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Roles of Cyclooxygenase (COX)-1 and COX-2 in Prostanoid Production by Human Endothelial Cells: Selective Up-Regulation of Prostacyclin Synthesis by COX-2

Gillian E. Caughey,* Leslie G. Cleland,* Peter S. Penglis,* Jennifer R. Gamble,† and Michael J. James*

The two cyclooxygenase (COX) isoforms, COX-1 and COX-2, both metabolize arachidonic acid to PGH₂, the common substrate for thromboxane A₂ (TXA₂), prostacyclin (PGI₂), and PGE₂ synthesis. We characterized the synthesis of these prostanoids in HUVECs in relation to COX-1 and COX-2 activity. Untreated HUVEC expressed only COX-1, whereas addition of IL-1β caused induction of COX-2. TXA₂ was the predominant COX-1-derived product, and TXA₂ synthesis changed little with up-regulation of COX-2 by IL-1β (2-fold increase). By contrast, COX-2 up-regulation was associated with large increases in the synthesis of PGI₂ and PGE₂ (54- and 84-fold increases, respectively). Addition of the selective COX-2 inhibitor, NS-398, almost completely abolished PGI₂ and PGE₂ synthesis, but had little effect on TXA₂ synthesis. The up-regulation of COX-2 by IL-1β was accompanied by specific up-regulation of PGI synthase and PGE synthase, but not TX synthase. An examination of the substrate concentration dependencies showed that the pathway of TXA₂ synthesis was saturated at a 20-fold lower arachidonic acid concentration than that for PGI₂ and PGE₂ synthesis. In conclusion, endothelial prostanoid synthesis appears to be differentially regulated by the induction of COX-2. The apparent PGI₂ and PGE₂ linkage with COX-2 activity may be explained by a temporal increase in total COX activity, together with selective up-regulation of PGI synthase and PGE synthase, and different kinetic characteristics of the terminal synthases. These findings have particular importance with regard to the potential for cardiovascular consequences of COX-2 inhibition. The Journal of Immunology, 2001, 167: 2831–2838.

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Abbreviations used in this paper: PGI₂, prostacyclin; TX, thromboxane; AA, arachidonic acid; ASA, acetylsalicylic acid; COX, cyclooxygenase; MAPK, mitogen-activated protein kinase.

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determinant of the dominance of PGI₂ and PGE₂ production when COX-2 was induced by IL-1β.

Materials and Methods

Materials
Human rIL-1β was from Genzyme (Cambridge, MA). AA, NS-398 (N-(2-cyclohexoxy-4-nitrophenyl) methanesulfonylamine, rabbit polyclonal Ab against human COX-2, and murine mAb against COX-1 were all purchased from Cayman Chemicals (Ann Arbor, MI). Peroxidase-labeled donkey anti-rabbit and goat anti-mouse Abs, ECL Western blotting system, [3H]PGE₂, [3H]-[6-keto-PGF₁α], and [3H]TXB₂ were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). PGH₂ and (4-[(4-fluorophenyl)-2-[(4-methyl-2-pyridyl)imidazolyl]oxy]phenyl) (SB203580) (Calbiochem) (San Diego, CA). Abs to the phosphorylated forms of p38 and p44/42 mitogen-activated protein kinases (MAPKs) and the mitogen-activated protein/extracellular signal-related kinase 1 inhibitor PD98059 were purchased from New England Biolabs (Beverly, MA). Rabbit anti-serum against PGE₂ and 6-keto-PGF₁α and mouse mAb against β-actin were obtained from Sigma (St. Louis, MO).

Cell culture
HUVECs were isolated as described (10). The cells were cultured on gelatin-coated culture flasks in medium M199 with Earle’s salts supplemented with 20% FCS, 25 μg/ml endothelial growth supplement (Genome Therapeutics, Waltham, MA), and 25 μg/ml heparin. Cells between passages 2 and 4 were plated in 24-well dishes (1.5 × 10⁴/ml) and allowed to reach confluence (24 h).

Cell stimulation
HUVECs were incubated with RPMI 1640 medium (containing 10 mM HEPES, 2 mM t-glutamine, 100 μM penicillin, and 100 μg/ml gentamicin), supplemented with 10% heat-inactivated FCS in the presence or absence of IL-1β (1 ng/ml, 37°C). For short-term stimulation (15 min) with either AA or PGH₂, cells were incubated in serum-free RPMI medium. To inhibit COX-1 activity, untreated cells were pretreated with aspirin (acetylsalicylic acid (ASA); 10 μM/mL) for 30 min, followed by washing (2) and then incubated in the appropriate medium with the test agents, according to the specified experiment. Other inhibitors were added 15 min before stimulation. Following the appropriate treatment, cell supernatants were collected and stored at −20°C until analysis for prostanoid measurement by RIA.

Prostanoid measurement
TXB₂, 6-keto-PGF₁α (the stable hydrolysis products of TXA₂ and PGI₂, respectively), and PGE₂ were measured by RIA using commercially available reagents, except for the TXB₂ antiserum, which was prepared as described previously (12).

Western blotting
Cell lysates were prepared by treating cells with ice-cold lysis buffer (HEPES-buffered HBSS, pH 7.4, 0.5% Triton X-100, 10 μg/ml leupeptin, 10 μg/ml aprotinin) and sample buffer (0.125 M Trizma base, pH 6.8, 20% glycerol, 4% SDS, 10% 2-ME), followed by 6 min at 37°C. Proteins were separated by 9% SDS-PAGE and then transferred onto a Sequi-Blot polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). After blocking the membranes with 5% fat-free dried milk in TBS (25 mMTris-Cl, 0.2 M NaCl, 0.15% Tween 20, pH 7.6), they were incubated with the appropriate primary Abs, followed by HRP-conjugated donkey anti-rabbit or sheep anti-mouse Ab. Equivalent protein loading and transfer efficiency were verified by staining for β-actin. Bound Abs were revealed with ECL reagent, according to the manufacturer’s protocol.

Statistical analysis
Results are expressed as mean ± SEM of triplicate incubations. Statistical significance was examined by Student’s t test, using p < 0.05 as the significance level.

Results
Effect of COX-2 induction on the synthesis of individual prostanooids
COX-2 was not detectable in unstimulated HUVECs (Fig. 1) and remained undetectable in the absence of IL-1β over the 24-h time period examined (data not shown). Treatment of HUVECs with IL-1β (1 ng/ml) for increasing times resulted in expression of COX-2, which was maximal by 16 h (Fig. 1). By comparison, COX-1 was expressed in unstimulated cells, and its expression did not change with IL-1β treatment (Fig. 1). A time course of prostanoid production by HUVECs in response to IL-1β treatment indicated very different synthesis profiles between TXA₂ and PGI₂ or PGE₂. TXA₂ synthesis was evident at the earliest time point of 2 h, when COX-1 was the only isoform detectable. As COX-2 was induced, there was a modest increase in TXA₂ production by ~2-fold. Even in the absence of IL-1β stimulation, TXA₂ synthesis increased with time (albeit less than IL-1β-stimulated HUVECs) (Fig. 2 A). By comparison, in the absence of IL-1β stimulation, synthesis of PGI₂ or PGE₂ was not detectable. Treatment of HUVECs with IL-1β resulted in little or no PGI₂ and PGE₂ synthesis up to 4 h, but with the induction of COX-2 by IL-1β, the production of these prostanooids increased by 54- and 84-fold, respectively (production at 2 h compared with that at 24 h) (Fig. 2, B and C). To determine more specifically the IL-1β-induced changes in synthetic capacity of each prostanooid pathway, HUVECs were treated with IL-1β at time intervals of 0, 8, and 24 h, washed, and then stimulated with AA (10 μM, 10 min, 37°C). This type of examination ensures constant substrate concentration. The results under these conditions were similar to those obtained with measurement of prostanooid accumulation, as described above. TXA₂ was the predominant prostanooid to be synthesized by untreated HUVECs (i.e., time 0), with PGI₂ and PGE₂ being minor products (Table I). At this time, COX-1 was the only isoform detectable (Fig. 1). With the induction of COX-2 by IL-1β, production of TXA₂ increased by ~2-fold at 20 h, whereas PGI₂ and PGE₂ synthesis increased by 31.6- and 39.3-fold, respectively (Table I). Therefore, it appears that TXA₂ is the major COX-1-derived product, but the induction of COX-2 results in a preferential increase in PGI₂ and PGE₂ synthesis.

Effect of specific inhibition of COX-1 or COX-2 on HUVEC prostanooid synthesis
To investigate the contributions of the COX isotypes to endogenously derived prostanooid synthesis, selective inhibition of COX-1 and COX-2 activities was required. We have previously documented that transient pretreatment of unstimulated monocytes with aspirin (ASA) results in irreversible inhibition of COX-1 activity, with no effect on activity or induction of COX-2 (11). Inhibition of COX-1 activity by ASA resulted in significant inhibition of TXA₂ synthesis by IL-1β-treated HUVECs (42%), but had
or absence (dashed line) of IL-1 at least.

TABLE I. Effect of exogenous AA on prostanoid synthesis by HUVECs

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Prostanoid Production (ng/4.5 × 10⁶ HUVECs)</th>
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<tr>
<td></td>
<td>TXB₂</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2.34 ± 0.16</td>
</tr>
<tr>
<td>IL-1β + ASA-wash</td>
<td>1.35 ± 0.28⁹</td>
</tr>
<tr>
<td>IL-1β + NS-398</td>
<td>1.75 ± 0.36⁹</td>
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</table>

⁹ HUVECs (4.5 × 10⁶) were treated with IL-1β (1 ng/ml) for 0, 8, or 20 h, washed, and incubated in serum-free medium, and AA was added (10 μM, 15 min, 37°C). Prostanoids were measured by RIA; results presented are mean ± SEM of triplicate incubations.

Effects of exogenous AA on prostanoid synthesis by HUVECs are shown in Table I. The treatment with the selective COX-2 inhibitor, NS-398, resulted in almost complete inhibition of IL-1β-induced PGI₂ and PGE₂ synthesis (91% and 92.5%, respectively), but only slightly reduced TXA₂ production (Table II).

The effect of specific COX inhibition under conditions of constant substrate concentrations was also examined. Following the 20-h incubation in the presence or absence of IL-1β, cells were then washed and incubated with 10 μM AA for 15 min. In untreated cells, inhibition of COX-1 activity resulted in significant inhibition of synthesis of TXA₂ (82%), the predominant COX-1-derived prostanoid synthesized by HUVECs (Fig. 3A). Inhibition of COX-1 activity by ASA pretreatment had no significant effect on the synthesis of PGI₂ or PGE₂, but TXA₂ levels were inhibited by 35% (Fig. 3). By contrast, selective inhibition of COX-2 activity by NS-398 (0.5 μM) resulted in almost complete inhibition of PGI₂ and PGE₂ production (Fig. 3, B and C), but synthesis of TXA₂ was inhibited by only 23% (Fig. 3A). Neither ASA nor NS-398 affected the amount of COX-2 protein expressed in response to IL-1β (Fig. 4). These data suggest that while the majority of TXA₂ synthesis is COX-1 dependent, COX-2 can contribute to the synthesis of TXA₂ by IL-1β-treated HUVECs. By comparison, synthesis of both PGI₂ and PGE₂ appears to be predominantly COX-2 derived.

Regulation of terminal synthases by IL-1β

It is possible that the preferential up-regulation of endothelial PGI₂ and PGE₂ synthesis by IL-1β may have resulted from increased amount or activity of PGI synthase and PGE synthase, respectively. To examine this possibility, we studied the effects of IL-1β on the conversion of exogenous PGH₂ to TXA₂, PGI₂, and PGE₂ by the respective terminal synthases. Addition of PGH₂ (10 μM) to untreated HUVECs resulted in production of all prostanoids being examined. Treatment of the cells with IL-1β (1 ng/ml) for

Table II. Effect of selective inhibition of COX-1 or COX-2 activity on IL-1β-stimulated prostanoid synthesis

<table>
<thead>
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<td>1.75 ± 0.36⁹</td>
</tr>
</tbody>
</table>

⁹ HUVECs (4.5 × 10⁶) were preincubated in the presence or absence of aspirin (10 μg/ml) for 30 min followed by two washes (ASA-wash). Cells were then incubated with IL-1β (1 ng/ml, 37°C) with or without NS-398 (0.5 μM) for 20 h. Cell supernatants were collected for prostanoid measurement by RIA; results presented are mean ± SEM of triplicate incubations.

* p < 0.05, by comparison to IL-1β-treatment only. Representative results from at least five separate experiments are shown.

**TABLE II.** Effect of selective inhibition of COX-1 or COX-2 activity on IL-1β-stimulated prostanoid synthesis

**TABLE I.** Effect of exogenous AA on prostanoid synthesis by HUVECs
20 h, before addition of PGH₂, did not alter the production of TXA₂, but synthesis of PGI₂ and PGE₂ significantly increased by ~10- and 6-fold, respectively (Fig. 5). These results indicate that treatment of HUVECs with IL-1β results in increased COX-2 expression and increased expression of PGI synthase and PGE synthase, but not TX synthase.

Kinetic activities of the terminal synthases

We have reported recently that the kinetic properties of the terminal synthases are an important determinant of prostanoid production in human monocytes (13). Therefore, we examined the kinetic properties of TX synthase, PGI synthase, and PGE synthase in HUVECs, in response to increasing substrate (AA) availability. Addition of exogenous AA at doses up to 5 μM to untreated HUVECs, i.e., only COX-1 present, resulted in a dose-dependent increase in TXA₂ production. However, no further increases in TXA₂ production were observed with higher doses of AA (10–100 μM), suggesting saturation of TX synthase or COX-1 at 5 μM AA (Fig. 6A). By contrast, both PGI₂ and PGE₂ synthesis increased dose dependently with increasing AA concentrations, up to at least 50 μM. This indicates that COX-1 is not saturated at 5 μM AA (Fig. 6A). The concentration of AA required to achieve half-maximal stimulation of TXB₂ synthesis was 1.2 μM compared with PGI₂ and PGE₂, which were 21.9 and 23.6 μM, respectively. Furthermore, at substrate concentrations up to 5 μM, the apparent rate constant of TX synthase was greater than that for PGI or PGE synthase. Similar dose responses to AA were observed in IL-1β-stimulated endothelial cells, in which COX-2 was induced (Fig. 6B). TXA₂ production increased dose dependently at doses of AA up to 10 μM, after which no further increases in TXA₂ production were observed. However, synthesis of PGI₂ and PGE₂ increased dose dependently with increasing AA concentrations, up to at least 50 μM. The concentration of AA required to achieve half-maximal...
TXA₂ synthesis was 2.6 μM, which was considerably less than that required for half-maximal production of PGI₂ (8.5 μM) and PGE₂ (13.2 μM).

Regulation of COX-2 induction by p38 and p44/42 MAPKs

The MAPK cascade is one of the major signaling pathways leading from cellular activation to gene transcription. Induction of COX-2 has been reported to be mediated by both the p38 and p44/42 MAPK pathways in various cell types, in response to either LPS or cytokine stimulation (14–16). Therefore, we examined the effect of IL-1β addition (1 ng/ml) on p38 and p44/42 activation in HUVECs. As shown in Fig. 7, IL-1β induced phosphorylation of both p38 and p44/42 MAPK in a time-dependent manner. Activation of p38 MAPK peaked at 15 min after exposure to IL-1β, and maximal activation of p44/42 MAPK was observed 30 min post-IL-1β treatment. In the absence of IL-1β, there was no detectable phosphorylation of either p38 or p44/42 MAPK (data not shown).

Although it appears that IL-1β can activate both the p38 and p44/42 MAPK pathways in HUVECs, we wanted to establish the potential roles of these MAPKs in the induction of COX-2 expression by HUVECs in response to IL-1β. Addition of SB 203580 (p38 MAPK inhibitor) or PD 98059 (inhibitor of mitogen-activated protein/extracellular signal-related kinase 1 activation) had no effect on prostanooid synthesis by untreated HUVEC (data not shown). However, addition of either SB 203580 or PD 98059 to IL-1β-treated HUVECs resulted in significant inhibition of both PGI₂ and PGE₂ synthesis (Table III). By comparison, addition of

**FIGURE 6.** Dose response with exogenous AA. Untreated HUVECs (4.5 × 10⁵) (A) or IL-1β-treated HUVECs (4.5 × 10⁵) (B) were cultured in serum-free medium in the presence of increasing concentrations of AA (0–100 μM) for 15 min, 37°C. Cell supernatants were then collected for prostanooid measurement by RIA, as described in Materials and Methods.

**FIGURE 7.** Time courses of p38 and p44/42 MAPK activation by IL-1β. HUVECs (4.5 × 10⁵) were incubated with IL-1β (1 ng/ml) for 0, 5, 15, and 30 min. Cells were then processed for Western blot analysis, as described in Materials and Methods. Results are representative of three separate experiments.
either of these inhibitors had no effect on TXA₂ production (Table III). Recently, doubts have been raised concerning the selectivity of the MAPK inhibitors SB 203580 and PD 98059. In particular, they have been reported to interfere directly with AA metabolism via the COX pathway in platelets (17). However, we did not observe inhibition of COX-1-derived prostanoids, namely TXA₂, indicating that at the concentrations used, neither compound inhibits COX-1 or TX synthase activities. Western blot analysis demonstrated that both SB 203580 and PD 98059 inhibited IL-1β-induced COX-2 induction (Fig. 8). Thus, IL-1β appears to up-regulate COX-2 expression in endothelial cells through a mechanism involving both the p38 MAPK and the p44/42 MAPK pathways. The p38 MAPK pathway has been reported to be involved in the regulation of COX-2 mRNA stability (16, 18), while the p44/42 MAPK pathway has been shown to regulate COX-2 at the transcriptional level (16). Whether this specificity of COX-2 regulation by these MAPKs occurs in our cell system is yet to be elucidated.

Discussion

Up-regulation of COX-2 in a cell contributes to the total cellular COX activity. It would be anticipated that this would result in a general and uniform increase in prostandoid synthesis. However, in the present study, we demonstrated that the profile of prostandoid synthesis in HUVECs is dependent on the specific isotype of COX that was present. In unstimulated HUVECs, which express COX-1, but no detectable COX-2, TXA₂ was the predominant prostandoid synthesized, with both PGI₂ and PGE₂ being relatively minor products. When COX-2 was induced by IL-1β, the synthesis of PGI₂ and PGE₂ increased substantially, whereas only a modest increase in TXA₂ production was observed. These differential changes in prostandoid synthesis were observed when production arising from either endogenous or exogenous AA was measured. Selective inhibition of either COX-1 or COX-2 supported the apparent dependencies of TXA₂ synthesis of COX-1 and PGI₂ and PGE₂ synthesis on COX-2 in endothelial cells.

Similar associations have been observed in rat peritoneal macrophages. COX-1 was linked with TXA₂ production, whereas the induction of COX-2 by LPS shifted prostandoid synthesis to favor PGE₂ (5, 19) and PGI₂ synthesis (5). Differences in the subcellular distributions of COX-1 and COX-2 were proposed as an explanation for the different prostandoid synthesis profiles associated with the different COX isozymes (5). However, this is unlikely, as COX-1 and COX-2 are reportedly to be located within the same subcellular locations (20).

Both PGI synthase and PGE synthase have been shown to be inducible enzymes. Expression of PGI synthase was increased by shear stress in HUVECs (7), and inflammatory stimuli have been reported to up-regulate PGE synthase activity in rat peritoneal macrophages and A549 cell line (19, 21, 22). With regard to PGE synthase, a cytosolic constituitive and a membrane-associated inducible form have recently been identified (21, 23). Based on co-expression studies in transfected HEK293 cells, cytosolic constituitive PGE synthase and membrane-associated inducible PGE synthase are reported to be functionally linked with COX-1 or COX-2, respectively (21, 23). In the present study, up-regulation of endothelial COX-2 was accompanied by specific up-regulation of the terminal synthases, PGI synthase and PGE synthase, but not TX synthase. Although this may explain in part the selective increase in PGI₂ and PGE₂ production with cell stimulation, other factors appear to be involved. Synthesis of PGI₂ and PGE₂ increased by 50- to 80-fold with endogenous AA, or 31- to 39-fold with exogenous AA, whereas PGI and PGE synthase activities increased only by 6- to 10-fold. To account for the magnitude of the increased ratios of PGI₂/TXA₂ and PGE₂/TXA₂ with COX-2 induction, we propose that different kinetic characteristics of the terminal synthases may be involved also. Examination of increasing concentrations of substrate in either untreated or IL-1β-treated cells demonstrated that at the lower doses of AA (≤10 μM), TXA₂ synthesis exceeded that of PGI₂ and PGE₂, suggesting that TX synthase has a higher rate constant than that for PGI and PGE synthase. However, at doses of AA >10 μM, synthesis of TXA₂ did not increase, whereas synthesis of both PGI₂ and PGE₂ increased. Because production of PGI₂ and PGE₂ was responsive to doses of AA >10 μM and up to 50 μM, this demonstrates that COX was not saturated. Therefore, the lack of responsiveness of TXA₂ synthesis indicates saturation of TX synthase at AA concentrations <10 μM. These results are in accordance with the differences in KM values reported for TX and PGE synthase in human monocytes, which were 1 and 17 μM, respectively (13).

Consideration of the kinetic characteristics of the terminal synthases allows an explanation of findings without invoking linkage effects.
of COX isotypes with terminal syntheses in different subcellular locations. Thus, it is proposed that under conditions of low total COX activity, as observed when COX-1 only is present, TXA₂ production predominates due to a higher rate constant of TX synthase. Upon cell stimulation, total COX activity increases due to COX-2 induction. TX synthase becomes rapidly saturated with PGH₂, whereas PGI and PGE syntheses respond to the increased COX activity with increased synthesis of PGI₂ and PGE₂. In this explanation, the linkages between COX-1 and TXA₂ synthesis and between COX-2 and PGI₂/PGE₂ are apparent linkages only. Thus, a major determinant of increased PGI₂ and PGE₂ production over TXA₂ by IL-1β treatment is increased total COX activity in combination with different kinetic characteristics of the terminal syntheses. The selective increase in PGI₂ synthase and PGE synthase activity further augments the increases in PGI₂ and PGE₂ synthesis over that of TXA₂.

This study indicates that the role of endothelial COX-2 induction in vascular homeostasis is important due to its action of altering the ratio of prostanoids from a prothrombotic (high TXA₂/PGI₂) to an antithrombotic (high PGI₂/TXA₂) mixture. Although the induction of COX-2 appears to be important in many physiological processes, the induction of COX-2 has generally been associated with production of deleterious prostanoids due to the involvement of COX-2 in inflammatory disorders such as rheumatoid arthritis and osteoarthritis (24). Consequently, there has been rapid development of selective COX-2 inhibitors. These have been shown to suppress unwanted inflammation in patients with rheumatoid arthritis and osteoarthritis, with decreased upper gastrointestinal side effects compared with conventional agents. The selective COX-2 inhibitors are now in clinical use (24, 25). However, little is known regarding the physiological role of COX-2 in the vasculature, and there is evidence to suggest that it may be cardioprotective (26). COX-2 knockout mice are reported to develop cardiac fibrosis (27), and administration of COX-2 inhibitors abolished the cardioprotective effect of ischemic preconditioning in a model of myocardial infarction. In this model, up-regulation of myocardial COX-2 by ischemia was associated with PGI₂ and PGE₂ synthesis (28). Our study indicates that induction of COX-2 in the endothelium would result in increased synthesis of PGI₂, but not TXA₂, favoring an antithrombotic state. Therefore, up-regulation of COX-2 by these cells may represent an important protective mechanism against vascular injury or insult. This could result in an exacerbation of the potential for thrombotic complications with the use of selective COX-2 inhibitors. Additionally, COX-2 inhibitors do not suppress COX-1-derived TXA₂ production by platelets, unlike conventional nonsteroidal anti-inflammatory drugs, and this would further alter the TXA₂/PGI₂ balance toward a prothrombotic state. Two recent studies examining the effects of selective COX-2 inhibition in healthy volunteers have demonstrated inhibition of systemic PGI₂ production without inhibition of platelet-derived TXA₂ production synthesis (29, 30). Systemic PGI₂ synthesis is measured by urinary excretion of 2,3 dimor-6 keto PGF₁α, and is believed to reflect mainly blood vessel-derived PGI₂ synthesis. COX-2 may be up-regulated in large straight tracts of vasculature that are exposed to the biomechanical stimulus of uniform laminar flow (9, 31), and this may account for the COX-2-derived PGI₂ production. However, the situation in vivo remains unclear, because COX-2 was not detected in healthy arterial and venous tissues, but was highly expressed in atherosclerotic lesions (32–35). The cardiovascular consequences of COX-2 inhibition are further emphasized by a recent large clinical trial in rheumatoid arthritis that reported a 4-fold increase in myocardial infarction in patients using a selective COX-2 inhibitor (36).

In summary, the results of this study indicate a mechanism through which the initial prothrombotic vascular response to injury by endothelial cells becomes self-limiting, through the induction of COX-2 and the increased production of PGI₂. The results further suggest that the changing total cellular COX activity in conjunction with the kinetic properties of the terminal prostanoid syntheses and the selective induction of PGI₂ synthase, but not TX synthase, mediate this response. Furthermore, preliminary results from specific COX-2 inhibition in healthy volunteers and clinical arthritis trials support the contention that vascular COX-2 is an important protein for maintaining vascular homeostasis.

References


