Rate of Vasoconstrictor Prostanoids Released by Endothelial Cells Depends on Cyclooxygenase-2 Expression and Prostaglandin I Synthase Activity

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Abstract—This study was undertaken to investigate the enzymatic regulation of the biosynthesis of vasoconstrictor metabolites of arachidonic acid (AA).1–7 From the Laboratory of Inflammation Mediators, Institute of Research of Santa Creu i Sant Pau Hospital, Barcelona, Spain. Received March 13, 1998; accepted May 18, 1998. © 1998 American Heart Association, Inc.

Endothelium modulates the response of vascular smooth muscle to hormones, neurotransmitters, and platelet products by releasing relaxing factors, such as NO and prostaglandin (PG) I$_2$ (also termed prostacyclin), and contracting factors, such as endothelin-1, angiotensin II, and vasoconstrictor metabolites of arachidonic acid (AA).1–7

PGI$_2$, PGF$_{2\alpha}$, and PGE$_2$ are the main eicosanoids detected in vitro in the incubates of endothelial cells under different experimental conditions, which include exogenously added AA and several agonists. PGD$_2$, 12-hydroxyheptadecatrienoic acid (HHT), 15-HETE, and 11-HETE have also been detected in minor amounts.8–11 All these eicosanoids are derived from

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cyclooxygenase (COX) activity in human umbilical vein endothelial cells (HUVECs). COX-2 is the common precursor of the prostanoids whose formation from AA is catalyzed by 2 COX isoenzymes encoded by different genes: COX-1 and COX-2. COX-1 is expressed in a constitutive manner, and COX-2 is the isoenzyme inducible by mitogens and is overexpressed in inflammatory processes. Interleukin (IL)-1, together with tumor necrosis factor, plays a pivotal role in the inflammatory response and orchestrates a pleiad of secondary events that orient endothelial cells toward a proinflammatory and prothrombotic function by inducing adhesion molecules, endothelin-1, and coagulation factors. A characteristic activity of IL-1 is to induce COX-2 in several cell types, including endothelial cells.

PGH₃ is the most potent platelet antiaggregatory agent, and it exhibits antiadhesive and smooth muscle–relaxing properties. PGH₃ also undergoes spontaneous or enzymatic transformation toward PGF₂α, PGF₂β, and PGD₂. A growing amount of data support the concept that untransformed PGH₃ is released in vivo by the vascular endothelium under different circumstances. The predominance of relaxing prostanoids, such as PGI₂, or contracting prostanoids, such as PGE₂ and PGF₂α, released by endothelial cells will primarily depend on COX activity but also on the secondary pathways that yield the different prostanoids. PGI₂ release as a consequence of the exposure of endothelial cells to IL-1 has been widely reported. Nevertheless, when COX activity is overexpressed, PGIS activity could be the limiting step in the biosynthesis of PGI₂, and other prostanoids could be more stimulated by IL-1.

Although release of PGH₃ by endothelium both in vivo and ex vivo has been suggested, its regulation has not been systematically studied, and whether transformation of PGH₃ into PGI₂, PGF₂α, PGF₂β, and PGD₂ in endothelial cells is spontaneous or enzymatically catalyzed remains unclear. In fact, PGIS is the only enzyme implicated in prostanoid biosynthesis that has been found in endothelial cells in addition to COX. The objective of the present work was to evaluate the hypothesis that the release of vasoconstricting prostanoids is regulated by 2 enzyme activities, COX and PGIS, and that these prostanoids are released predominantly under inflammatory conditions, such as after exposure to IL-1.

Materials and Methods

Cell Culture and Treatment

Endothelial cells were isolated from human umbilical veins and cultured as previously described. When HUVECs cultured with 20% of FBS reached confluence, they were seeded into 6-well plates and maintained without heparin and endothelial cell growth factor for 48 hours before the addition (or not) of 10 U/mL human recombinant IL-1β (50,000 U/µg; purity, >98%; Boehringer Mannheim S.A.) in medium 199 containing 4% FBS and maintained for the indicated period of time until incubation with [¹⁴C]AA or histamine. Human dermal fibroblasts were isolated and cultured as described previously.

Release of Prostanoids From Endogenous AA in Resting and IL-1β–Treated HUVECs

Cells cultured in 6-well plates were incubated at 37°C in 2.5 mL medium 199 containing 4% FBS and 10 U/mL IL-1β. At the indicated periods of time, prostanoids released into the medium were evaluated as the sum of all the eicosanoids formed through the biosynthesis of PGI₂, and other prostanoids could be more stimulated by IL-1.

Preparation of Washed Platelet Suspensions

Peripheral venous blood was drawn from healthy donors who had received no medication in the 2 weeks before extraction. Washed platelet suspensions were prepared as previously described. Platelet density was 2×10⁹ platelets/mL for the PGH₂ trapping experiments and 1.7×10⁹ platelets/mL for the study of COX-1 inhibition. COX-inactivated platelet suspensions were obtained by incubating platelet-rich plasma with 200 μmol/L acetylsalicylic acid (ASA) dissolved in ethanol (final concentration of ethanol, 0.5% [vol/vol]) for 10 minutes at room temperature before the washing procedure.

Release of Prostanoids From Endogenous AA in Resting and IL-1β–Treated HUVECs

Cells cultured in 6-well plates were incubated at 37°C in 2.5 mL medium 199 containing 4% FBS and 10 U/mL IL-1β. At the indicated periods of time, prostanoids released into the medium were evaluated after 250–µL aliquots of media were collected and placed in tubes containing indomethacin (final concentration, 10 µmol/L). Samples were kept at −80°C until analysis of PGE₂, PGF₂α, and 6-keto-PGF₁α by RIA (PGF₂α was from Amersham Ibérica; PGE₂, and 6-keto-PGF₁α were from Advanced Magnetics Inc).

Formation of Eicosanoids From Exogenous [¹⁴C]AA and Determination of COX Activity

After the indicated period of time of exposure to 10 U/mL IL-1β, cells were incubated at 37°C in 0.5 mL medium 199 containing 10 mmol/L HEPES, and 25 µmol/L [¹⁴C]AA (55 to 58 mCi/mmol [¹⁴C]AA, Amersham Ibérica) was added in 5 μL of ethanol. At the indicated periods of time, the reactions were stopped by adding 1N HCl to yield pH 3 followed by 1 vol of cold methanol. COX activity was evaluated as the sum of all the eicosanoids formed through the COX pathway.

PGH₂ is an unstable prostaglandin that is converted into PGI₂ by mild reducing agents, such as SnCl₂. Therefore, to estimate the PGH₂ released, we calculated the difference between the PGI₂ peak of the samples from cells incubated as previously described and cells incubated in the presence of 200 µmol/mL SnCl₂. Samples were kept at −80°C until analysis. HPLC analysis of eicosanoids was performed as previously described.

Release of Prostanoids From Endogenous AA in IL-1β–Treated HUVECs in Response to Histamine

Cells untreated and treated with 10 U/mL IL-1β for the indicated periods of time were incubated at 37°C in the presence of 0.5 mL medium 199 containing 10 mmol/L HEPES and 50 µmol/L histamine. After 10 minutes, supernatants were removed and placed in liquid N2. Samples were then stored at −80°C until analysis of PGE₂, PGF₂α, and 6-keto-PGF₁α by RIA.

COX-1– and COX-2–Specific mRNA Analysis

COX-1– and COX-2–specific mRNA levels were determined as previously described.
PGIS mRNA Analysis

Total RNA was isolated by phenol chloroform extraction according to the protocol described by Chomczynski and Sacchi and quantified spectrophotometrically by absorption at 260 and 280 nm. The specific levels of PGIS mRNA were determined by means of a quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Briefly, 1 μg of total RNA was reverse-transcribed into cDNA in the presence of 50 U reverse transcriptase of murine leukemia virus in a reaction buffer containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 5 mmol/L MgCl₂, 2.5 μmol/L random hexamers, 20 U of RNAsin (Geneamp RNA PCR kit, Perkin-Elmer), and 1 mmol/L deoxynucleotide triphosphates (Promega) in a final volume of 20 μL. The reaction was stopped by heating for 5 minutes at 99°C and then 5 minutes at 5°C.

The primers used for the amplification of the PGIS-specific cDNA were purchased from Oxford Biomedical Research, Inc. The cDNA for GAPDH was amplified and used as the internal control, and the sense and antisense primers for GAPDH were 5'-CCACCATGGCAGAATTCGAGGCA-3' and 5'-TCTAGACGGCAAGGCGTCGCTACC-3', respectively. The PCR was carried out in a DNA Thermal Cycler 480 (Perkin-Elmer) with a reaction mixture (100 μL) containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2.5 mmol/L MgCl₂, 0.2 μmol/L deoxynucleotide triphosphates, 0.25 μmol/L for PGIS and 1 μmol/L for GAPDH sense or anti-sense primers, 2.5 U Taq polymerase (Perkin-Elmer), and 4 μCi of [³H]deoxycytidine triphosphate (48 to 71 Ci/mmol, Amersham International). Aliquots were removed after 27, 30, and 35 cycles in order to test the linearity of the amplification. The profile of the amplification cycles was 94°C for 1 minute, 55°C for 2 minutes, and 72°C for 90 seconds. Amplification products were separated in a 1.5% low-melting-point agarose gel (GIBCO BRL) containing ethidium bromide (0.5 μg/mL, Perkin-Elmer). The bands were visualized under UV light and cut with a circular template. Radioactivity associated to the specific band was determined as previously described and normalized with respect to GAPDH.

Western Blotting of COX-1 and COX-2

Lysates of control cells and HUVECs treated with IL-1β for the indicated period of time were prepared as previously described. Total protein equivalents of each sample were submitted to SDS-PAGE and transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore). After blocking nonspecific binding of antibody, membranes were incubated with a specific polyclonal antibody for COX-1 and COX-2 (Oxford Biomedical Research). Positive controls were performed by using isolated COX-1 from ram seminal vesicles (Oxford Biomedical Research) or COX-2 purified from sheep placenta (Cayman Chemical). Detection was performed by using the ECL System (Amersham) according to the manufacturer's instructions. To estimate the relative levels of each COX protein, the films were scanned in order to obtain digital images. The digital images of the bands were quantified, with the use of the BioImage analysis software (Foster Findlay Associates Ltd), by determining the sum of the gray density value of all pixels inside a rectangular drawn to encompass each band (the same-sized rectangle for all bands in a particular gel), and a ratio of gray density to rectangular area was obtained for each band. Results were then expressed as arbitrary units or normalized to the darkest band.

Trapping of HUVEC-Derived PGH₃ by Platelets

HUVECs in 6-well plates, untreated and treated with 10 U/mL IL-1β for 24 hours, were incubated at 37°C for 0.450 mL medium 199 containing 10 mmol/L HEPES and 25 μmol/L [¹⁴C]AA. After 0.5 minutes, 10⁴ COX-inactivated platelets in 50 μL were added to the wells, and the reactions were stopped after another 9.5 minutes, as described previously. Inactivation of COX in the platelet suspensions was monitored previously by incubating 0.5-μL aliquots of the washed platelet suspensions with 25 μmol/L [¹⁴C]AA at 37°C for 5 minutes.

Trapping of PGH₃ from endogenous AA derived from resting and IL-1β-treated HUVECs by platelets was achieved by adding 50 μL of a platelet suspension containing 10⁴ COX-inactivated platelets to HUVECs 0.5 minutes after cell challenge with 50 μmol/L histamine. After 10 minutes of the addition of histamine, supernatants were removed and placed in liquid N₂. Samples were then kept at −80°C until the analysis of 6-keto-PGF₁α and thromboxane (TxB₂) B₃ by RIA (Tk₂B, Advanced Magnetics Inc).

Incubation With Isolated COX-1 and COX-2 and Obtaining of [¹⁴C]-Labeled Endoperoxides

COX-1 (5 U/0.5 mL) isolated from ram seminal vesicles (Oxford Biomedical Research) or COX-2 purified from sheep placenta (Cayman Chemical) was incubated in 100 mmol/L Tris-HCl (pH 8.5) containing 2 mmol/L phenol and 5 μmol/L [¹³C]AA. Samples were incubated at 37°C for 5 minutes, after which the reaction was stopped by adding 1N HCl to yield pH 3 followed by 1 vol cold methanol. The products were subjected to HPLC analysis.

For the experiments concerning PGIS inactivation, an incubation of 180 U/mL of isolated COX-1 with 25 μmol/L [¹³C]AA at 30°C for 1 minute was used as a source of [¹³C]-labeled endoperoxides. The reaction was stopped by adding an excess of indomethacin (final concentration, 10 μmol/L). In these conditions, the remaining untransformed [¹³C]AA was <10%, and the percentage of nondegraded endoperoxides, evaluated by the difference of PGF₂α after addition of 1 vol methanol or 1 vol methanolic solution of 400 μg/mL SnCl₂, was always >80%. Hence, the incubation mixture was immediately used as a source of labeled endoperoxides without further manipulation.

Effect of COX-2–Selective Inhibition on the Release of PGH₂

IL-1β-treated HUVECs expressed both COX-1 and COX-2 in all experimental conditions. Therefore, in order to determine the concentration of the selective COX-2 inhibitor SC-58125,44 (Laboratorio Almirall-Prodessfarma) to be used with HUVECs, cultures of PMA-treated human dermal fibroblasts and the HUV-EC-C line were used for COX-2 tests, and cultures of the HEL line and fresh human platelets were used for COX-1 tests. Fibroblasts and HUV-EC-C attached in 6-well plates, a 0.5-μL aliquot of HEL suspension (5×10⁵ cells/mL), or a 0.15-μL platelet suspension incubated at 37°C in the presence of the indicated concentration of SC-58125 for 5 minutes. [¹⁴C]AA (25 μmol/L) was later added, and cells were incubated for another 5 minutes. Reactions were stopped, and COX-derived eicosanoids were analyzed as described above. COX activity was evaluated as the sum of all the chromatographic peaks corresponding to COX-derived eicosanoids.

As a result of these experiments, SC-58125 concentrations of 1 and 10 μmol/L were chosen to study the effect of selective inhibition of COX-2 on the release of PGH₂ by HUVECs. Resting HUVECs and HUVECs treated with 10 U/mL IL-1β overnight were incubated with 1 and 10 μmol/L of SC-58125 for 5 minutes. HUVECs were then incubated in the presence of 25 μmol/L [¹³C]AA alone or in the presence of 200 μg/mL SnCl₂ for another 5 minutes as previously mentioned. The untransformed PGH₂ was then estimated as described above.

Effect of IL-1β on the Inactivation of PGIS

To determine the PGIS activity after treatment of HUVECs with IL-1β, cells cultured in 6-well plates were treated with 10 U/mL IL-1β for 6 hours, without any additive or in the presence of final concentrations of the following: 1 mmol/L deferoxamine (Sigma), 1% (vol/vol) DMSO (Sigma), 1 mmol/L captopril (Laboratorios CINDFA), 200 μmol/L pyrrolidinedithiocarbamate (PTC, Sigma), 1 mmol/L, 1,2,3-oxadiazolium, 5-amino-3-(4-morpholino)-chloride (SIN-1, Cayman), 100 μmol/L 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO, Cayman), 50 U/mL superoxide dismutase (Sigma), 500 U/mL catalase (Sigma), 1 mmol/L N⁶-monomethyl-L-arginine (L-NMMA, Cayman), 5 mmol/L mannitol (Merck), or 100 μmol/L tyrosine (Sigma) (all dissolved in culture medium) or 0.5 mmol/L phenylbutazone, 10 μmol/L indomethacin, or 50 μg/mL vitamin E (all from Sigma) dissolved in ethanol (final ethanol concentration, 0.25% [vol/vol]). Parallel incubations of HUVECs with the scavengers and inhibitors in the absence of IL-1β were performed as controls. The culture
medium was then removed, and HUVECs were treated with 100 μmol/L ASA in medium 199 for 10 minutes at 37°C. The ASA-containing medium was removed, and 200 μL of the solution containing the 14C-labeled endoperoxides was added. HUVECs were allowed to stand at 37°C for another 5 minutes. The final concentration of 14C-labeled endoperoxides was ≈18 μmol/L. 14C-labeled 6-keto-PGF1α was evaluated by HPLC as described. Resting (control) and IL-1β-treated HUVECs for each condition were always incubated with the same batch solution of 14C-labeled endoperoxides. Negative controls were performed using cells boiled for 15 minutes. Synthesis of NO in untreated and in IL-1β-treated cells was determined by assay of the culture supernatants for NO2− (nitrite) using the Griess reagent, which contains 1 vol of 10 g/L sulfanilamide in 50 mL/L H3PO4, and 1 vol of 1 g/L N-(1-naphthyl)ethylenediamine dihydrochloride in water (all from Sigma). An aliquot of 500 μL of the culture medium was reacted with an equal volume of the Griess reagent for 10 minutes at room temperature in the dark. The optical densities of the assay samples were measured at 550 nm. Concentrations of NO2− were determined from a standard curve prepared using NaNO2 (Sigma) diluted in culture medium.

Statistics
Sigma-Stat software was used for statistical analysis. Statistical significance between groups was assessed using the Student t test for 2 groups. One-way ANOVA and the Student-Newman-Keuls test were used to compare >2 groups. Correlations were evaluated by linear regression. A value of P<0.05 was considered significant.

Results
AA Metabolism in HUVECs
As expected, resting and IL-1β–treated HUVECs incubated with exogenous [14C]AA produced 6-keto-PGF1α, PGE2, and PGF2α as major eicosanoids, and PGD2, HHT, 15-HETE, and 11-HETE were minor compounds.

COX-1 and COX-2 Expression
The time course of mRNA and protein expression of COX-1 and COX-2 was measured by incubating HUVECs with or without 10 U/mL of IL-1β during various time intervals. Specific mRNAs encoding for both COX-1 and COX-2 were induced by IL-1β in a time-dependent manner (Figure 1), although only the increase of COX-2 was statistically significant (P<0.05). The levels of COX-1 mRNA were slightly enhanced, whereas for COX-2 they were enhanced 12.5-fold by IL-1β. The maximum levels of COX-2 mRNA were observed between 3 and 6 hours of exposure to IL-1β.

The Western blot analysis of the COX isoforms present in HUVECs is shown in Figure 2. In the absence of IL-1β, the antibody against COX-1 recognized a band in HUVEC samples of ≈70 kDa corresponding to the migration of purified COX-1 from ram seminal vesicles. The antibody against COX-2 did not detect the purified COX-1 from ram seminal vesicles and had a slight reaction with a protein band of the unstimulated cells. In contrast, after 1 hour of IL-1β treatment, the antibody against COX-2 peptide recognized a protein band in HUVEC samples of ≈70 kDa corresponding to the migration of purified COX-2 from sheep placenta. The densitometric analysis of the bands showed that after 3 hours, IL-1β significantly increased the expression of COX-2 in a time-dependent manner, whereas modifications in the protein band corresponding to COX-1 over time were minimal. COX-2 expression reached a maximum level between 6 and 9 hours of IL-1β exposure, followed by a slight decrease at 24 hours. Figure 2 shows that the total COX activity also increased in a time-dependent manner with the IL-1β treatment, although it was only statistically significant after 6 hours of exposure to the cytokine. It was notable that whereas COX-2 protein levels decreased after 24 hours of exposure to IL-1β, the total activity continuously increased until 24 hours.

Evaluation of Release of Prostanoids by HUVECs in Response to IL-1β
Release of prostanoids by resting and 10 U/mL IL-1β–treated cells was determined by monitoring the most significant
prostanoids over 24 hours: PGE$_2$, PGF$_2\alpha$, and 6-keto-PGF$_1\alpha$. Results in Figure 3 show that after 24 hours, treatment with IL-1\(\beta\) caused a higher increment in the quantity of PGF$_2\alpha$ (≈20-fold) and PGE$_2$ (≈16.5-fold) than of 6-keto-PGF$_1\alpha$ (≈3.5-fold) accumulated in the medium compared with no treatment. Levels of 6-keto-PGF$_1\alpha$ in the medium were abruptly enhanced 1 hour after exposure of cells to IL-1\(\beta\), whereas PGE$_2$ and PGF$_2\alpha$ were released into the medium more slowly, following the pattern of COX-2 expression. We should point out that 6-keto-PGF$_1\alpha$ was the only compound whose accumulated quantity in the culture medium was significantly enhanced (≈7-fold) 1 hour after exposure to IL-1\(\beta\), when COX-2 protein and activity was not enhanced (Figure 2). Release of PGE$_2$, PGF$_2\alpha$, and 6-keto-PGF$_1\alpha$ by control cells was quantitatively slight, and the 3 progression curves had similar patterns.

**Biosynthesis of Eicosanoids From Exogenous AA**

To observe the differences concerning synthesis of the different eicosanoids between those cells that overexpress COX-2 and resting cells, progression curves after the addition of labeled substrate were evaluated, and results are shown in Figure 4. The maximum levels of 6-keto-PGF$_1\alpha$ and PGF$_2\alpha$ were similar in both resting and IL-1\(\beta\)-stimulated HUVECs (10 U/mL IL-1\(\beta\) for 24 hours). Nevertheless, the amount of PGI$_2$ increased to a maximum 10 minutes after substrate addition in resting HUVECs, whereas the synthesis of PGI$_2$ practically ceased 1 minute after addition in the cells treated with IL-1\(\beta\). Furthermore, the amount of 6-keto-PGF$_1\alpha$, produced after 10 minutes by IL-1\(\beta\)-treated cells was significantly lower than that produced by control cells. HHT was produced in a greater amount in IL-1\(\beta\)-treated than in resting HUVECs, whereas the synthesis of PGI$_2$ practically ceased 1 minute after addition in the cells treated with IL-1\(\beta\). In contrast, an increasingly higher production of PGE$_2$, PGD$_2$, and HETEs was observed in IL-1\(\beta\)-treated cells compared with control cells (Figure 4).
Results presented in Figure 4 suggest that IL-1\(\beta\) does not exert any effect on PGIS expression. To confirm this, PGIS mRNA levels were determined by RT-PCR, and results are shown in Figure 5. Results from 4 experiments indicated that PGIS mRNA was not increased in HUVECs exposed overnight to IL-1\(\beta\) compared with control cells.

Characterization of the Biosynthetic Pathway of PGE\(_2\) and PGD\(_2\) in HUVECs

The slow time course of PGE\(_2\), PGD\(_2\), and HETE formation and the fact that they were the only eicosanoids that increased as a consequence of the induction of the COX-2 expression when an excess of exogenous substrate was supplied suggested that all of these eicosanoids could be formed in HUVECs by the exclusive intervention of COX. To verify that transformation of PGH\(_2\) into PGE\(_2\) and PGD\(_2\) was not enzymatic, 2 different experimental approaches were carried out.

Figure 6 shows representative chromatograms corresponding to samples of HUVECs and isolated COX incubated with exogenous \([^{14}\text{C}]\text{AA}\). Isolated COX-1 and COX-2 yield a ratio of PGE\(_2\) to PGD\(_2\) of 4.2±0.8 and 4.4±1.2 (mean±SD, n=5), respectively, similar to the ratio obtained in samples from HUVECs (3.9±0.99) (n=10). No 6-keto-PGF\(_{1\alpha}\) was detected in the isolated COX incubations. In addition, the presence of SnCl\(_2\) in the incubations of HUVECs with \([^{14}\text{C}]\text{AA}\) caused a complete diversion of the transformation of HUVEC-derived PGH\(_2\) toward PGF\(_{1\alpha}\) toward PGE\(_2\) plus PGD\(_2\) (Figure 6).

Comparison Between the Metabolism of Exogenous and Endogenous AA as a Function of Time of Incubation With IL-1\(\beta\)

After treatment of HUVECs with 10 U/mL IL-1\(\beta\) for different periods of time, cells were incubated in the presence of
25 µmol/L [14C]AA or 50 µmol/L histamine. Histamine-induced release of endogenous AA and the formation of the major PGs were evaluated by RIA. Results in Figure 7 show some differences between the effect of IL-1β on the metabolism of exogenous and endogenous AA. When substrate was supplied exogenously, we observed a slight reduction in the ability of HUVECs to form PGI2 (evaluated as 6-keto-PGF1α) and PGF2α as a function of time of incubation with IL-1β. In contrast, the ability to form PGE2 and PGD2 increased with the time of exposure to the cytokine and correlated with COX-2 expression. Whereas formation of PGI2 and PGF2α from endogenous substrate increased suddenly after 1 hour of incubation with IL-1β and a sustained ability to form these PGs was then observed, statistically significant correlations were observed only between the increment in COX-2 protein expression and the increment in PGE2 and PGD2 formation from exogenous substrate and between the increment in COX-2 protein expression and the increment in PGE2 formation in response to histamine (formation of PGD2 from endogenous substrate was not determined; Figure 7).

**Effect of IL-1β on the Release of Untransformed PGH2 by HUVECs From Exogenous and Endogenous Substrate**

The amount of untransformed PGH2 released by HUVECs from exogenous [14C]AA was estimated as the difference in the labeled PGF2α between the samples incubated in the presence and absence of SnCl2. After any period of time of exposure to IL-1β, the formation of 6-keto-PGF1α and HETEs from exogenous substrate was only minimally modified by the presence of SnCl2, whereas formation of PGI2 and PGF2α from exogenous substrate increased concomitantly. HHT levels were also substantially reduced in the presence of SnCl2 but were not totally suppressed (not shown). Figure 8 shows the estimated release of PGH2 and PGI2 (evaluated as 6-keto-PGF1α) relative to the resting cells as a function of time of incubation with IL-1β. The increment in the release of PGH2 into the medium was statistically correlated with the increase in COX-2 protein and with the decrement in PGI2 formation (Figure 8). The ratio of PGH2 to PGI2 increased ∼4-fold as a consequence of treatment with IL-1β.

PGE2, PGF2α, and 6-keto-PGF1α released from endogenous sources in HUVECs stimulated with histamine were also evaluated, after several periods of time of exposure to IL-1β, both in the absence and presence of SnCl2. Formation of 6-keto-PGF1α was lower in the presence of SnCl2 than in its absence (Figure 9), in contrast with results from the experiments adding exogenous [14C]AA. As expected, PGE2 levels were substantially lower in the presence of SnCl2 but were not totally suppressed. In addition, the production of PGF2α was similar both in the absence and in the presence of SnCl2 in particular when the activity of COX was maximum. Data from exogenous substrate experiments indicated that SnCl2 did not modify COX and PGIS activities. Hence, the lower levels of prostanooids observed in the presence of SnCl2 should be due to a less

**Figure 6.** Representative chromatograms of samples from IL-1β–treated HUVECs (24 hours with 10 U/mL) incubated with 25 µmol/L of [14C]AA for 10 minutes in the absence and in the presence of SnCl2 and from 5 U of COX isolated from ram seminal vesicles incubated with 5 µmol/L [14C]AA for 5 minutes.

**Figure 7.** Production of the major prostanoids formed by HUVECs from exogenous and endogenous substrate as a function of time of exposure to IL-1β (top panels). Cells were treated with 10 U/mL IL-1β for the indicated periods of time. They were later incubated with 25 µmol/L [14C]AA or with 50 µmol/L histamine for 10 minutes. Eicosanoids were analyzed by HPLC or RIA. Values are mean±SD (n=3). Correlations between the IL-1β–increased prostanoids and the increment in COX-2 are also shown (bottom panels). OD indicates optical density.
effective release of AA. This led to a decreased formation of PGH<sub>2</sub> and therefore a decreased production of PGI<sub>2</sub>. The less effective mobilization of endogenous AA would also explain why PGF<sub>2α</sub> did not increase as much as expected as a consequence of the diversion of the PGH<sub>2</sub> toward formation of PGF<sub>2α</sub> instead of PGE<sub>2</sub> and PGD<sub>2</sub>. Hence, although the results indicated that untransformed PGH<sub>2</sub> was also released from endogenous AA, the estimation of the amount of PGH<sub>2</sub> released was not possible when this indirect method was used.

To evaluate the untransformed PGH<sub>2</sub> released by HUVECs from endogenous sources, PGH<sub>2</sub> was trapped by ASA-treated platelets, and its transformation by Tx synthase was evaluated. First, platelets treated with 200 μmol/L ASA were added to the wells of HUVECs and incubated in the presence of [14C]AA. ASA-treated platelets did not produce any eicosanoid derived from COX activity. Preliminary experiments served to choose the appropriate time to add the platelet suspensions to the HUVECs. COX-inactivated platelets were added to the HUVECs treated with IL-1β at increasing periods of time after the addition of 25 μmol/L [14C]AA to the HUVECs. The maximum conversion of labeled PGH<sub>2</sub> into labeled TxB<sub>2</sub> and HHT was observed when 10<sup>8</sup> platelets were added to HUVECs 0.5 minutes later than the addition of [14C]AA (not shown). Therefore, a delay of 0.5 minutes between the addition of [14C]AA and platelet suspensions was chosen for the following experiments.

Results in the Table show that the presence of platelets in the incubation mixture did not modify the amount of PGI<sub>2</sub> produced by HUVECs from endogenous or exogenous AA. ASA-treated platelets trapped HUVEC-derived PGH<sub>2</sub> from both exogenous and endogenous AA. HHT in platelets is formed by the action of Tx synthase. The ratio of HHT to TxB<sub>2</sub> formed in our conditions was evaluated by incubating suspensions of platelets untreated with ASA with 25 μmol/L [14C]AA and determining both compounds by HPLC. This ratio was 1.47±0.18 (mean±SD, n=5), which allowed us to estimate the amount of PGH<sub>2</sub> trapped by platelets from HUVECs incubated with [14C]AA and those incubated with 50 μmol/L histamine were comparable, indicating a substantial release of PGH<sub>2</sub> from endogenous AA.
Effect of the Selective Inhibition of COX-2 on the Release of PGH₂

To select the most suitable concentration of the selective inhibitor in our experimental system, concentration-dependent inhibition curves on both COX-1 and COX-2 whole-cell systems were obtained by using human platelets and HEL line suspensions for COX-1 and the HUV-EC-C line and human dermal fibroblasts treated with PMA for COX-2. The [14C]AA concentration used was the same as that in HUVEC experiments. The COX activity in these systems was measured as the sum of all COX-derived compounds. As a result of these experiments, we chose 1 and 10 μmol/L SC-58125, since at these concentrations, inhibition of COX-2 was high and COX-1 was not substantially inhibited (see Figure 10). As expected, the inhibition of the release of PGH₂ evaluated using the SnCl₂ indirect method was consistent with a major COX-2 origin.

Inactivation of PGIS During Incubation of HUVECs With IL-1β

After treatment of HUVECs with 10 U/mL IL-1β for 6 hours, cells were incubated with [14C]labeled endoperoxides, and the formation of PGF₁α as 6-keto-PGF₁α was measured. A significant decrease in the formation of PGF₁α was observed in cells after IL-1β treatment compared with resting cells. This decrease was similar to that observed when cells were incubated with [14C]AA (Figure 11). These results clearly indicate that PGIS was partially inactivated during the treatment with IL-1β.

To approach the mechanism of inactivation of PGIS in response to IL-1β, cells were incubated with several radical scavengers and inhibitors during exposure to IL-1β. Vitamin E and extracellular addition of catalase and superoxide dismutase did not exert any effect. In contrast, the Fe³⁺-chelating agent deferoxamine and the free radical scavengers PDTC and DMSO totally reverted the effect of IL-1β. Mannitol and captopril also significantly prevented the effect of the cytokine. Indomethacin and phenylbutazone partially avoided the effect of IL-1β, but no statistical significance was achieved. The effect of IL-1β was totally suppressed by the NO synthase L-NMMA but not by extracellular addition of l-tyrosine. This was consistent with a significant 1.5-fold increase of the NO⁻ levels in the culture medium of IL-1β-treated cells compared with control cells (492.3 ± 117 and 320.5 ± 63.3 pmol/10⁶ cells for 6 hours, respectively; mean ± SD, n = 6, P < 0.05). In contrast, the presence the NO scavenger carboxy-PTIO dramatically increased the PGIS inhibitory effect of IL-1β. The effect of carboxy-PTIO was significantly prevented by DMSO but not by l-tyrosine. The inhibitors or scavengers referred in Figure 11 did not exert any statistically signif-

### Table 1: Production of PGF₁α (Evaluated as 6-Keto-PGF₁α) and Amounts of PGH₂ Produced by IL-1β-Stimulated HUVECs That Was Trapped by ASA-Treated Platelets

<table>
<thead>
<tr>
<th>Compound</th>
<th>Exogenous AA</th>
<th>Endogenous AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HUVECs</td>
<td>HUVECs + Platelets</td>
</tr>
<tr>
<td>TxB₂</td>
<td>ND</td>
<td>289.7 ± 105.4</td>
</tr>
<tr>
<td>HHT</td>
<td>266.5 ± 105.4</td>
<td>613.8 ± 143.5</td>
</tr>
<tr>
<td>PGH₂ trapped*</td>
<td>(637.0 ± 206.3)</td>
<td>...</td>
</tr>
<tr>
<td>6-Keto-PGF₁α₉₉</td>
<td>451.85 ± 91.7</td>
<td>492.5 ± 15.8</td>
</tr>
</tbody>
</table>

ND indicates not detected. HUVECs, pretreated with 10 U/mL IL-1β for 24 hours, were incubated with [14C]AA for exogenous AA experiments (n = 3) and with 50 μmol/L histamine for endogenous AA experiments (n = 4) 0.5 minutes before the addition of 100 × 10⁶ platelets pretreated with ASA. Reactions were stopped, and the eicosanoid was analyzed as described in Materials and Methods. Values are mean ± SD and are expressed as pmol/10⁶ HUVECs for 10 minutes.

*Considering the PGH₂ transformed by platelets into TxB₂ plus HHT.
‡Estimated by the following approximation: TxB₂(HUVECs + platelets) + HHT(HUVECs + platelets) - HHT(platelets).

Figure 10. Effect of the selective COX-2 inhibitor SC-58125 on the release of PGH₂ (bars). HUVECs treated with 10 U/mL IL-1β overnight were incubated with and without the indicated concentrations of SC-58125 for 5 minutes before the addition of 25 μmol/L [14C]AA. The release of PGH₂ was then estimated by the indirect method of the SnCl₂. Error bars represent individual values (n = 3). Inhibition curves of SC-58125 of COX-2 and COX-1 in whole-cell systems are also shown. The HUV-EC-C line and human dermal fibroblasts treated with PMA were used as COX-2 systems. In the experimental conditions used, COX-1 was slightly expressed in both fibroblasts and the HUV-EC-C line (not shown). The HEL line and human platelets were used as the COX-1 system. COX activity was evaluated as a sum of all COX-derived eicosanoids formed after incubation of cells with [14C]AA. Values are mean ± SD (n = 4).
by isolated COX. (3) The presence of SnCl₂ totally diverted exogenous AA-derived PGH₂ toward PGF₂α, instead of PGE₂ and PGD₂.

In agreement with other reports, we observed that release of PGI₂ by HUVECs was increased in cells exposed to IL-1β compared with resting cells. However, the release of PGF₂α and PGE₂ was substantially more induced by IL-1β than was the release of PGI₂. The mobilization of AA from cell lipids induced by IL-1β could account for the fact that the cytokine-induced release of PGI₂ occurred (Figure 3) before an appreciable increase in the COX expression and activity (Figure 2). In contrast, a slow PGF₂α and PGE₂ accumulation in the medium was observed as COX-2 increased. This indicated that a COX-2-dependent excess of PGH₂, which was not transformed into PGI₂, was formed during the incubation with IL-1β. On the other hand, the ability of cells to form PGE₂ and PGD₂ from exogenous AA was greatly enhanced with the expression of COX-2 (Figure 7). The same phenomenon was observed when mobilization of endogenous AA was induced by histamine; the ability to synthesize PGE₂ (in this case PGD₂ was not measured) also correlated with COX-2 expression (Figure 7). This was consistent with the data reported by Bull et al, who found that PGE₂ was the most enhanced eicosanoid in response to histamine after pretreatment of dermal microvascular endothelial cells with IL-1β. The fact that the increased ability to form PGI₂ in response to histamine was already observed after 1 hour of IL-1β-exposure, when COX activity was not appreciably induced, suggests that IL-1β exerted priming in the histamine-induced AA mobilization.

Since biosynthesis of PGE₂ and PGD₂ are nonenzymatic in HUVECs, PGH₂ could be transformed into a mixture of these position isomers outside the cell. The ratio of untransformed [¹⁴C]AA-derived PGH₂ to PGI₂ released by HUVECs as a function of time of exposure to IL-1β overlapped with the increase in the COX activity and COX-2 expression. This indicates that the presence of COX-2 causes a dramatic increase in the ratio of PGH₂ to PGI₂ released by HUVECs when exogenous substrate is supplied. The fact that the COX-2-selective inhibitor SC-58125 inhibited, in a concentration-dependent manner, the release of PGH₂ at concentrations that did not appreciably inhibit COX-1 also supports this conclusion (Figure 10). A substantial release of PGH₂ formed from endogenous AA could be demonstrated by trapping with ASA-treated platelets. In contrast to that which occurred in the exogenous substrate experiments, SnCl₂ was unable to completely suppress PGE₂ formation when IL-1β-treated cells were stimulated with histamine. This may be due to the endogenous and exogenous AA having access to the COX located in different intracellular compartments. PGH₂ generated from endogenous sources could be partially converted nonenzymatically into PGE₂ inside the cell.

The formation of PGF₂α by 2-electron reduction of PGH₂ could occur nonenzymatically inside or partially outside the cells. Our results are not conclusive with respect to these points. Nevertheless, unlike 6-keto-PGF₁α, formation of PGF₂α showed a slow progression curve consistent with a nonenzymatic reduction of PGH₂. In contrast with that which

**Figure 11.** Effect of several radical scavengers and inhibitors on the inactivation of PGIS during the exposure of HUVECs to IL-1β. Cells were treated with 10 U/ml IL-1β for 6 hours in the absence of any drug or in the presence of final concentration of 1 mmol/L captopril, 500 U/mL catalase, 100 μmol/L carboxy-PTIO (C-PTIO), 1 mmol/L deferoxamine, 1% (vol/vol) DMSO, 10 μmol/l indomethacin, 5 mmol/L mannitol, 1 mmol/L L-NMMA, 0.5 mmol/L phenylbutazone, 200 μmol/L PDTC, 50 U/mL superoxide dismutase (SOD), 100 μmol/L tyrosine (L-Tyr), 50 μmol/L vitamin E, or the indicated combinations of substances. Parallel incubations in the absence of IL-1β were performed as controls. α-Tocopherol indicates α-tocopherol. Cells were then incubated with [¹⁴C]endoperoxides as described in Materials and Methods. Results are expressed as percentage of increment with respect to their own controls. Values are mean±SD; the numbers in parentheses indicate the number of experiments. *P<0.05 compared with their own controls; #P<0.05 compared with the cells without scavengers or inhibitors (none); and @P<0.05 compared with C-PTIO alone.

**Discussion**

In 1978, Marcus et al suggested that PGE₂ and PGD₂ could be formed nonenzymatically in HUVECs. Their suggestion was based on the observation that PGH₂ incubated in the absence of any drug or in the presence of final concentration of 1 mmol/L captopril, 500 U/mL catalase, 100 μmol/L carboxy-PTIO (C-PTIO), 1 mmol/L deferoxamine, 1% (vol/vol) DMSO, 10 μmol/l indomethacin, 5 mmol/L mannitol, 1 mmol/L L-NMMA, 0.5 mmol/L phenylbutazone, 200 μmol/L PDTC, 50 U/mL superoxide dismutase (SOD), 100 μmol/L tyrosine (L-Tyr), 50 μmol/L vitamin E, or the indicated combinations of substances. Parallel incubations in the absence of IL-1β were performed as controls. α-Tocopherol indicates α-tocopherol. Cells were then incubated with [¹⁴C]endoperoxides as described in Materials and Methods. Results are expressed as percentage of increment with respect to their own controls. Values are mean±SD; the numbers in parentheses indicate the number of experiments. *P<0.05 compared with their own controls; #P<0.05 compared with the cells without scavengers or inhibitors (none); and @P<0.05 compared with C-PTIO alone.

Our results indicate that HUVECs do not transform PGH₂ into PGE₂ and PGD₂ enzymatically. This concept is supported by the following: (1) Progression curves indicate that the formation of PGE₂ and PGD₂ is substantially slower than that of the other prostanoids and overlap those of 15-HETE and 11-HETE, whose formation does not require later enzymatic steps. (2) The ratio of PGE₂ to PGD₂ formed by HUVECs from exogenous substrate was almost identical to that yielded...
occurred with PGE$_2$ and PGD$_2$, PGF$_{2\alpha}$ did not increase with the COX activity when an excess of exogenous substrate was supplied. This was consistent with an enzymatic and/or an electron donor–dependent synthesis. These facts taken together strongly suggest that formation of PGF$_{2\alpha}$ in endothelial cells is limited by the presence of some cell-derived electron donor rather than by an enzymatic activity posterior to COX. The cellular nature of the electron donor is suggested by the fact that the relative quantity of PGF$_{2\alpha}$ formed in the incubations with isolated COX was negligible. We can speculate that a slow turnover of such an electron donor(s) could account for the predominant formation of PGF$_{2\alpha}$, when the slow release of prostanooids caused by the presence of IL-1$\beta$ was measured (Figure 3). In contrast, when an excess of exogenous AA was supplied, or a high quantity of AA was suddenly released by the action of histamine, a great amount of PGH$_2$ was rapidly formed. As a consequence, this factor(s) could be rapidly depleted and finally exhausted, thus limiting the transformation of PGH$_2$ into PGF$_{2\alpha}$. Under such conditions, PGE$_2$ and PGD$_2$ would be the predominant nonenzymatic transformations of PGH$_2$ (Figures 4 and 7).

These considerations lead to the idea that COX and PGIS are the only enzymes involved in the biosynthesis of prostanooids in endothelial cells. Moreover, progression curves indicate that PGE$_2$, PGD$_2$, and PGF$_{2\alpha}$ were formed in detectable amounts, mainly when PGIS became inactive, which occurred faster in the IL-1$\beta$-treated cells than in the resting HUVECs (Figure 4). The faster inactivation of PGIS under COX-stimulated conditions yielding an excess of PGH$_2$ is consistent with the “suicide” behavior of PGIS. The fact that the levels of mRNA for PGIS were not induced by IL-1$\beta$ indicates that the only enzyme activity regulated at the transcriptional level was COX, particularly COX-2. This was consistent with the fact that the PGi, that formed when an excess of exogenous substrate was supplied did not increase according to the increment of COX-2 protein and activity. Moreover, PGi, formation ability was reduced as a function of time of treatment with IL-1$\beta$, reaching a minimum 6 to 9 hours after the addition of IL-1$\beta$ (Figure 8), when the expression of COX was maximum (Figure 2).

The partial inactivation of PGIS as a consequence of exposure of HUVECs to IL-1$\beta$ was further confirmed by supplying $^{14}$C-labeled endoperoxides and analyzing the formation of labeled 6-keto-PGF$_{1\alpha}$. The inhibition of PGIS observed after exposure to IL-1$\beta$ using labeled endoperoxides as substrate was comparable to that obtained the addition of $^{14}$C-labeled AA (Figure 10). The partial inactivation of PGIS also contributed to the accumulation of an excess of PGH$_2$, and the increase of PGH$_2$ released by HUVECs correlated not only with COX-2 expression but also with PGIS inactivation (Figure 8).

Evidence that the HO$_{-}$ radical was involved in the inactivation of PGIS was provided by the following: (1) The use of DMSO, PDTC, mannitol, and captopril, powerful HO$_{-}$ scavengers, partially or totally antagonized the effect of IL-1$\beta$. (2) Iron-dependent HO$_{-}$ formation from O$_2$$^-$$,$ generating systems such as Fenton or Haber-Weiss reactions was inhibited by deferoxamine, a powerful Fe$^{3+}$ chelator, which also blocked the inactivation of PGIS induced by IL-1$\beta$. (3) $\alpha$-Tocopherol did not antagonize the IL-1$\beta$–induced inactivation of PGIS, since $\alpha$-tocopherol did not react efficiently with HO$_{-}$. Catalase and superoxide dismutase added to the extracellular phase did not exert any effect on the inactivation of PGIS, probably because of the fast intracellular generation of HO$_{-}$ from H$_2$O$_2$ generated by dismutation of O$_2$ or from homolytic fission of ONOOH, with the extracellular presence of these enzymes being not effective.

Peroxidase activity as a source of free radicals has been reported to be involved in the inactivation of PGIS. Deferoxamine and PDTC are also inhibitors of the peroxidase activity of COX. Since most of the COX inhibitors do not exert any effect on peroxidase activity, the fact that indo- methacin and phenylbutazone partially (but without statistical significance) inhibited the inactivation of PGIS by IL-1$\beta$ would indicate that PGG$_2$ contributed to the production of the inactivating oxygen-derived free radicals.

Another explanation has been given by Zou and Ullrich, who found that peroxynitrite irreversibly inhibits PGIS. Our results from L-NMMA experiments, which inhibited inactivation of PGIS by IL-1$\beta$, are consistent with those of these authors, who suggested that NO$^-$ formation is the limiting step in the peroxynitrite production in endothelial cells and may be induced by the inflammatory cytokines. Actually, exposure to IL-1$\beta$ caused a significant 1.5-fold increase in the NO$_2$ released by HUVECs in terms of NO$_2$; although we did not evaluate the expression of the inducible NO synthase. The fact that the NO$^-$ scavenger carboxy-PTIO significantly increased the inhibitory effect of IL-1$\beta$ in the present study was in apparent contradiction with this. Nevertheless, carboxy-PTIO equimolarly reacts with NO$^-$ to yield 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl plus NO$_2$; Homolytic fission of peroxynitrous acid, formed by protonation of peroxynitrite, yields NO$_2$ and HO$_2$; Zou et al recently reported that nitration of an active site–related tyrosine could account for the PGIS inactivation produced by peroxynitrite. These authors proposed an O-O homolytic cleavage in the peroxynitrite molecule caused by the heme iron of the PGIS. This could finally yield NO$_2$–nitrating species, which may attack tyrosine or tyrosinate. Nevertheless, nitration of tyrosyl residues by NO$_2$ is also possible through the formation of tyrosyl radicals, which could be favored by HO$_{-}$. Our results from carboxy-PTIO experiments are consistent with an inactivation of the PGIS mediated by NO$_2$. The fact that the presence of DMSO in addition of carboxy-PTIO decreased the ratio of inactivated PGIS, when compared with carboxy-PTIO alone, suggests that HO$_{-}$ was also involved. Consistently, another mechanism by which deferoxamine could prevent the effect of IL-1$\beta$ on PGIS inactivation may be the direct reaction with peroxynitrite. We also observed that SIN-1, which releases both NO$^-$ and O$_2$ as a source of peroxynitrite, inactivated PGIS in both resting and IL-1$\beta$–treated HUVECs. In summary, our results about the exact mechanism of the partial inactivation of PGIS caused by the exposure of HUVECs to IL-1$\beta$ are not conclusive, but they strongly suggest that both NO$^-$ (which could generate peroxynitrite that undergoes homolytic cleavage of the O-O bond to yield NO$_2$) and HO$_{-}$ were involved.
Present results provide a biochemical explanation for the production and release of the contractile prostanoids, PGH₂ and PGF₂α, by endothelial cells. Predominance of relaxing or constricting prostanoids will depend on both COX and PGIS activities. An impairment of endothelial cell function occurs under inflammatory conditions, and COX activity is increased by means of the induction of COX-2 and by partial inactivation of PGIS. Under such conditions, endothelial cells could produce an excess of PGH₂ that could exert proinflammatory and prothrombotic activities before its conversion to other prostanoids inside and outside the endothelium. PGH₂ from endothelial cells enriched in COX-2 could also be converted into TxA₂ by platelets and macrophages. MicrovesSEL expression of COX-2 could contribute to the platelet activation observed in the disseminated intravascular coagulation, usually associated with systemic inflammatory responses. By use of an antagonist of the PGH₁/TxA₂ receptor, it has been shown that endogenous PGH₁ decreases the thrombotic activity of tissue plasminogen activator.⁶¹ PGH₁ and TxA₂ stimulate proliferation of vascular smooth muscle cells in vitro.⁶²–⁶⁶ Expression of COX-2 could also contribute to the myointimal hyperplasia observed in atherosclerosis by promoting the formation of PGH₂ (and transcellular TxA₂) and hydroxy linoleic acids,¹⁹ which elicit smooth muscle cell mitogenesis.⁶⁷

In summary, our results show that resting HUVECs and HUVECs exposed to IL-1β produce only PGH₁ and PGI₂ enzymatically. Production of PGF₂α, however, can clearly be linked to the presence of a limiting intracellular electron donor. Overexpression of COX-2 turns the endothelial cell toward a phenotype characterized by an increased production of PGH₁, which cannot be totally transformed into PGI₂, partly because of its inactivation. Despite the fact that caution and more research is required to assess the biological relevance of our findings in vivo, the present data increase our knowledge about the metabolism of AA by endothelial cells and about the possible biological role of the induction of COX-2 in the vascular endothelium.

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