBs in ECs and to demonstrate LB which they were induced. These are the initial studies to demonstrate induction of LBs in ECs and to demonstrate LB induction during exposure to hypoxia in any cell type. These results imply that in ECs, LBs are structurally distinct inducible sites for synthesis of eicosanoid mediators.

LIPID BODIES (LBs) are non-membrane-bound, lipid-rich cytoplasmic inclusions found in many cell types including endothelial cells (ECs) (6, 7, 12, 28). Well described structurally and to some degree biochemically, neither the precise function of LBs nor the factors responsible for LB induction have been completely clarified (5, 6, 18, 29, 34). However, LBs are more numerous and prominent in many types of leukocytes both in vitro and in vivo during inflammatory responses. LB formation appears to be a specific cellular response stimulated by cis fatty acids and diglycerides and mediated by protein kinase C (PKC) and phospholipase C (6, 33). By ultrastructural and morphological criteria, induction of LBs is not associated with cellular damage (5–7, 12, 18, 28, 29, 33, 34). Moreover, LBs seem to be a major intracellular repository of fatty acids, including arachidonic acid, and may represent a nonmembrane site of eicosanoid formation (3, 4, 6).

Little information is available concerning LBs in ECs. However, ECs are rich in arachidonic acid and are a major source of eicosanoid products (13, 22, 24). Alterations in membrane lipids and eicosanoid products have been demonstrated in these cells during many cellular stimuli or stresses including exposure to hypoxia (2, 10, 17, 19, 23, 25); furthermore, we have demonstrated that alterations in eicosanoid products are dependent both on the level and duration of hypoxia and the vascular bed from which the ECs emanate (10).

In the current study we investigated induction of LBs in ECs from systemic and pulmonary vascular beds during exposure to acute and chronic hypoxia. We then evaluated potential mechanisms of LB induction, whether LBs could be induced by other forms of cellular stress, and whether LBs might play a role in endothelial eicosanoid production.

MATERIALS AND METHODS

Materials

MEM and trypsin-EDTA were from Gibco-BRL (Grand Island, NY). Tissue culture plastic was from Falcon Plastics (Los Angeles, CA) and Costar (Cambridge, MA). Bovine calf serum was from HyClone (Logan, UT). Sealed chambers for hypoxic incubations were from Billups-Rothenburg (Del Mar, CA). All reagents were from Sigma (St. Louis, MO) unless otherwise noted.

EC Cultures

Bovine aortic and pulmonary arterial ECs (BAECs and BPAECs, respectively) were obtained as previously described (1, 10, 15, 25, 26). Briefly, ECs were harvested by lightly scraping the luminal surface of longitudinally opened vessels. Cells were suspended and maintained in MEM containing 5% heat-inactivated bovine calf serum. From isolation the cells were incubated in 95% air-5% CO2 at 37°C or, if used in experiments with chronic hypoxia, in a humidified sealed chamber maintained at 37°C gassed with 3% O2-5% CO2-92% N2. The cells were maintained in the same O2 environment throughout experiments.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: H. W. Farber, Pulmonary Center, Boston Univ. School of Medicine, 715 Albany St., R-304, Boston, MA 02118 (E-mail: hfarber@lung.bumc.bu.edu).
throughout the culture period (2–4 mo). Chambers were regassed every 24 h; the partial pressure of O\textsubscript{2} in the culture medium was measured using a pH/blood gas analyzer and was similar to that previously reported (1, 10, 11, 15, 25, 26). Confluent ECs were passed after treatment with 0.25% trypsin-EDTA. EC purity was assessed by phase microscopic "cobblestone appearance," the presence of von Willebrand factor, angiotensin-converting enzyme activity, and uptake of fluorescent acetylated low-density lipoprotein (1, 10, 15, 25, 26). All experiments were performed using BAECs and BPAECs of passages 3–12; individual experiments used identical cell lines of identical passage numbers.

**EC Viability**

EC monolayers were assessed for injury as previously described (1, 10, 11, 16, 27). In the current experiments, a combination of phase-contract microscopic appearance, adherent cell counts, trypan blue exclusion, or \textsuperscript{51}Cr release was employed. For adherent cell counts, at specified times medium was removed and adherent cells were counted by hemocytometer or Coulter counter after trypsinization. For trypan blue exclusion, at specified times medium was removed and after a 5-min incubation period trypan blue exclusion was assessed by light microscopy. For \textsuperscript{51}Cr release, at specified times radioactivity in the medium and the ECs was measured, and \textsuperscript{51}Cr release was expressed as the percentage above the control.

**LB Staining and Enumeration**

In initial studies ECs grown on glass coverslips were fixed by exposure to osmium tetroxide (OsO\textsubscript{4}) vapor and then stained for 2–4 h in Oil red O [6 parts 0.5% (wt/vol) Oil red O in isopropanol, 4 parts water], rinsed briefly in isopropanol, and then mounted with von Apathy's medium (28). When it became apparent that LBs were induced in ECs by exposure to hypoxia, contrast with LBs was augmented by staining ECs sequentially with ferrocyanide \([K_4Fe(CN)_6]\)-reduced OsO\textsubscript{4} thioicarbohydrate, and OsO\textsubscript{4} (6, 32). Slides were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer of pH 7.4 (15 min), rinsed twice in cacodylate buffer (3 min), stained in 1.5% \(K_4Fe(CN)_6\) in 1% aqueous OsO\textsubscript{4} (32 min), rinsed three times in water, immersed in 1% thioicarbohydrate (5 min), rinsed three times in water, stained in 1% OsO\textsubscript{4} in 0.1 M cacodylate (3 min), rinsed with water, and then dried and mounted in von Apathy's medium. LBs were enumerated with phase-contrast microscopy \((\times400)\) in 25–50 consecutively scanned ECs by a single investigator (L. M. Scarfo). Because she also performed most of the experiments, she was not always blinded to the conditions. In those instances, the number of LBs was confirmed by one of the other authors either at the time of experiment or from photographs of the experiments.

**LB Induction**

Enumeration of LBs and potential mechanisms for LB induction were investigated under various common cellular stresses.

**Response to acute hypoxia or hyperoxia.** Normoxic BAECs or BPAECs exposed to 40, 10, 3, or 0% O\textsubscript{2} for \(\leq48\) h were compared with BAECs or BPAECs maintained in 21% O\textsubscript{2} for the same times.

**Adaptive response to chronic hypoxia.** BAECs or BPAECs cultured long term (2–4 mo) in 3% O\textsubscript{2} were compared with BAECs or BPAECs cultured for the same times in standard culture conditions.

**Response of chronically hypoxic cells to acute hypoxia.** Chronically hypoxic BAECs or BPAECs exposed acutely to 0% O\textsubscript{2} for \(\leq48\) h were compared with BAECs or BPAECs remaining in 3% O\textsubscript{2} for the same times.

**Reoxygenation.** Normoxic BAECs or BPAECs exposed to 0% O\textsubscript{2} for \(\leq48\) h were returned to 21% O\textsubscript{2} for \(\leq48\) h. BAECs or BPAECs cultured long term in 3% O\textsubscript{2} were returned to 10, 21, or 40% O\textsubscript{2} for \(\leq48\) h.

**Heat stress.** BAECs or BPAECs were sealed with Parafilm and placed in a water bath at specific temperatures (46–52°C) for 15 min. We previously demonstrated that these temperatures are sufficient to alter EC metabolism as evidenced by induction of heat-shock proteins (HSPs) (26).

**Glucose deprivation.** BAECs or BPAECs were cultured in glucose-free medium (GIBCO-BRL) for up to 7 days, sufficient time to detect alterations in EC metabolism as evidenced by induction of glucose-regulated proteins (GRPs) (29).

**Inhibitors and agonists.** In neutrophils, a diglyceride activator of PKC, 1-oleoyl-2-acetyl-rac-glycerol (OAG), has been shown to stimulate LB formation, and an inhibitor of PKC activation, 1–0-hexadecyl-2–0-methyl-rac-glycerol (HMG), has been shown to block LB formation (33). Moreover, in specific types of eosinophils, induction of LBs has been blocked by an inhibitor of tyrosine kinase (5). For inhibitor and agonist studies in ECs, BAECs or BPAECs were incubated with various concentrations of PKC inhibitor HMG (3–30 \(\mu\)M), PKC agonist OAG (10–100 \(\mu\)M), tyrosine kinase inhibitor genistein (10–100 \(\mu\)M), or vehicle (DMSO) for 0.5–1 h before exposure to the appropriate cellular stress.

**PGH Synthase Staining**

BAECs or BPAECs grown on glass slides in normoxia (21% O\textsubscript{2}) or chronic hypoxia (3% O\textsubscript{2}) were exposed to 0% O\textsubscript{2} for 24 h. After exposure the slides were blocked with 30% FCS and stained for 1.5 h with control antibody MOPC-195 or with MabC, a monoclonal antibody that recognizes both isoforms of PGH synthase (Cayman Chemicals, Ann Arbor, MI). Cells were then biotinylated with a second antibody that was avidin conjugated with glucose oxidase and photographed as previously described (6).

**Data Analysis**

All experiments were performed 4–12 times; data were expressed as means \(\pm\) SE LB/EC or as a mean net \(\pm\) SE LB/EC after subtracting the LB/EC found during control incubations. Results were qualitatively similar and reproducible in all EC lines examined and were independent of passage number or primary cell line of ECs used. In some figures data are representative examples of at least four experiments. Students t-test or one-way ANOVA followed by the Student-Newman-Keuls multiple comparison test was used to compare means. Differences were considered significant at \(P < 0.05\).

**RESULTS**

**EC viability.** Using a combination of previously described parameters (1, 11, 16, 26, 27), we found no evidence of injury in ECs exposed to acute hypoxia, chronic hypoxia, or acute on chronic hypoxia (data not shown). Thus, as in previous studies under these conditions, ECs maintained cellular viability and function (1, 11, 16, 26, 27).

**Response to acute hypoxia or hyperoxia.** Normoxic BAECs and BPAECs contained few LBs: depending on
the primary cell line, we observed 2–10 LB/EC (Figs. 1 and 2). Exposure to 0% O₂ caused marked induction of LBs in a similar fashion in both cell types. Results are described as the mean number of LB/EC (± SE), n = 4–10 for each condition. For BAECs at 21% O₂, LB/EC was 6.1 ± 1.0, and at 0% O₂ for 24 h, LB/EC was 32.4 ± 2.7 (P < 0.05). For BPAECs at 21% O₂, LB/EC was 8.2 ± 1.6, and at 0% O₂ for 24 h, LB/EC was 33.8 ± 3.1 (P < 0.05). Induction of LBs became significant after 12 h of 0% O₂: LB induction increased until 24 h, after which no further increase in LBs was observed up to 48 h exposure (Fig. 3). Exposure to less severe hypoxia (10 or 3% O₂) also increased the number of LBs, although to a lesser degree in each case than that observed at 0% O₂. In that case, for BAECs at 21% O₂, LB/EC was 6.1 ± 1.0; at 10% O₂ for 24 h, LB/EC was 13.6 ± 0.9 (P < 0.05); and at 3% O₂ for 24 h, LB/EC was 22.1 ± 1.7 (P < 0.05). For BPAECs at 21% O₂, LB/EC was 8.2 ± 1.6; at 10% O₂ for 24 h, LB/EC was 17.2 ± 1.4 (P < 0.05); and for 3% O₂ for 24 h, LB/EC was 25.4 ± 1.8 (P < 0.05). In contrast, exposure to mild hyperoxia (40% O₂) decreased the number of LBs. For BAECs at 21% O₂, LB/EC was 6.1 ± 1.0, and at 40% O₂ for 24 h, LB/EC was 2.0 ± 0.2 (P < 0.05). For BPAECs at 21% O₂, LB/EC was 8.2 ± 1.6, and at 40% O₂ for 24 h, LB/EC was 2.6 ± 0.4 (P < 0.05) (see Figs. 1 and 2).

Adaptive response to chronic hypoxia. There was a notable difference in the number of LBs in the two EC types cultured long term (2–4 mo) in 3% O₂. In chronically hypoxic BAECs, the number of LBs was markedly increased compared with the normoxic counterparts (Figs. 1 and 3): at 21% O₂, LB/EC was 6.1 ± 1.0, and at 3% O₂ long term, LB/EC was 30.9 ± 2.4 (P < 0.05). In contrast, in chronically hypoxic BPAECs, there was no increase in the number of LBs; in fact, there were even fewer LBs than in the normoxic counterparts (Figs. 2 and 3): at 21% O₂, LB/EC was 8.2 ± 1.6, and at 3% O₂ long term, LB/EC was 2.7 ± 0.7 (P < 0.05).

Response of chronically hypoxic cells to acute hypoxia. Again, there was a marked difference in response to hypoxia dependent on EC type. In chronically hypoxic BAECs there was no significant change in the number of LBs during exposure to further hypoxia (Fig. 3). In contrast, in chronically hypoxic BPAECs in which there was a much lower number of LBs, exposure to further hypoxia (0% O₂) caused a dramatic increase: the number of LBs increased significantly

![Figure 1](http://jalpheart.physiology.org/)
after a 4-h exposure, reached a plateau at 12–16 h, and did not increase further up to a 48-h exposure (Fig. 3).

Reoxygenation. After the return of BAECs or BPAECs exposed acutely to 0% O₂ for 48 h or more under hypoxia, the number of LBs returned to control levels within 24 h (data not shown). Exposure of BAECs or BPAECs cultured long term in 3% O₂ to 10, 21, or 40% O₂ for 48 h again produced disparate results depending on the EC type. Exposure of BAECs cultured long term in 3% O₂ to 10, 21, or 40% O₂ caused a decrease in the number of LBs in an O₂-concentration fashion. For 3% O₂ long term, LB/EC was 30.9 ± 2.4; for 3% O₂ long term followed by: 1) 10% O₂ for 24 h, LB/EC was 7.6 ± 0.7 (P < 0.05); 2) 21% O₂ for 24 h, LB/EC was 5.4 ± 3.3 (P < 0.05); and 3) 40% O₂ for 24 h, LB/EC was 2.6 ± 0.6 (P < 0.05). In contrast, exposure of BPAECs cultured long term in 3% O₂ to 10, 21, or 40% O₂ caused variable results depending on the acute O₂ concentration to which the ECs were exposed: for 3% O₂ long term, LB/EC was 2.7 ± 0.7; for 3% O₂ long term followed by: 1) 10% O₂ for 24 h, LB/EC was 5.8 ± 1.1 (P < 0.05); 2) 21% O₂ for 24 h, LB/EC was 3.5 ± 1.0 (P < 0.05); and 3) 40% O₂ for 24 h, LB/EC was 1.2 ± 0.6 (P < 0.05).

Heat stress. Despite induction of HSPs, exposure to a sublethal increase in temperature (46–52°C) did not alter the number of LBs in either normoxic BAECs or BPAECs. For BAECs at 37°C, LB/EC was 6.5 ± 0.6; at 46°C, LB/EC was 6.1 ± 0.9; and at 52°C, LB/EC was 6.6 ± 1.0. For BPAECs at 37°C, LB/EC was 7.6 ± 0.8; at 46°C, LB/EC was 7.8 ± 1.0; and at 52°C, LB/EC was 7.4 ± 0.6 (n = 4 for each condition).

Glucose deprivation. Despite induction of GRPs, culture in glucose-free medium for ≤7 days did not alter the number of LBs in either normoxic BAECs or BPAECs (n = 4 for each condition). For BAECs, control LB/EC was 6.2 ± 0.8; after glucose deprivation for 7 days, LB/EC was 6.4 ± 0.5. For BPAECs, control LB/EC was 8.0 ± 1.0; after glucose deprivation for 7 days, LB/EC was 7.8 ± 0.9.

Inhibitors and agonists. The PKC inhibitor HMG did not alter the number of LBs in normoxic BAECs or BPAECs; in contrast, at all concentrations tested (3–30 μM) HMG decreased induction of LBs during exposure to hypoxia by ~50% (Table 1). At all concentrations tested (25–100 μM) the PKC agonist OAG increased the number of LBs in normoxic BAECs or BPAECs by ~50%; OAG did not alter the induction of LBs during exposure to hypoxia (Table 1). The tyrosine kinase inhibitor genistein did not alter the number of LBs in normoxic BAECs or BPAECs; in contrast, at the
inflammatory response, alterations in eicosanoid formation, particularly in ECs, are a common finding during exposure to this cellular stress. For example, we have previously demonstrated that exposure to acute hypoxia causes a rapid and transient increase in prostacyclin and thromboxane production (10). Exposure to longer periods of hypoxia alters this response and in both cases is dependent on the duration of hypoxia and the vascular bed from which the ECs emanate. Further study of this phenomenon in ECs has demonstrated that although there are marked changes in EC phospholipids during acute and chronic hypoxia, changes in phospholipase are not as extensive and likely do not explain the alteration in cyclooxygenase metabolites seen during exposure to hypoxia (2, 25). Other studies and our current observations (Figs. 4 and 5) add to speculation that a significant amount of cyclooxygenase metabolism does not occur at the plasma membrane but is induced by a specific stimulus or cellular stress within cytoplasmic LBs (4, 6, 29, 31).

Despite the wealth of information available on the biochemistry and pharmacology of arachidonic acid metabolites, questions remain regarding the subcellular sites from which arachidonic acid is mobilized. For example, it is widely believed that phospholipids of cell membranes represent the major source of arachidonic acid entering the cyclooxygenase or lipoxygenase pathways of oxidation. However, little direct evidence exists to substantiate this claim. For example, several studies in various cell types have failed to demonstrate plasma membrane-associated PGH or cyclooxygenase (31). The

**Table 1. Effect of HMG, OAG, and genistein on hypoxia-induced LB formation**

<table>
<thead>
<tr>
<th>Condition</th>
<th>BAEC</th>
<th>BPAEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia, 21% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.1±0.7</td>
<td>7.3±0.8</td>
</tr>
<tr>
<td>+3 μM HMG</td>
<td>5.7±0.9</td>
<td>7.4±0.6</td>
</tr>
<tr>
<td>+30 μM HMG</td>
<td>5.9±0.4</td>
<td>6.9±0.5</td>
</tr>
<tr>
<td>Acute hypoxia, 0% O&lt;sub&gt;2&lt;/sub&gt;×24 h</td>
<td>30.7±2.1*</td>
<td>34.2±3.2*</td>
</tr>
<tr>
<td>+3 μM HMG</td>
<td>17.2±1.6†</td>
<td>17.8±1.9†</td>
</tr>
<tr>
<td>+30 μM HMG</td>
<td>15.8±1.4†</td>
<td>16.6±1.7†</td>
</tr>
<tr>
<td>Normoxia, 21% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.5±0.3</td>
<td>6.9±1.1</td>
</tr>
<tr>
<td>+50 μM OAG</td>
<td>9.4±0.6*</td>
<td>10.6±1.4*</td>
</tr>
<tr>
<td>+100 μM OAG</td>
<td>10.3±0.4*</td>
<td>11.6±1.0*</td>
</tr>
<tr>
<td>Acute hypoxia, 0% O&lt;sub&gt;2&lt;/sub&gt;×24 h</td>
<td>30.1±1.8*</td>
<td>32.9±2.9*</td>
</tr>
<tr>
<td>+50 μM OAG</td>
<td>29.6±1.9*</td>
<td>34.2±2.6*</td>
</tr>
<tr>
<td>+100 μM OAG</td>
<td>30.9±2.3*</td>
<td>33.4±3.2*</td>
</tr>
<tr>
<td>Normoxia, 21% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.7±0.8</td>
<td>7.0±1.4</td>
</tr>
<tr>
<td>+25 μM genistein</td>
<td>6.1±0.2</td>
<td>7.0±1.0</td>
</tr>
<tr>
<td>+100 μM genistein</td>
<td>5.6±0.5</td>
<td>6.8±0.9</td>
</tr>
<tr>
<td>Acute hypoxia, 0% O&lt;sub&gt;2&lt;/sub&gt;×24 h</td>
<td>32.3±2.8*</td>
<td>31.6±2.0*</td>
</tr>
<tr>
<td>+25 μM genistein</td>
<td>16.5±1.8†</td>
<td>18.2±1.6†</td>
</tr>
<tr>
<td>+100 μM genistein</td>
<td>7.2±1.3†</td>
<td>8.4±1.8†</td>
</tr>
</tbody>
</table>

Results are means (no. of lipid bodies per endothelial cell) ± SE. HMG, 1-hexadecyl-2,0-methyl-rac-glycerol; OAG, 1-oleoyl-2-acet-y1-rac-glycerol; LB, lipid body; EC, endothelial cell; BAEC, bovine aortic ECs; BPAEC, bovine pulmonary arterial ECs. BAEC and BPAEC were grown on glass slides under designated conditions, exposed to osmium tetroxide (4% aqueous solution) vapors for 1 h, and stained with Oil red O and isopropanol for 3 h. LBs were visualized and quantified by direct phase microscopy (×400). See MATERIALS and METHODS for details. n = 4–8 for each condition; *P < 0.05 compared to normoxic EC; †P < 0.05 compared to acutely hypoxic EC (0% O<sub>2</sub>×24 h).
current study supports a growing body of experimental evidence that implicates non-membrane-bound cytoplasmic LBs in eicosanoid metabolism in mammalian cells (6, 7, 20, 21, 28, 29, 30, 32). Together with previous studies that have demonstrated 1) ultrastructural localization of radiolabeled arachidonic acid to LBs (7, 20, 21, 28, 30, 32); 2) biochemical demonstration of substantial amounts of radiolabeled arachidonyl phospholipids in isolated LBs (32); 3) PG endoperoxide synthase (cyclooxygenase) protein and enzymatic activity in isolated LBs (6, 8, 9); and 4) principal leukotriene-forming enzymes in isolated LBs (6), they provide evidence that LBs are uniquely constituted for the production of eicosanoids.

We found that induction of LBs in response to hypoxia differed depending on the origin of the ECs and the degree of hypoxia. Although the response of normoxic pulmonary and systemic ECs was similar during exposure to acute changes in O2 content, there was a marked difference in the LBs in these cell types during chronic hypoxia. In chronically hypoxic BAECs the increase in LBs observed during acute hypoxia per-
sisted, whereas in hypoxic BPAECs the number of LBs decreased to even less than the number found in normoxic BPAECs. Furthermore, there was no change in the number of LBs in chronically hypoxic BAECs during exposure to further hypoxia, whereas in chronically hypoxic BPAECs exposure to further hypoxia caused a similar multifold induction as observed in the normoxic counterparts. The explanation for this difference between pulmonary and systemic ECs is not clear but is interesting in light of previous characteristics we have reported about ECs exposed to hypoxia. Although we have described numerous metabolic alterations in hypoxic ECs, when compared, most of these alterations have been similar in both pulmonary and systemic ECs (1, 14, 15, 27, 35). Interestingly, only changes in lipid-associated metabolic functions appear dissimilar. For example, we have observed differences in induction of an eicosanoid lipid neutrophil chemoattractant (11), PG metabolism (10), phospholipid distribution (25), and now, induction of LBs in hypoxic pulmonary and systemic ECs. These findings suggest that differences in eicosanoid-related metabolic functions between pulmonary and systemic ECs serve a specific purpose. Further studies will be necessary to determine the mechanism and significance of these metabolic differences.

Induction of LBs appeared unique among the major cellular stresses, namely change in O₂ concentration, temperature, or substrate. Although the degree of each cellular stress we used in these studies is sufficient to induce specific stress proteins in ECs: hypoxia-associated proteins (HAPs), HSPs, or GRPs, respectively (27), only exposure to hypoxia was able to induce these cytoplasmic organelles under these conditions. Thus it appears that induction of LBs is a distinctive response of ECs to the stress of decreased ambient O₂ rather than a uniform response to a variety of cellular stresses. Furthermore, this may represent another facet of unique EC responses to hypoxia that contribute to the increased tolerance of ECs compared with other mammalian cell types.

We found that induction of LBs, at least during acute hypoxia in normoxic ECs, was likely mediated by PKC and tyrosine kinase. The PKC agonist OAG induced LBs in normoxic cells, whereas both the PKC inhibitor HMG and the tyrosine kinase inhibitor genistein suppressed LB induction by hypoxia. Previous studies in leukocytes have also demonstrated that induction of LBs is mediated by the action of PKC (33). The role of tyrosine kinase-dependent signaling is not as clear, however, and may not be contributory in all circumstances in which LBs are induced. For example, in eosinophils from individuals with hypereosinophilic syndrome or in eosinophils cultured with granulocyte macrophage colony-stimulating factor, tyrosine kinase-dependent signaling appears to be important in the induction of LBs; however, it does not appear to mediate induction in normal eosinophils (5). Together these studies suggest that PKC pathways are important in the induction of LBs but that tyrosine kinase-depen-
dent pathways may be differentially recruited depending on the stimulus or the cell type.

In summary, these are the initial studies to demonstrate induction of LBs in ECs and the initial report and characterization of LB induction during exposure to hypoxia. These studies have confirmed findings in other cell types that these cytoplasmic organelles are structurally distinct, inducible sites for the synthesis of eicosanoid mediators. They also suggest that at least in ECs during exposure to hypoxia, PKC-dependent and tyrosine kinase-dependent pathways are important in LB induction. Further studies are under way to determine the similarities and differences in induction and metabolic function of these organelles in ECs compared with other cell types.

The authors thank Daniel M. Tzizik for technical assistance. This work was supported by National Institutes of Health Grants AI-22571 (to P. F. Weller) and HL-45537 (to H. W. Farber).

REFERENCES

12. Galli SJ, Dvorak AM, Peters SP, Schulman ES, MacGlashan DW Jr, Isomura T, Pyne K, Harvey VS, Hammel I,


