Histamine Directly and Synergistically with Lipopolysaccharide Stimulates Cyclooxygenase-2 Expression and Prostaglandin I\textsubscript{2} and E\textsubscript{2} Production in Human Coronary Artery Endothelial Cells

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Histamine Directly and Synergistically with Lipopolysaccharide Stimulates Cyclooxygenase-2 Expression and Prostaglandin I2 and E2 Production in Human Coronary Artery Endothelial Cells

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Although histamine plays an essential role in inflammation, its influence on cyclooxygenases (COX) and prostanoid homeostasis is not well understood. In this study, we investigated the effects of histamine on the expression of COX-1 and COX-2 and determined their contribution to the production of PGE2, prostacyclin (PGI2), and thromboxane A2 in human coronary artery endothelial cells (HCAEC). Incubation of HCAEC monolayers with histamine resulted in marked increases in the expression of COX-2 and production of PGI2 and PGE2 with no significant change in the expression of COX-1. Histamine-induced increases in PGI2 and PGE2 production were due to increased expression and function of COX-2 because gene silencing by small interfering RNA or inhibition of the catalytic activity by a COX-2 inhibitor blocked prostanoid production. The effects of histamine on COX-2 expression and prostanoid production were mediated through H1 receptors. In addition to the direct effect, histamine was found to amplify LPS-stimulated COX-2 expression and PGE2 and PGI2 production. In contrast, histamine did not stimulate thromboxane A2 production in resting or LPS-activated HCAEC. Histamine-induced increases in the production of PGE2 and PGI2 were associated with increased expression of mRNA encoding PGE2 synthase and PGI2 synthase. The physiological role of histamine on the regulation of COX-2 expression in the vasculature is indicated by the findings that the expression of COX-2 mRNA, but not COX-1 mRNA, was markedly reduced in the aortic tissues of histidine decarboxylase null mice. Thus, histamine plays an important role in the regulation of COX-2 expression and prostanoid homeostasis in vascular endothelium. The Journal of Immunology, 2007, 179: 7899–7906.

Histamine is one of the major constituents of the mast cell that is released into the extracellular milieu upon degranulation. Histamine and other mast cell mediators are known to regulate vasodilation and bronchoconstriction (1, 2), and to modulate the functions of other cell types, including monocytes and macrophages (3, 4), eosinophils (5, 6), T lymphocytes (7), neutrophils (8), and endothelial cells (9). A direct association between mast cell-derived histamine and vascular inflammation is evident from the finding that the coronary arteries of patients with ischemic heart disease contain more mast cells and histamine than normal vessels (10), and patients with variant angina have elevated levels of histamine in the coronary circulation (11). The fact that histamine is able to stimulate endothelial cell production of such proinflammatory cytokines as IL-6 and IL-8 (9, 12, 13) indicates that this mast cell product can act as an important inflammatory agent in addition to its well-recognized vasoactive functions.

Cyclooxygenase-1 (COX-1)3 and COX-2 belong to a family of enzymes that catalyze the oxygenation of arachidonic acid to PGG2/H2, which are used by specific PG synthases to generate PGE2, PGD2, PGF2α, prostacyclin (PGI2), and thromboxane (TX) A2. Among the PGs, PGI2 and TXA2 have earned considerable interest because of their involvement in cardiovascular diseases (14, 15). PGI2 is a major PG produced by endothelial cells, and is a potent vasodilator and an inhibitor of leukocyte adhesion and platelet aggregation. Hence, PGI2 is thought to play a protective role in atherothrombosis. TXA2, in contrast, is a potent inducer of vasoconstriction, platelet activation, and platelet adhesion. Because PGI2 and TXA2 act on vascular endothelium in opposing manners, their relative concentrations in the microenvironment and systemic circulation are critical for vascular homeostasis and athrogenesis.

It is well recognized that innate immune dysregulation plays an important role in the pathogenesis of many inflammatory diseases. Recently, we have shown that histamine stimulates TLR2 and TLR4 expression in endothelial cells and enhances inflammatory responses to Gram-positive and Gram-negative bacterial cell wall components (16). Thus, histamine is not only able to induce inflammatory responses directly, but also has the potential to amplify infection-associated activation of endothelial functions (16). The objectives of this study were to determine the role of histamine in the regulation of COX-2 gene expression in vitro and in vivo.

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3 Abbreviations used in this paper: COX, cyclooxygenase; EGM-2 MV, endothelial cell growth medium; ELA, enzyme immunoassay; H1R, histamine H1 receptor; H2R, histamine H2 receptor; H3R, histamine H3 receptor; HCAEC, human coronary artery endothelial cell; HDC, histidine decarboxylase; PGI2, prostacyclin; siRNA, small interfering RNA; TX, thromboxane.

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to evaluate its regulatory function on the production of PGE_2, PGI_2, and TXA_2 in naive and LPS-stimulated human coronary artery endothelial cells (HCAEC). This report demonstrates that histamine directly and synergistically with LPS stimulates the expression of COX-2 and amplifies the production of PGI_2 and PGE_2 without affecting basal or LPS-induced TXA_2 production in HCAEC. In addition, the study presents first evidence that the aortic tissues of histidine decarboxylase (HDC) null (HDC^−/−) mice express markedly reduced levels of COX-2 mRNA without change in the level of expression of COX-1 mRNA. These findings support the concept that histamine plays a key role in the regulation of COX-2 expression and prostanooid homeostasis in the vasculature.

Materials and Methods

Materials

HCAEC, endothelial cell growth medium (EGM-2 MV), trypsin-EDTA, and trypsin-neutralizing solution were purchased from Cambrex. Enzyme immunoassay (EIA) kits for 6-keto-PGF_1 alpha, PGE_2, and TXB_2, and COX-2, COX-1, and goat anti-rabbit IgG HRP Abs were obtained from Cayman Chemical. Actin, rabbit anti-goat IgG Abs, and ECL chemiluminescence reagent were purchased from Santa Cruz Biotechnology. Histamine dihydrochloride, fexofenadine hydrochloride, famotidine, 2-[(3-trifluoromethyl) phenyl] histamine dimaleate, and Escherichia coli (O111:B4) LPS were purchased from Sigma-Aldrich. RNeasy mini kit was purchased from Qiagen. High-capacity cDNA Achieve kit, SYBR Green PCR Master Mix kit, and all the gene-specific primers were purchased from Applied Biosystems.

Radioimmunoprecipitation assay lysis and extraction buffer was a kit, and all the gene-specific primers were purchase from Applied Biosystems. Enzyme immunoassay (EIA) kits for 6-keto PGF_1 alpha, PGE_2, and TXB_2, and COX-2, syntheasize, forward, 5'-GCCACATAGCTCTATAAGCTTGAAAC-3' and reverse, 5'-GGTCAATGGGAACCCCTGTAATCT-3'; beta-actin, forward, 5'-CCACGTCCTACCGATTGATT-3' and reverse, 5'-AGTTGGCTACCTACGAGTTTTC-3'; PGE_2 synthise, forward, 5'-CCTGGGCTTGTACTTCTT-3' and reverse, 5'-AGTTGAATCAGCGGCGCAA-3'; TXA_2 synthase, forward, 5'-GTGTCGTGTCCTCCCTTTCTA CCT-3' and reverse, 5'-CTGATCTCGCCGCCCCTTA-3'.

Real-time PCR was performed using the ABI 7500 Real-Time PCR system (Applied Biosystems) equipped with a 96-well optical reaction plate. The amplification reactions were performed in 25 μl total volume containing 20 μl of SYBR Green PCR Master Mix and 5 μl of cDNA of each sample. All real-time experiments were run in triplicate, and a mean value was used for the determination of mRNA levels. COX-2 or COX-1 mRNA levels from each treatment were normalized to the corresponding amount of beta-actin mRNA levels. Negative controls, containing water instead of sample cDNA, were used in each real-time plate.

Semiquantitative RT-PCR analyses of the expression of COX-1 and COX-2 mRNA in aortic tissues of wild-type and HDC^−/− mice

Aortic tissues were harvested from two HDC^−/− mice and two wild-type controls. The protocols used in this study were in accordance with the guidelines approved by the institutional review boards of the University of Kansas Medical Center and Vanderbilt University. After exsanguination, thoracic aortas were dissected and rinsed with cold PBS. Total RNA was extracted using TRIzol reagent. Total RNA (1 μg) was used for reverse-transcription reaction using random oligonucleotide primers, according to the manufacturer’s instruction (Invitrogen Life Technologies). Aiquots of the reverse-transcription product were PCR amplified using gene-specific sense and antisense primers. The PCR cycle parameters were as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s for the 1st cycle, followed by 94°C for 5 min and 72°C for 10 min for 45 cycles. The PCR products were electrophoresed on agarose gels (1.5%) and stained with ethidium bromide for imaging. The sequences of primers used are as follows: COX-1, forward, AGGAGATGGCTGCTGATTTGG and reverse, AAT CTGGCTTTCTCTCCCT and reverse, CACCATAGAATACGTCGG (product size 861 bp); CAAGAGACCGAGCAATCAAG (product size 246 bp). The sequences of primers used in the real-time PCR analyses of various genes are as follows: COX-1, forward, 5'-GCCAGTAACTCCTGTGTTACT-3' and reverse, 5'-GGCCGAGCCGGACACA-3'; COX-2, forward, 5'-AGTTGGCTACCTACGAGTTTTC-3' and reverse, 5'-CCTGGGCTTGTACTTCTT-3'; beta-actin, forward, 5'-CCACGTCCTACCGATTGATT-3' and reverse, 5'-AGTTGAATCAGCGGCGCAA-3'; PGE_2 synthase, forward, 5'-GCCACATAGCTCTATAAGCTTGAAAC-3' and reverse, 5'-AGTTGGCTACCTACGAGTTTTC-3'; mPGE_2 synthase-1, forward, 5'-CTGATCTCGCCGCCTTA-3'.

Western blot analyses

Confluent HCAEC monolayers were incubated with selected stimuli for indicated times at 37°C. The cells were then washed with ice-cold PBS twice and lysed in radioimmunoprecipitation assay buffer containing 25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, and 0.1% SDS, supplied with 1% (v/v) protease inhibitor mixture at 4°C. Cell debris was removed by centrifugation of the lysate at 13,000 × g for 15 min at 4°C. Aliquots of supernatants normalized to protein concentrations were mixed with equal volumes of 2x SDS sample buffer and heated to 100°C for 5 min. Samples were resolved on 10% SDS-PAGE and transferred onto a nitrocellulose membrane. After blocking in TBST (20 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20) containing 5% nonfat milk overnight, membranes were washed thrice in TBST and incubated with primary Ab for h in blocking solution at room temperature. After washing thrice, membranes were incubated with HRP-conjugated secondary Abs for 1 h and washed three times, and the signals were detected using ECL reagents.

Immunoblot data quantitation

The chemiluminescent signals were quantified by using Bio-Rad ChemiDoc XRS imager, and COX-1- and COX-2-specific signals in each lane were normalized to the actin signal. For quantifying magnitude change in treated cells over untreated cells, the actin-normalized value for medium-treated cells was considered as 1, and fold increase over unstimulated cells was calculated.

Statistical analysis

Analyses were done using SPSS 14.0 (SPSS). For overall comparisons among treatment groups, a significant factorial ANOVA was followed up using Student-Newman-Keuls posthoc tests. For multiple comparisons
with a control (e.g., medium alone), we used Dunnett’s test. The test for synergy was declared significant if the mean value obtained for the combined treatment group was significantly greater than the sum of the upper limits of the 95% confidence intervals for the means of the two separately treated groups. Results are reported as mean ± SEM. For all analyses, the comparison was considered significant if \( p < 0.05 \).

Results

**Histamine induces COX-2, but not COX-1, mRNA expression**

To examine whether histamine regulates the expression of COX-1 or COX-2 gene expression in HCAEC, a time course of its effect on the mRNA expression was monitored by real-time PCR. In previous studies, 10 \( \mu M \) concentration of histamine was found to be sufficient to induce near-maximal stimulation of inflammatory responses in endothelial cells (9, 16). Therefore, HCAEC were incubated with 10 \( \mu M \) histamine for 1, 2, 4, and 8 h to evaluate the kinetic profile of COX-1 and COX-2 mRNA expression. As shown in Fig. 1A, incubation of HCAEC with histamine resulted in a 11-fold increase in COX-2 mRNA expression within the first hour of treatment. Histamine-stimulated COX-2 mRNA expression was transient, decreased progressively during continued incubation, and reached the basal level by 8 h. In contrast, the expression of COX-1 mRNA was not altered by treatment of HCAEC with histamine for 1–8 h. Histamine-induced COX-2 mRNA expression was concentration dependent between 0.1 and 100 \( \mu M \), and an increase was evident at a concentration as low as 0.1 \( \mu M \) (Fig. 1B). The concentration-dependent response of HCAEC to histamine did not attain a plateau even at a concentration of 100 \( \mu M \). However, considering the relatively low levels of histamine in normal tissues and in circulation (1–3 nM) (17), concentrations greater than 100 \( \mu M \) were not tested in this experiment.

**Histamine induces COX-2 protein expression**

To determine whether histamine-induced COX-2 mRNA expression is associated with increased expression of COX-2 protein, Western blot analyses were conducted after incubating HCAEC with histamine for different time periods. As shown in Fig. 2, A and B, only low-level expression of COX-2 protein was noted in resting HCAEC (medium-treated controls). Histamine-induced expression of COX-2 protein was detectable within 2 h after initiating the incubation, stayed at steady state between 4 and 8 h, and returned to baseline by 24 h (Fig. 2A). Incubation of HCAEC with histamine (0.1–10 \( \mu M \)) led to a concentration-dependent increase in the expression of COX-2 protein (Fig. 2B). The level of expression of COX-1 protein was not affected by incubation with various concentration of histamine or by the duration of incubation (Fig. 2, C and D).

**Histamine stimulates production of PGI2 and PGE2**

In view of our finding that histamine stimulated the expression of COX-2 mRNA and protein in HCAEC, we asked whether the newly expressed protein is functionally active. It is well established that COX-2 catalyzes the conversion of arachidonic acid to PGI2, which in turn is rapidly converted to PGE2, PGD2, PGF2\(_\alpha\), PGl\(_2\), and TXA2 by the terminal synthases. In this study, we determined the production of PGI2 because of its well-recognized vasodilatory and immunomodulatory functions. Accordingly, HCAEC monolayers were incubated with selected concentrations of histamine (1, 10, and 100 \( \mu M \)) for 4, 8, and 12 h, and the production of PGI2 was monitored by quantifying the levels of 6-keto PGF\(_{1\alpha}\) in culture supernatants. As shown in Fig. 3A, incubation of HCAEC with histamine for 8 or 12 h led to a statistically significant increase in the production of PGI2 that was concentration and time dependent. To determine whether histamine-stimulated PGI2 production is indeed associated with the increased function of COX-2, HCAEC were pretreated with COX-2-specific inhibitor NS-398 for 30 min before stimulation with histamine, and PGI2 production was measured after 12 h. As shown in Fig. 3B, NS-398 caused a ∼50% reduction of the basal PGI2 production

![FIGURE 1.](image1)

**FIGURE 1.** Histamine induces COX-2, but not COX-1 mRNA expression in HCAEC. HCAEC monolayers were incubated with histamine (10 \( \mu M \)) for 1–8 h (A) or with 0.1–100 \( \mu M \) histamine for 2 h (B). After the incubation, total RNA was extracted, reverse transcribed, and analyzed by real-time PCR using specific primers, as described in Materials and Methods. The amplicons were normalized for \( \beta \)-actin and assigned a value of 1 for medium-treated cells. The magnitude of changes of mRNA expression in histamine-treated cells was calculated by comparing with the message in medium-treated HCAEC. The results presented are representative of three experiments with similar results. Each value presented is the mean ± SEM of triplicate determinations. * Indicates \( p < 0.05 \) when compared with medium-treated cells.

![FIGURE 2.](image2)

**FIGURE 2.** Histamine induces COX-2, but not COX-1 protein expression in HCAEC. HCAEC monolayers were incubated with histamine (10 \( \mu M \)) for 2–24 h (A) or with 0–10 \( \mu M \) histamine for 4 h (B). After washing with PBS, total cell lysates were prepared and subjected to Western blot analyses, as described in Materials and Methods. The results presented are representative of three experiments with similar results. Data presented in C and D are mean ± SEM of fold increases in protein expression as determined by chemiluminescence image quantifications of three Western blot analyses. * Indicates \( p < 0.05 \) when compared with medium-treated cells.

![FIGURE 3.](image3)

**FIGURE 3.** Histamine induces PGI2 and PGE2 production. A, Histamine-induced COX-2 gene expression in HCAEC. B, Incubation of HCAEC with histamine resulted in a concentration-dependent increase in COX-2 mRNA expression within the first hour of treatment. Histamine-stimulated COX-2 mRNA expression was transient, decreased progressively during continued incubation, and returned to the basal level by 24 h. C, Incubation of HCAEC with histamine (0.1–10 \( \mu M \)) led to a concentration-dependent increase in the expression of COX-2 protein (Fig. 2B). The level of expression of COX-1 protein was not affected by incubation with various concentration of histamine or by the duration of incubation (Fig. 2, C and D).
and complete inhibition of histamine-induced PGI₂ production. To further confirm the association between histamine-induced COX-2 expression and PGI₂ production, HCAEC were transfected with COX-2-specific siRNA or negative control siRNA, and COX-2 mRNA expression and PGI₂ production were determined. As evident from Fig. 3C, transfection of HCAEC with active siRNA resulted in the inhibition of constitutive and histamine-induced COX-2 mRNA expression by ~70% and 75%, respectively. In agreement with the siRNA-induced decrease in COX-2 mRNA expression, both the basal and histamine-stimulated production of PGI₂ were decreased proportionately (Fig. 3D).

**Histamine-induced COX-2 gene expression and PGI₂ production in HCAEC are mediated via histamine H1 receptor (H₁R)**

Previous studies from our laboratory have shown that histamine-induced production of IL-6 and IL-8 (9) as well as the expression of TLRs (16) in human endothelial cells are mediated via H₁R. To determine whether histamine-induced COX-2 expression and PGI₂ production are mediated through H₁R, HCAEC monolayers were incubated with histamine in the presence of H₁R antagonist fexofenadine or the histamine H₂ receptor (H₂R) antagonist famotidine, and mRNA expression and PGI₂ production were monitored. As shown in Fig. 4A, incubation of HCAEC with 10 μM histamine for 2 h resulted in a 7-fold increase in COX-2 mRNA expression, which was completely abrogated by the H₁R antagonist fexofenadine. In contrast, the H₂R antagonist famotidine did not affect histamine-stimulated COX-2 gene expression. The involvement of H₁R in histamine-stimulated COX-2 expression is further confirmed by the finding that an H₁R-specific agonist was able to mimic the effect of histamine and was inhibited by fexofenadine, but not by famotidine (data not shown). In addition, the H₁R agonist dimaprit failed to stimulate COX-2 expression (data not shown). Neither fexofenadine nor famotidine had any effect on the basal expression of COX-2.

Next we determined whether histamine-stimulated PGI₂ production is also mediated via H₁R activation. Once again, as in the case of COX-2 expression, histamine-induced production of PGI₂ was completely inhibited by the H₁R antagonist, fexofenadine, and not by the H₂R antagonist, famotidine (Fig. 4B).

**Histamine amplifies LPS-induced expression of COX-2 mRNA and protein**

Having documented the direct effect of histamine on COX-2 expression, we examined whether histamine is capable of modulating the expression of COX-2 that was initiated by other inflammatory agents. To determine the potential synergy between histamine and LPS on expression of COX-2, HCAEC monolayers were incubated...
for 2 h with LPS (100 ng/ml) in the presence and absence of 10 μM histamine, and the expression of COX-2 mRNA and protein was determined (Fig. 5). The results presented in Fig. 5A demonstrate that histamine and LPS independently stimulated COX-2 mRNA expression by 6- and 12-fold, respectively. Interestingly, in addition to its direct effect, histamine was found to amplify LPS-stimulated COX-2 gene expression, as indicated by the 26-fold increase over the constitutive level of expression, a significantly synergistic effect. The Western blot analyses of COX-1 and COX-2 proteins confirm that the amplifying effect of histamine on LPS-stimulated COX-2 expression is not only limited to the transcriptional level, but is also evident at the translational level (Fig. 5B). The quantification of COX-2 signals shows that incubation of HCAEC with histamine, LPS, or LPS plus histamine resulted in 8-, 15-, and 25-fold increases in COX-2 expression, respectively (Fig. 5C). In contrast, no significant changes in the level of expression of COX-1 protein were noted after incubation with histamine, LPS, or LPS plus histamine.

Histamine amplifies LPS-induced endothelial production of PGI₂ and PGE₂, but does not affect TXA₂ production

To examine whether histamine-induced COX-2 expression as well as its amplification by LPS are translated into functional activities, we monitored the kinetics of the production of PGI₁, PGE₂, and TXA₂ by HCAEC. HCAEC monolayers were incubated with histamine (10 μM), LPS (100 ng/ml), or a combination of histamine (10 μM) and LPS (100 ng/ml) for 4–48 h, and the production of 6-keto PGF₁α, PGE₂, and TXB₂ (stable product of TXA₂) was monitored. Incubation of HCAEC with histamine or LPS caused stimulation of PGI₂ (Fig. 6A) and PGE₂ production (Fig. 6B) in a time-dependent fashion. As in the case of COX-2 mRNA and protein expression, histamine markedly enhanced LPS-induced production of PGI₂ and PGE₂ at all time points tested. The ability of histamine to amplify the LPS effect was evident even at 48 h after stimulation of HCAEC, which indicates the existence of a substantial synergy between histamine and LPS in COX-2-mediated PGI₂ and PGE₂ production. As shown in Fig. 6C, histamine did not stimulate TXA₂ production at any of the time periods tested. Although incubation of HCAEC with LPS for 24 and 48 h resulted in significant increases in TXA₂ synthesis, unlike in the case of PGI₂ and PGE₂, histamine failed to modulate TXA₂ synthesis either in the presence or absence of LPS (Fig. 6C).
Histamine stimulates the expression of PGI$_2$ synthase and PGE$_2$ synthase, but not TXA$_2$ synthase, mRNA expression

To determine whether histamine-induced increases in PGI$_2$ and PGE$_2$ production are associated with changes in PGI$_2$ synthase and PGE$_2$ synthase gene expression, HCAEC monolayers were incubated with histamine (10 μM) for 2, 8, and 12 h, and the mRNA levels were quantified by real-time PCR. As shown in Fig. 7A, incubation of HCAEC with histamine resulted in time-dependent increase in PGI$_2$ synthase mRNA expression and attained a 3- to 4-fold increase in PGE$_2$ synthase mRNA expression, with a maximum of 40% at 8 h (Fig. 7B). In contrast, TXA$_2$ synthase mRNA expression in HCAEC was minimally affected by histamine treatment (Fig. 7C).

HDC is the only enzyme that generates histamine from the amino acid histidine through a single enzymatic reaction. Because the HDC$^{-/-}$ mouse cannot synthesize histamine, this model was used to test the in vivo role of histamine on COX-1 and COX-2 gene expression. Accordingly, the expression of COX-1 and COX-2 mRNA in aortic tissues of wild-type and HDC$^{-/-}$ mice was determined. The semiquantitative RT-PCR data presented in Fig. 8 demonstrate that aortic tissues of wild-type controls (lanes 1 and 2) constitutively express significant amounts of COX-2 mRNA, whereas the tissues from HDC$^{-/-}$ mice express markedly lower levels (Fig. 8, lanes 3 and 4). The level of expression of COX-2 mRNA in the aortic tissues of HDC$^{-/-}$ mice was comparable to that of wild-type controls. The deficient expression of COX-2 in HDC$^{-/-}$ mice aortic tissues and the in vitro data showing increased expression of COX-2 mRNA and protein in histamine-stimulated HCAEC suggest that histamine plays a key role in the regulation of the COX-2 pathway.

**Discussion**

The importance of the mast cell and its products in vascular inflammation is well recognized (18, 19), and is suggested by increased levels of histamine in the coronary circulation (11) and increased synthesis of this amine in atherosclerotic lesions (20). Increasing interest is now focused on the role of COX-2 and prostanoid homeostasis in the pathogenesis of atherosclerosis (21). Previous reports from our laboratory have documented the ability of mast cell proteases and histamine to interact with endothelial cells and enhance inflammatory responses, as determined by the production of IL-6 and IL-8 (9, 22, 23) and expression of TLR2 and TLR4 (16). In the present study, we demonstrate that histamine, acting through H$_R$, selectively induces the expression of functionally active COX-2 and stimulates the production of PGI$_2$ and PGE$_2$ in HCAEC. The increases in PGI$_2$ and PGE$_2$ production by histamine-treated HCAEC were found to be associated with increased expression of mRNA encoding PGI$_2$ and PGE$_2$ synthases, the terminal enzymes involved in the conversion of PGH$_2$ to PGI$_2$ and PGE$_2$ (24, 25). The finding that pretreatment of HCAEC with COX-2-specific inhibitor NS-398 or transfection of the cells with gene-silencing siRNA inhibited histamine-stimulated prostanoid production documents that COX-2 expression contributes to the increase in prostanoid production. Interestingly, histamine did not stimulate COX-1 and TXA$_2$ synthase gene expression or the production of TXA$_2$. The preferential effect of histamine on the induction of COX-2 expression with resultant production of PGE$_2$ and PGI$_2$ and its lack of influence on COX-1...
expression and TXA2 production support the concept of a distinct coupling pattern of COX-2 with PGE2 and PGI2 synthases and that of COX-1 with TXA2 synthase (25, 26). It is intriguing that, despite the coexistence of both COX-1 and COX-2 in HCAEC, histamine is able to segregate its influence on COX-2/PGE2/PGI2 pathway and not on COX-1/TXA2 pathway. It is also of interest that the increase in the production of prostanooids is not always associated with parallel induction of the expression or activity of a distinct PG synthase (27). Because a physiological balance in the production of PGI2 and TXA2 by endothelial cells is critical for the maintenance of vascular integrity and control of thrombosis (28, 29), the histamine-induced shift of prostanooid equilibrium in favor of PGI2 production is noteworthy and supports its well-recognized vasodilatory and vasoprotective function.

It is well recognized that the cellular responses of histamine are mediated through a family of histamine receptors (H1, H2, H3, and H4) variably expressed in different cell types (30). Human endothelial cells predominantly express H1R, which are involved in histamine-mediated hypersensitivity reactions and inflammatory responses (9, 31). The present finding that histamine-induced COX-2 expression and PGI2 production are inhibited by the H1R antagonist, fexofenadine, and not by the H2R antagonist, famotidine, suggests that histamine-mediated prostanooid homeostasis in HCAEC is regulated via H1R activation. These findings are in agreement with our previous reports demonstrating a distinct role of H1R in histamine-mediated proinflammatory cytokine production and TLR expression (16) and of others on histamine-induced released of PGI2 in HUVECs (32, 33). It should be noted that the stimulatory effect of histamine on COX-2 expression is not always mediated via H1R, but is rather dependent on the cell type. For instance, histamine has been shown to stimulate COX-2 expression and generation of PGE2 in colon cancer cells through H1R (34). However, the lack of involvement of H2R in histamine-mediated prostanooid synthesis in HCAEC is further confirmed by the fact that dimaprit, an H2R-specific agonist, failed to stimulate the production of PGI2 (data not shown). Therefore, H1R seems to be an important player in the regulation of inflammatory responses and prostanooid homeostasis in vascular endothelium.

In addition to its direct effect, histamine was able to synergize the effect of LPS on the expression of COX-2 and production of PGE2 and PGI2 without modulating TXA2 production. The failure of histamine to produce TXA2 was not due to the lack of TXA2 synthase activity because LPS-treated HCAEC generated significant amounts of TXA2 after 24 and 48 h of treatment (Fig. 6C). Because the production of TXA2 is thought to be coupled to COX-1 and TXA2 synthase (25) and histamine does not stimulate the expression of COX-1 (Figs. 1 and 2) or TXA2 synthase in HCAEC (Fig. 7C), the lack of TXA2 production by histamine-treated HCAEC is predictable. The fact that histamine failed to modulate TXA2 production either directly or in the presence of LPS suggests a novel mechanism by which this mast cell mediator regulates prostanooid homeostasis in HCAEC. Such a selective release of PGI2 without altering TXA2 synthesis is seen in hypoxia-induced COX-2 expression in HUVECs (35). It is noteworthy that, although TXA2 is predominantly produced by mature human platelets, which express only COX-1 (36), TXA2 is produced in other cell types, such as monocytes and macrophages, which express both COX-1 and COX-2 (37). However, distinct contribution of COX-1 and COX-2 to the production of a particular prostanooid in response to specific inflammatory signals has not been established. Thus, the present finding that LPS, but not histamine, was able to induce TXA2 production by HCAEC suggests that the type of inflammatory agent encountering the cells influences the pattern of PG production.

The ability of histamine to synergize LPS-induced COX-2 expression and prostanooid production underscores the potential role of this mast cell mediator to amplify infection-associated inflammatory responses in vascular endothelium. It is well recognized that TLRs are critical components of the innate immune system and each of these TLRs recognizes a distinct pathogen-associated molecule to initiate the inflammatory response (38–43). Among the TLRs identified to date, TLR4, in association with its accessory molecules, recognizes LPS (43–47). Recently, we demonstrated that histamine has the ability to stimulate the expression of TLR4 mRNA and protein, and amplify LPS-induced production of cytokines in HUVECs (16). Therefore, although the effect of histamine on TLR4 expression in HCAEC was not examined in this study, it is reasonable to suggest that the amplification of LPS-induced COX-2 expression and enhancement in the production of PGI2 and PGE2 is due to increased expression of functionally active TLR4. The assumption is further supported by the finding that, although histamine alone is a poor inducer of NF-κB translocation in endothelial cells, it markedly enhances LPS-induced NF-κB activation, an index of amplified TLR4 activation (9). It is noteworthy that dysfunctional TLR4 polymorphism, which affects the extracellular domain of the receptor, is associated with a reduction in systemic levels of proinflammatory mediators (48) and cardiovascular events (49). Furthermore, compared with control subjects, individuals with TLR4 polymorphism who presented with significantly lower intima-medium thickness in the carotid arteries had a 65% reduction of 11-dehydro-TXB2 in the urine, indicating decreased systemic production of TXA2 in these individuals.

The circulating levels of PGI2 and TXA2 are pivotal for the normal functioning of the cardiovascular system (21, 50), and the syntheses of both PGI2 and TXA2 are increased in patients with atherosclerosis (14, 15). PGI2 is an antithrombotic and vasodilator molecule that can decrease vascular remodeling and cholesterol uptake (51, 52). This is particularly evident from the fact that disruption of the prostacyclin receptor gene leads to increased intima-medium ratio in response to vascular injury and promotes initiation and progression of atherosclerosis in hyperlipidemic mouse (53, 54), suggesting a protective role for prostacyclin in vascular remodeling. TXA2, in contrast, is a prothrombotic and vasoconstricting agent, and enhances vascular remodeling (51, 52). Because PGI2 and TXA2 exert opposing influences in the cardiovascular system, the up-regulation of the expression of COX-2, PGE2 synthase, and PGI2 synthase in HCAEC by histamine emphasizes its importance in the maintenance of vascular integrity.

In conclusion, the present study demonstrates that incubation of HCAEC with histamine leads to increased expression of COX-2, with resultant enhancement in the production of PGE2 and PGI2. We also present evidence that histamine-induced production of PGE2 and PGI2 can be attributed to increased gene expression of PGE2 and PGI2 synthases. Interestingly, histamine did not affect TXA2 synthase gene expression or TXA2 production. In addition to the direct effect, histamine is capable of amplifying LPS-stimulated expression of COX-2 and production of PGE2 and PGI2, potentially via histamine-stimulated expression of TLR4 (16). Both the direct and synergizing effects of histamine on endothelial cell activation are found to be mediated via H1R activation. The role of histamine in the regulation of COX-2 expression in the vasculature is further supported by the finding that the aortic tissue of HDC+/− mouse, which is deficient in histamine, has significantly reduced expression of COX-2 mRNA without a change in the levels of COX-1 mRNA. These results underscore the important role of histamine in prostanooid homeostasis in the vasculature.