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

Xiaoyu Tan, Suzanne Essengue, Jaya Talreja, Jeff Reese, Daniel J. Stechschulte and Kottarappat N. Dileepan

*J Immunol* 2007; 179:7899-7906; doi: 10.4049/jimmunol.179.11.7899

http://www.jimmunol.org/content/179/11/7899

References

This article cites 54 articles, 19 of which you can access for free at: http://www.jimmunol.org/content/179/11/7899.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Histamine Directly and Synergistically with Lipopolysaccharide Stimulates Cyclooxygenase-2 Expression and Prostaglandin I2 and E2 Production in Human Coronary Artery Endothelial Cells

Xiaoyu Tan,* Suzanne Essengue,* Jaya Talreja,* Jeff Reese,† Daniel J. Stechschulte,* and Kottarappat N. Dileepan2*

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 and PGI₂ synthases. 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, PGE₂ₐ₃, 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 and

Copyright © 2007 by The American Association of Immunologists, Inc. 0022-1767/07/$2.00

*Division of Allergy, Clinical Immunology, and Rheumatology, Department of Medicine, University of Kansas Medical Center, Kansas City, KS 66160; †Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN 37232

Received for publication April 4, 2007. Accepted for publication September 20, 2007.

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

³ Abbreviations used in this paper: COX, cyclooxygenase; EGM-2 MV, endothelial cell growth medium; EIA, enzyme immunoassay; H₁R, histamine H₁ receptor; H₂R, histamine H₂ receptor; HCAEC, human coronary artery endothelial cell; HDC, histidine decarboxylase; PGI₂, prostacyclin; siRNA, small interfering RNA; TX, thromboxane.
to evaluate its regulatory function on the production of PGE₂, PGF₂α, and TXA₂ 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 PGF₂α and PGE₂ without affecting basal or LPS-induced TXA₂ 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 transfection neutralization solution were purchased from Cambrex. Enzyme immunoassay (EIA) kits for 6-keto PGF₁α, PGE₂, and TXB₂, 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, tamoxifen, 2-(3-trifluoromethyl) phenyl histamine dimaleate, and Escherichia coli (011: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 product of Pierce Biotechnology. Transfection reagent siPort Amine, COX-2 small interfering RNA (siRNA), and negative control siRNA were obtained from Ambion.

Culture of HCAEC

HCAEC were grown in EGM-2 MV containing 1 µg/ml hydrocortisone acetate, 50 ng/ml gentamicin, 50 µg/ml amphotericin B, and the recommended concentrations of human epidermal growth factor, vascular endothelial growth factor, human fibroblast growth factor-β, recombinant insulin-like growth factor-1, ascorbic acid, and 5% FBS, as described previously (9, 16). At confluence, the cells were detached from the culture flasks using trypsin-EDTA, washed twice, and resuspended in fresh EGM-2 MV. The cells used in all experiments were between three and six passages.

EIA determinations of the production of PGE₂, PGF₂α, and TXA₂

HCAEC (1.5 × 10⁵) were plated onto each of the wells of a 96-well cell culture plate and allowed to adhere for 16–18 h. Following adherence, selected concentrations of the activating stimuli or medium were added to the monolayers and incubated for indicated time periods in a final volume of 0.2 ml at 37°C in 5% humidified CO₂. After the indicated incubation period, culture supernatants were harvested, appropriately diluted, and assayed for 6-keto PGF₁α (for PGI₂), PGE₂, and TXB₂ (for TXA₂) levels by EIA.

Transfection and siRNA

Gene-specific siRNA or negative control siRNA was introduced by using the siPORT Amine transfection reagent, according to the manufacturer’s instructions. Briefly, 50–150 µl of the transfection reagent mixture containing 0.75 µl of siPORT Amine reagent, 100 nM siRNA, and OPTIMEM I was added to each of the wells of a 24-well cell culture plate. An aliquot of the HCAEC suspension containing 0.5 × 10⁵ cells was then added to the transfection mixture and incubated at 37°C in 5% CO₂ for 24 h. After the incubation, medium containing the transfection reagent was discarded, and the cells were maintained in fresh medium for another 24 h. The cells were subsequently treated with histamine (10 µM) for 2 h. Total RNA was extracted from HCAEC monolayers by real-time PCR analysis. An aliquot of 12.5 µl above transfection reagent mixture and 1.3 × 10⁴ cells were plated into each of the wells of a 96-well culture plate. The cells were treated the same as described above. Cell culture supernatants were collected after 12-h histamine treatment for PGI₂ EIA analysis.

Real-time PCR

After incubating HCAEC monolayers with medium or the stimulating agents, supernatant was removed from the cell culture dish and total RNA was isolated using RNeasy mini kit, according to the manufacturer’s protocol. Total RNA (1 µg) was reverse transcribed into first-strand cDNA using high-capacity cDNA Achieve kit following the manufacturer’s procedure. The primers used for SYBR Green real-time PCR were designed using the Primer Express Software v3.0 (Applied Biosystems). The sequences of primers used in the real-time PCR analyses of various genes are as follows: COX-1, forward, 5'-GGCCATGTCCCTCTGTGTTCT-3' and reverse, 5'-GGCCGAAGCCTGTTGAGAA-3'; COX-2, forward, 5'-AGGTGCGTGGTGTAGAGAA-3' and reverse, 5'-GGTGTCATTCTCTCTTTCCT-3'; β-actin, forward, 5'-CCAGCTTACCCAGTTGAGATG-3' and reverse, 5'-ATGCCGGACGGCTGTTC-3'; PGI₂, synthase, forward, 5'-GGCCATAGCTCTAAGGCTGAAC-3' and reverse, 5'-AGGTTCCTACACGACTTGG-3'; mPGE₂ synthase-1, forward, 5'-CCTGGCTGTCTCTGCTTT-3' and reverse, 5'-AGTGCATCCAGCGCGAAC-3'; TXA₂, synthase, forward, 5'-GTCAGCGTTCATTCCCA-3' and reverse, 5'-CTGATCTCAGGCCCTTACA-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 triplelicate, 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 β-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). Aliquots 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, AGGAGATGCGTCTGAGTGTGG and reverse, AAT CTGACCTTCCAGTCTTTCC and reverse, CACCATTAGACATCTGCGG (product size 861 bp); rpL7, forward, TCAATGGAGTAAGCCAAAGGCTGGGTG and reverse, CAAGAGACCGAGCAATCAAG (product size 246 bp).

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 for 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 1 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 XR5 imager, and COX-1- and COX-2-specific signals in each lane were normalized to the actin signal. For quantifying magnitude change in treatment 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 post hoc tests. For multiple comparisons
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 μM concentration of histamine was found to be sufficient to induce near-maximum stimulation of inflammatory responses in endothelial cells (9, 16). Therefore, HCAEC were incubated with 10 μM 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 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 μM, and an increase was evident at a concentration as low as 0.1 μM (Fig. 1B). The concentration-dependent response of HCAEC to histamine did not attain a plateau even at a concentration of 100 μM. However, considering the relatively low levels of histamine in normal tissues and in circulation (1–3 nM) (17), concentrations greater than 100 μM were not tested in this experiment.

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 PGH2, which in turn is rapidly converted to PGE2, PGD2, PGF2α, PGI2, 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 μM) for 4, 8, and 12 h, and the production of PGI2 was monitored by quantifying the levels of 6-keto PGF1α 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 expression 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 3. Histamine-induced COX-2 expression is functionally active and contributes to the increased production of PGI₂. HCAEC monolayers were incubated with medium or histamine (1.0–100 μM) for 4, 8, and 12 h (A) or with 10 μM histamine in the presence or absence of 1.0 μM COX-2 inhibitor NS-398 (B), and the production of PGI₂ was determined by quantifying 6-keto PGF₁α by EIA. To determine the association of COX-2 mRNA expression and PGI₂ production, HCAEC monolayers were transfected with COX-2-specific siRNA or negative control siRNA for 24 h. The cells were then treated with 10 μM histamine for 2 h for the determination of COX-2 mRNA expression by real-time PCR (C) or 12 h for determination of PGI₂ production (D). Data presented in A and B are mean ± SEM of quadruplicate determinations of a representative experiment of three. Each value presented in C and D is the mean ± SEM of triplicate determinations of a representative experiment of two. * Indicates p < 0.05 when compared with values for medium-treated cells (A), cells treated without NS-398 (B), and reagent control-treated cells (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 H₁R 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

FIGURE 4. H₁R antagonist blocks histamine-induced COX-2 mRNA expression and associated PGI₂ production in HCAEC. HCAEC monolayers were preincubated for 30 min with the H₁R antagonist, fexofenadine (Fex, 10 μM), or the H₂R antagonist, famotidine (Fam, 10 μM), followed by incubation with medium or histamine (His, 10 μM) for 2 h for the analyses of COX-2 mRNA expression by real-time PCR (A) or for 24 h for the production of PGI₂ (B). Each value presented in A is the mean ± SEM for triplicate determinations of a representative experiment of three. Each value presented in B is the mean ± SEM for quadruplicate determinations of a representative experiment of three. * Indicates p < 0.05 when compared with values for medium-treated cells.
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, and 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 PGI₂, 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 PGI2 synthase and PGE2 synthase, but not TXA2 synthase, mRNA expression

To determine whether histamine-induced increases in PGI2 and PGE2 production are associated with changes in PGI2 synthase and PGE2 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 PGI2 synthase mRNA expression and attained a 3- to 4-fold increase in 8 h before returning to basal levels by 12 h posttreatment. The expression of PGE2 synthase mRNA in response to histamine was of lesser magnitude, and it required 8 h to attain a 40% increase (Fig. 7B). In contrast, TXA2 synthase mRNA expression in HCAEC was minimally affected by histamine treatment (Fig. 7C).

The aortic tissues of HDC−/− mice are deficient in COX-2 mRNA expression with no change in COX-1 mRNA

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-1 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 H1R, selectively induces the expression of functionally active COX-2 and stimulates the production of PGI2 and PGE2 in HCAEC. The increases in PGI2 and PGE2 production by histamine-treated HCAEC were found to be associated with increased expression of mRNA encoding PGI2 and PGE2 synthases, the terminal enzymes involved in the conversion of PGH2 to PGI2 and PGE2 (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 TXA2 synthase gene expression or the production of TXA2. The preferential effect of histamine on the induction of COX-2 expression with resultant production of PGE2 and PGI2 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 prostanoids is not always associated with parallel induction of the expression or activity of a distinct PG production (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 prostanoïd 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 H₁ antagonist, fexofenadine, and not by the H₂R antagonist, famotidine, suggests that histamine-mediated prostanoïd 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 release 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 prostanoïd 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 prostanoïd 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 prostanoïd 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 prostanoïd 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 prostanoïd 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, TLR-4, in association with its accessory molecules, recognizes LPS (43–47). Recently, we demonstrated that histamine has the ability to stimulate the expression of TLR-4 mRNA and protein, and amplify LPS-induced production of cytokines in HUVECs (16). Therefore, although the effect of histamine on TLR-4 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 TLR-4. 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 TLR-4 activation (9). It is noteworthy that dysfunctional TLR-4 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 TLR-4 polymorphism who presented with significantly lower intima-media 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-media ratio in response to vascular injury and promotes initial 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 TLR-4 (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 prostanoïd homeostasis in the vasculature.