Inflammatory stress increases receptor for lysophosphatidylcholine in human microvascular endothelial cells

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Inflammatory stress increases receptor for lysophosphatidylcholine in human microvascular endothelial cells. Am J Physiol Heart Circ Physiol 285: H1786–H1789, 2003. First published June 12, 2003; 10.1152/ajpheart.00359.2003. The atherogenic serum lysophosphatidylcholine (LPC) is known to mediate vascular endothelial responses ranging from upregulation of adhesion molecules and growth factors to secretion of chemokines and superoxide anion. We investigated whether endothelial cells express receptors for LPC, which may account for their actions. Human brain microvascular (HBMEC) and dermal microvascular endothelial cells (HMEC) were prepared for RT-PCR analysis for possible expression of the G protein-coupled receptors, GPR4 and G2A, which are believed to be specific LPC receptors. Results indicated that HBMEC expressed high basal GPR4 mRNA, but stimulation with tumor necrosis factor-α (TNF-α) (100 U/ml) or H2O2 (50 μmol/l) for 2 h or overnight upregulated expression severalfold. In contrast, HMEC expressed high basal GPR4 mRNA, which was not further increased by either TNF-α or H2O2 stimulation. Another LPC receptor, G2A, was not detected in either endothelial cell type. Competition binding studies were made to evaluate specific binding of [3H]LPC to the intact endothelial cell monolayer. Basal specific [3H]LPC binding in HBMEC was approximately eight times lower than in HMEC; however, TNF-α or H2O2 stimulation increased [3H]LPC binding on HMEC but not HBMEC. The results indicated that GPR4 expression was consistent with specific [3H]LPC binding. Overall, we report that endothelial cells selectively expressed GPR4, a specific LPC receptor. Furthermore, GPR4 expression by HBMEC, but not HMEC, was increased by inflammatory stresses. We conclude that endogenous GPR4 in endothelial cells may be a potential G protein-coupled receptor by which LPC signals proinflammatory activities.

G protein-coupled receptors 4 and 2A; oxidants; tumor necrosis factor-α; specific binding

LYSOPHOSPHATIDYLCHOLINE (LPC) is a proinflammatory and atherogenic serum lysophospholipid that activates a variety of cell types, including the vascular endothelium (6, 8, 9, 13, 15, 24). Endothelial cells stimulated with LPC are shown to upregulate endothelial cell adhesion molecules such as ICAM-1, VCAM-1, and P-selectin (8, 14, 26) and growth factors (17). Moreover, LPC-activated endothelium secretes cytokines, matrix metalloproteinases, O2·−, as well as arachidonic acid (4, 7, 20, 21, 23). Thus LPC stimulates multiple inflammatory activities in the vascular endothelium.

LPC can activate several second messenger pathways, including protein kinase C, extracellular signal-regulated kinases, tyrosine kinases, and Ca2+ (2, 16, 19, 26), implicating engagement of transduction mechanisms. Recent evidence suggests that two cloned orphan G protein-coupled receptors, GPR4 and G2A, may be specific LPC receptors in immune and tumor cells (5, 25). In these reports, the ectopically expressed receptors demonstrated high binding affinity and specificity with LPC. The physiological function and endogenous expression of LPC receptors by the vascular endothelium, as well as the consequent pathological implications, have yet to be fully elucidated. The goal of the present study was to investigate the intriguing possibility that endothelium may express endogenous GPR4 and G2A by which LPC mediates inflammatory responses.

MATERIALS AND METHODS

For these studies, we cultured human microvascular endothelial cells from two different vascular beds, brain (HBMEC) and dermis (HMEC). HBMEC were grown in RPMI 1640 supplemented with 10% FBS, 10% NuSerum (Becton Dickinson; Bedford, MA), endothelial cell growth supplement (30 μg/ml), heparin (5 U/ml), 1 mmol/l sodium pyruvate, 1 mmol/l minimal essential media (MEM), nonessential amino acids, 1 mmol/l MEM vitamins, 1% l-glutamine, and 1% penicillin-streptomycin. HMEC were cultured in MCDB 131 medium (GIBCO-BRL; Gaithersburg, MD) supplemented with 5% FBS (Hyclone; Logan, UT), 10 μg/ml human epidermal growth factor, 1 mg/l hydrocortisone, 1% penicillin-streptomycin, and 1% l-glutamine. Both HBMEC (18) and HMEC (1, 11) express the endothelial phenotype and demonstrate endothelial functional characteristics.

The expression of GPR4 and G2A mRNAs in HBMEC and HMEC was determined by RT-PCR as described previously...
(10). Total RNA was extracted from confluent monolayers of HBMEC or HMEC treated for 2 h or overnight with either TNF-α (100 U/ml) or H2O2 (50 μmol/l), as well as from human lymphocytes as a positive control. Total RNA was reverse transcribed with oligo-dT primers, and PCR was performed with specific primer sets corresponding to GenBank sequences of human GPR4 (5' = CAT CGC CCT GTG CTG CA; 3' = AAG AGC ACG TGA TAG GGC GC) and G2A (5' = ATG TGC CCA ATG CTA CTG; 3' = GTT CAC CGT GGA CAG GCA CA). RT-PCR products were analyzed by 1.5% agarose gel electrophoresis.

Specific binding of LPC (1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine; Avanti Polar Lipids, Alabaster, AL) to endothelial cell surface was determined by competition binding assays. Confluent cell monolayers grown in 24-well culture dishes were treated overnight with either TNF-α (100 U/ml) or H2O2 (50 μmol/l). The cells were washed and incubated for 60 min at 4°C with HEPES buffer (pH 7.4, 0.1% BSA) containing 0.02 nmol [3H]LPC (L-1-[methyl-3H]lyso-3-phosphatidylcholine; 40–60 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO) or [3H]LPC plus a 200-fold molar excess of unlabeled LPC. After three washes with cold HEPES buffer, cells were lysed with 0.1 mol/l NaOH, radioactivity was counted, and specific binding from duplicate samples was calculated (as fmol LPC bound/10⁶ cells). Separate dishes of cells were treated in parallel for cell count determination.

RESULTS AND DISCUSSION

A major finding is that human endothelial cells from two different vascular beds, brain and skin, expressed the LPC receptor GPR4 mRNA (Fig. 1) but not G2A.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Treatment</th>
<th>Specific Binding, fmol[3H]LPC/10⁶ cells (mean ± SE)</th>
<th>Estimated Ligand-Occupied Sites/Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBMEC</td>
<td>None</td>
<td>1.75 ± 0.83</td>
<td>1,050</td>
</tr>
<tr>
<td>HBMEC</td>
<td>TNF-α</td>
<td>5.50 ± 0.82*</td>
<td>3,300</td>
</tr>
<tr>
<td>HBMEC</td>
<td>H2O2</td>
<td>9.30 ± 0.48*</td>
<td>5,580</td>
</tr>
<tr>
<td>HMEC</td>
<td>None</td>
<td>14.8 ± 3.7</td>
<td>8,910</td>
</tr>
</tbody>
</table>

LPC, lysophosphatidylcholine; HBMEC, human brain microvascular endothelial cells; HMEC, human microvascular endothelial cells. n = 3 studies. Estimated ligand-occupied sites per cell were determined by (mol[3H]LPC bound/cell) × (Avogadro’s number (6.02 × 10²³ molecules/mol)). HBMEC were treated with 100 U/ml TNF-α or 50 μmol/l H2O2 overnight. *P < 0.01 compared with untreated control.
mRNA (Fig. 2). This selectivity for GPR4 expression by endothelial cells is consistent with the report that GPR4 appears to have wide tissue distribution, including the ovary, lung, kidney, liver, brain, and lymph nodes (25). Our current finding suggests that this wide distribution in tissue expression extends to the vascular endothelium as well. In contrast, G2A was not expressed by either endothelial cell types but was detected in human lymphocytes serving as a positive control (Fig. 2). This observation is consistent with reports indicating that G2A is found predominantly in tissues rich in lymphocytes such as spleen and thymus and to a lesser extent in other tissues such as the heart and lung (22).

The pattern of constitutive GPR4 mRNA expression by the two endothelial cell types was considerably different. HBMEC expressed barely detectable levels of constitutive GPR4 (the predicted 419-bp product was detected only at increased PCR cycles; data not shown), whereas the basal expression of GPR4 mRNA in HMEC was higher than that in HBMEC. The findings suggest that HMEC may have greater surface expression of endogenous GPR4 protein than HBMEC. Our laboratory is at present developing an antibody to GPR4 to investigate this hypothesis.

We next determined whether inflammatory stress induces GPR4 expression in HBMEC and HMEC. Evidence to date indicates that LPC receptors appear to be inducible by a wide range of signals, including DNA-damaging reagents, stress, and apoptosis (22). HBMEC and HMEC were stimulated by the cytokine TNF-α or the oxidant H2O2 for 2 h or overnight. The results indicated that in HBMEC, but not HMEC, stimulation with TNF-α or H2O2 increased GPR4 mRNA over control within 2 h (Fig. 1), which was sustained overnight (data not shown). Subsequent sequencing of the purified bands in both forward and reverse directions (Research Resources Center, University of Illinois at Chicago) and BLAST 2.0 analysis (Basic Local Alignment Search Tool, NCBI) indicated that the GPR4 DNA sequences had 96–97% identity with gene database sequences, corresponding to E values <10−160. Thus results indicated that GPR4 was increased by TNF-α and H2O2 stimuli. Although reasons for the selective upregulation of GPR4 in HBMEC but not in HMEC are not apparent from this study, the findings do suggest that cerebral vascular endothelium appears to be highly sensitive to inflammatory stresses in the context of LPC receptor expression, which may lead to enhanced responsiveness to LPC. In contrast, neither HBMEC (Fig. 2) nor HMEC express G2A, the other LPC receptor; furthermore, TNF-α or H2O2 was ineffective in induction of its expression.

We also evaluated the relation of GPR4 expression and specific binding of LPC to endothelial cells following inflammatory stress. The binding studies were optimized to evaluate surface binding of LPC on intact cells. The results indicated that HBMEC have greater than eightfold lower basal [3H]LPC binding than HMEC (Table 1), which is consistent with the lower constitutive GPR4 mRNA detected. HBMEC stimulated by TNF-α or H2O2 overnight showed three- and fivefold increases of [3H]LPC binding over control, respectively (Table 1). The induced increases of [3H]LPC binding were consistent with the increased GPR4 expression found. Overall, the results suggest that [3H]LPC likely bound endogenous GPR4. It is possible that LPC binds to both GPR4 and other closely related receptors because GPR4 shares 30–50% sequence homology with other G protein-coupled receptors such as the platelet activating factor (PAF) receptor (3, 12). However, binding studies indicated that PAF did not compete with LPC binding in cells ectopically expressed with GPR4 (25), indicating that LPC likely did not bind to the PAF receptor. Interestingly, another bioactive phospholipid sphingosylphosphorylcholine has been shown to bind with GPR4 at higher affinity [Michaelis-Menten dissociation constant (Kd) = 36 nM] than LPC (Kd = 159 nM) when investigated in cells ectopically expressed with GPR4 (25), suggesting that endothelial cells may also be activated by SPC through GPR4.

In conclusion, our results indicated that of the known receptors for LPC to date, vascular endothelial cells selectively expressed GPR4 but not G2A. Furthermore, GPR4 expressed in brain endothelial cells was inducible by inflammatory stresses such as cytokines and oxidants, and this ability appears to be tissue specific. We conclude that the endogenous GPR4 expressed in the endothelium may provide a G protein-coupled receptor mechanism by which the highly proinflammatory LPC signals cellular responses.

DISCLOSURES

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REFERENCES


