Retinoic acid induces PGI synthase expression in human endothelial cells

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Abstract Retinoic acid (RA) exhibits anti-inflammatory, anti-tumor, and immuno-modulatory actions, and affects angiogenesis and thrombosis. Arachidonic acid (AA) metabolites are involved in all these processes. We explored the effect of RA on AA metabolism in human umbilical vein endothelial cells (HUVECs). 13-*cis*-RA increased the release of prostaglandin I_2 (PGI_2), both spontaneous and thrombin-induced, in terms of 6-oxo-$PGF_{1\alpha}$ analyzed by enzyme-immunoassay. Coincubation with 13-*cis*-RA and interleukin-1β resulted in a synergic increase in the release of PGI_2. Consistently, 13-*cis*-RA increased the ability of HUVECs to inhibit AA-induced platelet aggregation. 13-*cis*-RA did not induce cyclooxygenase-isoenzyme expression, determined by immunoblotting, or activity, evaluated by analyzing eicosanoids formed from exogenous labeled AA by HPLC. In contrast, RA induced PGI synthase (PGIS) activity and expression in terms of mRNA and protein determined by real-time PCR and Western blotting, respectively. Results from experiments with several species of RA and with retinoic acid receptor (RAR) and retinoid X receptor (RXR) antagonists showed that the effect of RA on PGIS expression was mediated by RAR. Actinomycin D and cycloheximide both inhibited RA-induced PGIS expression. Furthermore, RA increased PGIS transcriptional activity in transient transfection assays, an effect that was prevented by an RAR antagonist. These results reinforce the concept that RA could be beneficial for patients with cardiovascular risk.—Camacho, M., C. Rodríguez, J. Salazar, J. Martínez-González, J. Ribalta, J-R. Escudero, L. Masana, and L. Vila. **Retinoic acid induces PGI synthase expression in human endothelial cells. *J. Lipid Res.* 2008.** 49: **1707–1714.**

Supplementary key words prostacyclin synthase • prostaglandin I_2 • retinoic acid receptor • cyclooxygenase pathway

In the vascular tissue, prostaglandin I_2 (PGI_2, also called prostacyclin) is mainly produced by endothelial cells (ECs), and it generally counteracts the biological effects of thromboxane (Tx) A_2. PGI_2 inhibits platelet aggregation, relaxes vascular smooth muscle, and inhibits monocyte migration, lymphocyte adhesion, and smooth muscle cell (SMC) proliferation. Unlike nitric oxide, PGI_2 does not regulate the systemic circulation constitutively, and more likely functions on demand in response to local stimuli (1). PGH_2, formed from arachidonic acid (AA) by the catalytic action of cyclooxygenase (COX), is the common intermediate in the biosynthesis of two series of prostanoids. Two isoforms of COX have been fully characterized in humans to date. In general, COX-1 can be viewed as a constitutive enzyme, and prostanoids formed through the action of COX-1 mediate the so-called housekeeping functions, such as the regulation of renal function, maintenance of gastric mucosa integrity, and hemostasis. COX-2 is normally expressed in a number of tissues and cell types, such as endothelium or renal macula densa. However, it is induced in response to hormones, growth factors, pro-inflammatory cytokines, bacterial endotoxin, and tumor promoters, and is typically overexpressed at inflammatory sites, such as atherosclerotic lesions (as reviewed in Refs. 1–3).

The enzyme catalyzing the conversion of PGH_2 into PGI_2 is PGI synthase (PGIS, prostacyclin synthase). PGIS is a membrane-bound hemoprotein located in the endoplasmic reticulum, and according to its spectral characteristics, is a cytochrome P450 protein (4). Tyrosine-nitrating agents such as peroxynitrite and tetranitromethane cause tyrosine nitration and PGIS inactivation (5, 6). Activity of PGIS is also controlled by substrate-dependent suicide inactivation (7).

After treating human umbilical vein endothelial cells (HUVECs) with pro-inflammatory stimuli such as inter-

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leukin 1β (IL-1β), we observed that PGIS is inactivated faster in IL-1β-treated cells than in resting cells (8, 9). Inactivation dependent on hydroxyl and NO_2 radicals could also account for PGIS inactivation in inflammatory conditions (8, 10). Many aspects of cardiovascular disease have traditionally been explained on the basis of disturbances in the TXA_2 and PGI_2 balance. Nevertheless, depending on the activity ratio of COX to PGIS, ECs could release untransformed PGH_2 (8, 9, 11). The biological activities of PGH_2 are identical to those of TXA_2, because both bind to the same receptor. COX-2 selective inhibitors reduce circulating PGI_2 levels, indicating that COX-2 is involved in systemic production of PGI_2 (12). COX-2 is present after vascular damage and is highly expressed in atherosclerotic lesions and aortic aneurysms in animal models and human tissue (2). It probably plays a protective role in moderate vascular inflammation, promoting PGI_2 biosynthesis. Nevertheless, the scenario could be different if COX-2 were highly expressed in a severely inflamed area, potentially producing large amounts of other prostanoids, such as PGE_2 or PGH_2 (8, 13).

Natural and synthetic derivatives of vitamin A (retinoids) exert profound effects on growth, maturation, and differentiation of many cell types (14), and are currently used in cancer and several dermatological disease therapies (15, 16). Retinoids also exhibit putatively beneficial biological actions in the cardiovascular system. They increase NO synthesis, modulate hemostatic properties and inhibit endothelin-1 expression in ECs (17–19), inhibit actions of angiotensin II and TXA_2 receptor expression in vascular SMCs (20, 21), reduce monocyte tissue factor (TF) and TF/factor VIIa-dependent arterial thrombosis (22), and exhibit beneficial actions in cardiovascular disease models (23–31).

Because information regarding the effect of retinoic acid (RA) on prostanoid synthesis in ECs is scarce (32, 33), this work was focused on exploring the effect of RA on the COX-PGIS pathway in this cell type.

MATERIALS AND METHODS

Cell culture and treatment

ECs from human umbilical veins (HUVECs) were isolated and cultured as previously described (34). Cells in the first passage were cultured without heparin and EC growth factor for 48 h prior to addition (or not) of 1 μmol/l RA and 10 U/ml human recombinant IL-1β (Roche Applied Science; Barcelona, Spain), in M199 containing 1% FBS. Cells were then incubated for the indicated (in the Results section) period of time until mRNA analysis, thrombin stimulation, or enzyme activity evaluation. To study the involvement of RA receptors (RARs) in the effects of RA, cells were incubated in the presence or in the absence of the selective RAR pan-antagonist, LE540 (35), or the retinoid X receptor (RXR) pan-antagonist, PA452 (36), (kindly provided by Dr. Kagechika, School of Biomedical Science, Tokyo Medical and Dental University, Tokyo, Japan) at a concentration range reported for in vitro studies (37, 38) for 30 min prior to the addition of 1 μmol/l of 13-*cis*-RA for 24 h.

PGI_2 released in the conditioned medium from endogenous AA

6-oxo-$PGF_{1\alpha}$ (stable hydrolysis product of PGI_2) was analyzed by specific enzyme immunoassay (EIA) (Amersham Bioscience; Europe, GmbH), following the manufacturer's instructions.

PGI_2 formation, from endogenous AA, in response to thrombin

Cells were treated with the indicated species of RA (1 μmol/l) with or without 10 U/ml IL-1β for the indicated periods of time. They were then incubated with 1 U/ml of thrombin at 37°C for 10 min. Medium was removed, and they were stored at −80°C until 6-oxo-$PGF_{1\alpha}$ analysis by EIA.

Enzyme activity

Cells were treated with 1 μmol/l 13-*cis*-RA or untreated (control) for the indicated periods of time. COX activity was then evaluated by incubating cells with 25 μmol/l [1-^{14}C]AA (55–58 mCi/mmol; Amersham Bioscience) for 10 min as previously reported (8). COX activity was evaluated as the sum of all HPLC peaks corresponding to eicosanoids formed through the COX pathway. PGIS activity was evaluated as noted previously (8). HPLC analysis of eicosanoids was performed as described in a previous study (39).

PGIS, COX-1, and COX-2 mRNA analysis

For mRNA expression, cells were treated with 1 μmol/l of the indicated species of RA for the indicated periods of time and total RNA was extracted. mRNA expression was evaluated by real-time PCR as described (13). The amount of RNA was normalized to β-actin as endogenous control, and RNA of untreated cells was used as a calibrator sample.

COX-1, COX-2, and PGIS protein expression

Lysates of cells were prepared as previously reported (34). Total protein equivalents of each sample were subjected to SDS-PAGE and processed for Western blotting as previously described (13).

PGIS promoter construction

A 1,963 bp fragment of PGIS 5′ flanking region sequence was amplified by PCR. A *Kpn*I and an *Xho*I restriction site (underlined) were added to the forward (5′-atc ctcggt acc cgt tct atg gcc ttc tt-3′) and reverse (5′-aca tagctc gag tag cag cag cag caa cag-3′) primers used, respectively. The amplified product was cloned into the pGL3 basic vector (Promega) (pGL3/PTGIS-1963) and sequenced using the ABIPRISM Big Dye Terminator cycle sequencing kit v3.1 (Applied Biosystems).

Transient transfection assays

HUVECs were transfected using Lipofectin™ (Life Technologies) as previously described (40). Briefly, transfections were carried out with 1 μg/well of the construct pGL3/PTGIS-1963, 0.3 μg/well of pSVβ-gal, and 3 μl of Lipofectin™. The DNA/liposome complexes were added to the cells for 5 h. The media was then replaced by media containing 1% FBS, without heparin or EC growth factor, and left for 18 h prior to the addition (or not) of 1 μmol/l 13-*cis* RA in the presence or absence of 5 μmol/l of RAR antagonists for 24 h. Luciferase activity was measured in cell lysates using the Luciferase assay kit (Promega) and a luminometer (Anthos Lucy 1.0) following the manufacturer's instructions. Results were normalized by β-galactosidase activity using the Enzyme Assay System™ (Promega).

Platelet aggregation experiments

Platelet-rich plasma (PRP) was obtained from peripheral venous blood drawn from healthy nonsmoker adult donors who had not received any medication for 2 weeks prior to the extraction as described before (41). PRP was adjusted to 0.25×10^6 cells/μl with autologous platelet-poor plasma for aggregation assays.

In vitro platelet aggregation in response to 0.8 mmol/l AA (Diamed, Switzerland) was performed in an Automatic Platelet Aggregation Analyzer, PA-3220 (AGGRECORDER II, Menarini) as described previously (41, 42). Prior to aggregation onset, 50 μl of HUVEC suspensions was added to 400 μl of PRP and left to stand at 37°C for 5 min. The ratio of platelet to HUVEC was chosen in previous experiments, and total inhibition of platelet aggregation was observed when the platelet/HUVEC ratio was higher than 1×10^6, irrespective of the previous treatment of HUVECs. The platelet/HUVEC ratio was 4×10^6 for experiments with HUVECs untreated with IL-1β and 10×10^6 in those experiments performed with HUVECs treated with the cytokine. Percentage of change in relative transmittance (% of aggregation) was recorded for 5 min after addition of 50 μl AA solution. The area under the aggregation curve was the quantitative parameter considered. Quantitative results were expressed as percentage of the area under the transmittance curve relative to the area under the curve obtained with platelets alone.

HUVECs used in aggregation studies were obtained by detaching cells with nonenzymatic cell dissociation solution (Sigma) of cell monolayers treated with: none, 1 μmol/l 13-*cis*-RA for 48 h, 10 U/ml recombinant IL-1β for 48 h, or 1 μmol/l 13-*cis*-RA plus 10 U/ml IL-1β for 48 h. After the mentioned treatments, parallel HUVEC cultures were washed and incubated at 37°C for 20 min with 0.2 mmol/l acetyl salicylic acid (ASA) (Sigma) prior to their detachment, washing, and further addition to PRP.

Statistics

Sigma-Stat software was used for statistical analysis. Statistical significance between pairs of groups was assessed using Student's *t*-test, and ANOVA test was used for more than two groups. A *P* value below 0.05 was considered significant.

RESULTS

Fig. 1A shows the effect of 13-*cis*-RA on the release of PGI_2 (in terms of 6-oxo-$PGF_{1\alpha}$) by HUVECs. 13-*cis*-RA caused an increase in PGI_2 accumulation in the culture

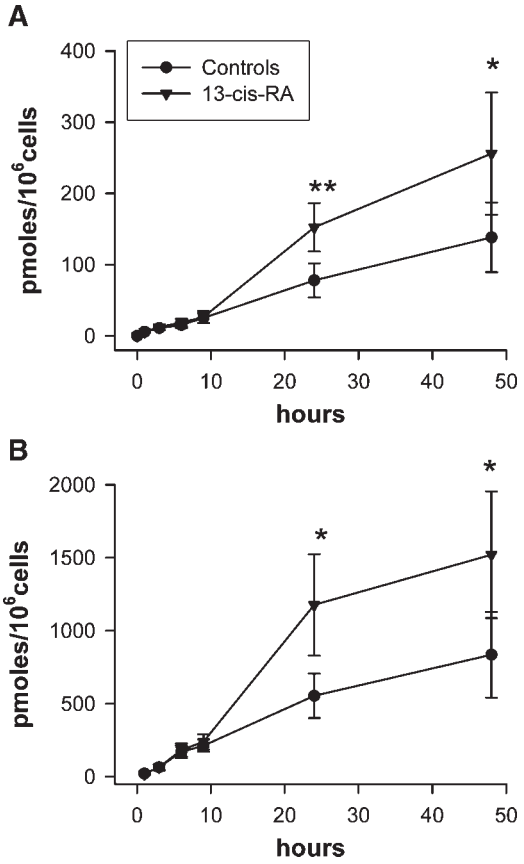

Fig. 1. Time course of 13-*cis*-RA-stimulated release of prostaglandin I_2 (PGI_2) by human umbilical vein endothelial cells (HUVECs). HUVECs were incubated without (control) or with 1 μmol/l of 13-*cis*-RA, in the absence (A) or in the presence (B) of 10 U/ml of interleukin-1β (IL-1β) for the indicated periods of time. Prostanoids accumulated in the medium were then analyzed by enzyme immunoassay (EIA); PGI_2 was measured as 6-oxo-$PGF_{1\alpha}$ stable metabolite of PGI_2 (n = 5, mean ± SEM). * $P < 0.05$, ** $P < 0.01$ when compared with control cells. RA, retinoic acid.

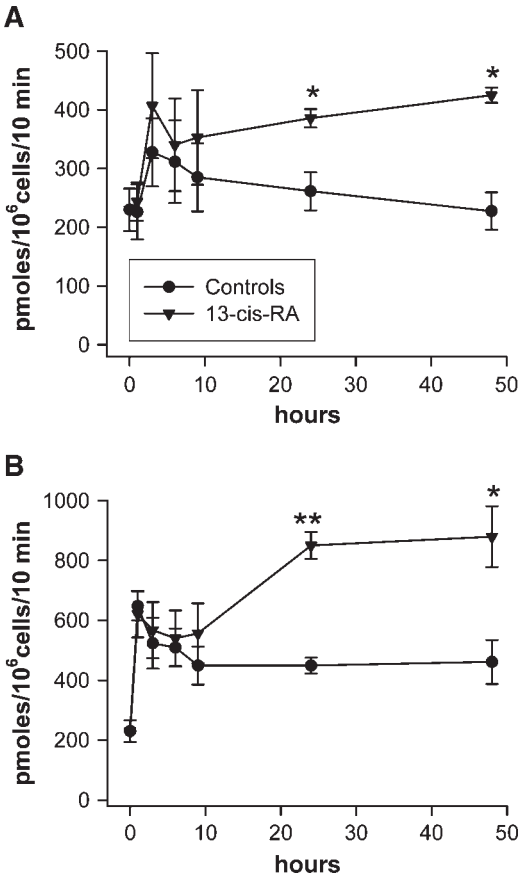

Fig. 2. Effect of 13-*cis*-RA on the release of PGI_2 by HUVECs in response to thrombin. HUVECs were incubated without (control) or with 1 μmol/l of 13-*cis*-RA in the absence (A) or in the presence (B) of 10 U/ml of IL-1β for the indicated periods of time. Culture medium was replaced and cells incubated with 1 U/ml of thrombin for 10 min. Prostanoids in the medium were analyzed by EIA (n = 5, mean ± SEM). * $P < 0.05$, ** $P < 0.01$ when compared with control cells.

medium when compared with controls without 13-*cis*-RA. 13-*cis*-RA also increased PGI_2 accumulation when cells were coincubated with IL-1β (Fig. 1B). As expected, IL-1β induced COX-2 expression (data not shown). To see the response of RA-treated cells to a strong phospholipase stimulus, after the treatment with 13-*cis*-RA in the presence or absence of IL-1β over several time periods, medium was replaced and cells were stimulated with thrombin for a short period of time (10 min). 6-oxo-$PGF_{1\alpha}$ in the medium was then measured (**Fig. 2**). The results given in Fig. 2 show that in this case also, 13-*cis*-RA significantly increased the ability of HUVECs to produce PGI_2,

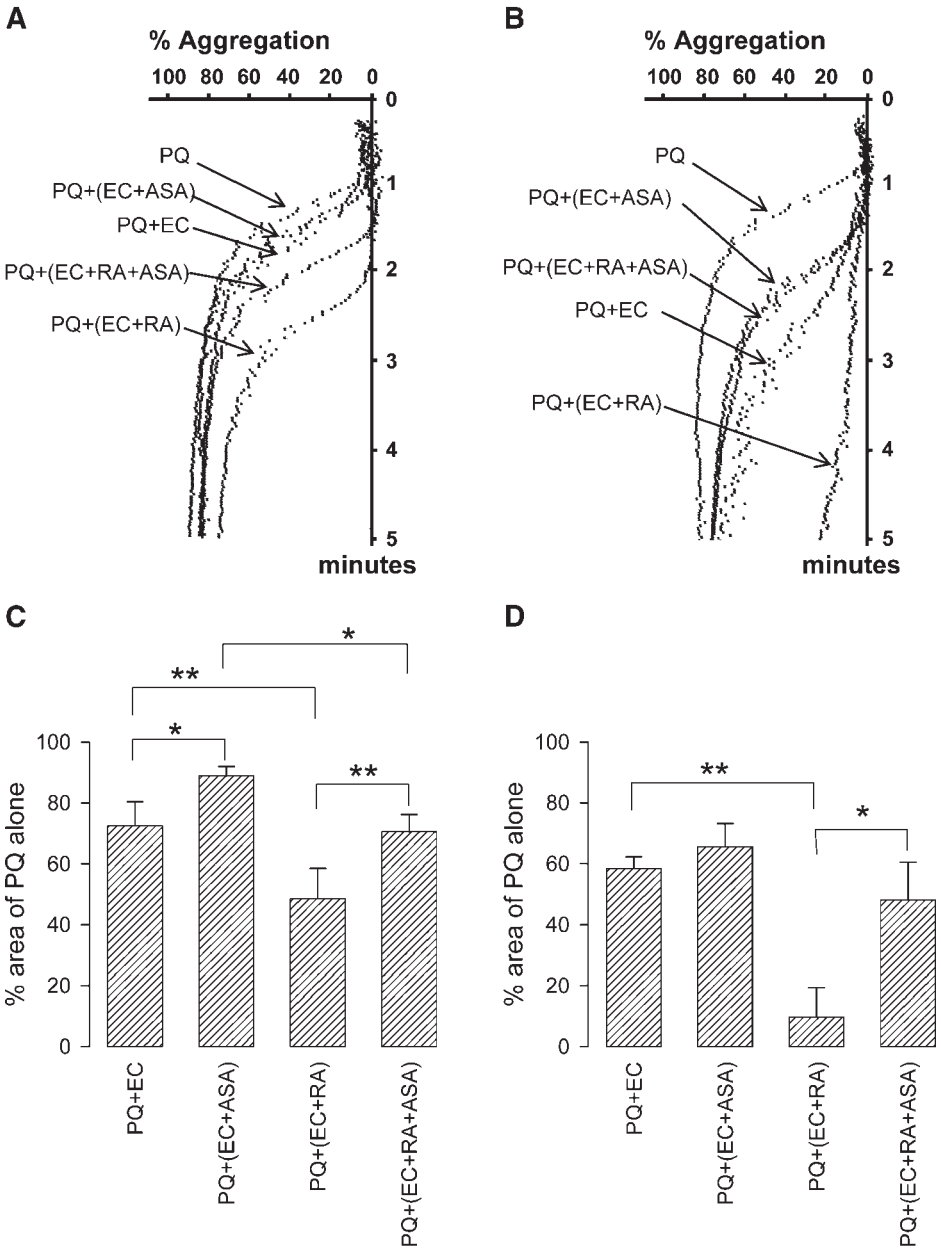

Fig. 3. Effect of 13-*cis*-RA treatment on HUVEC-modified platelet aggregation in response to AA. A: Representative curves of transmittance relative to platelet-poor plasma (% aggregation) of mixed preparations of PRP (0.4 ml, 0.2×10^6 platelet/μl) plus 50 μl HUVEC suspensions; PQ, PRP plus culture medium plus AA; PQ+EC, PRP plus HUVEC plus AA solution; PQ+(EC+ASA), PRP plus HUVECs pretreated with ASA, plus AA; PQ+(EC+RA), PRP plus HUVECs pretreated with 13-*cis*-RA, plus AA; PQ+(EC+RA+ASA), PRP plus HUVECs pretreated with 13-*cis*-RA and ASA, plus AA. Platelets/HUVECs ratio: 4×10^6. B: As indicated in A, but using HUVECs pretreated with IL-1β. Platelets/HUVECs ratio: 10×10^6. C: Percentage of the area under the relative transmittance of PQ curve in platelets treated as indicated in A. D: Percentage of the area under the relative transmittance of PQ curve in platelets treated as indicated in B. Bars are from four independent experiments and represent the mean ± SEM; AA, arachidonic acid; PRP, platelet-rich plasma; ECs, endothelial cells; ASA, acetyl salicylic acid. * $P < 0.05$, ** $P < 0.01$.

both alone and in combination with IL-1β, in a time-dependent fashion. This effect of 13-*cis*-RA was associated with an enhancement of the ability of HUVECs to inhibit platelet aggregation induced by AA (**Fig. 3**). The effect was observed in both IL-1β-untreated and IL-1β-treated HUVECs. In fact, it was remarkable that in three out of four experiments in which HUVECs were treated with IL-1β, 13-*cis*-RA totally inhibited platelet aggregation. Figure 3A, B shows representative transmittance records of platelet aggregation tests in the presence of IL-1β-untreated and IL-1β-treated HUVECs, respectively.

Biosynthesis of PGs by HUVECs was fully inhibited by the preincubation with ASA (results not shown). Pretreatment with ASA significantly reduced the effect on platelet aggregation of HUVECs under basal conditions (Fig. 3C). Interestingly, this effect was more pronounced with HUVECs exposed to RA. When HUVECs were pretreated with IL-1β, preincubation with ASA reversed significantly their inhibitory effect on platelet aggregation only when HUVECs were also exposed to 13-*cis*-RA (Fig. 3D).

13-*cis*-RA did not modify either COX activity or COX isoenzyme expression (**Fig. 4**). In contrast, PGIS expression in terms of mRNA, protein, and enzyme activity was significantly increased by the exposure of HUVECs to 13-*cis*-RA in a time-dependent manner. Protein expression and enzyme activity increased at least until 48 h of exposure to 13-*cis*-RA (**Fig. 5**).

Fig. 6A shows the effect on PGIS expression in HUVECs exposed for 48 h to several RA species. All natural RAs were able to induce PGIS expression. In contrast, RA-*p*-hydroxyanilide (4HRP), which activates neither RAR nor RXR, showed no effect on the induction of PGIS mRNA or activity. PA452, an RXR pan-antagonist, did not modify the effect of 13-*cis*-RA on PGIS expression, whereas LE540, an RAR pan-antagonist, concentration-dependently inhibited the effect of 13-*cis*-RA (Fig. 6B).

Results given in **Fig. 7** show that both actinomycin D and cycloheximide totally suppressed the effect of 13-*cis*-RA on

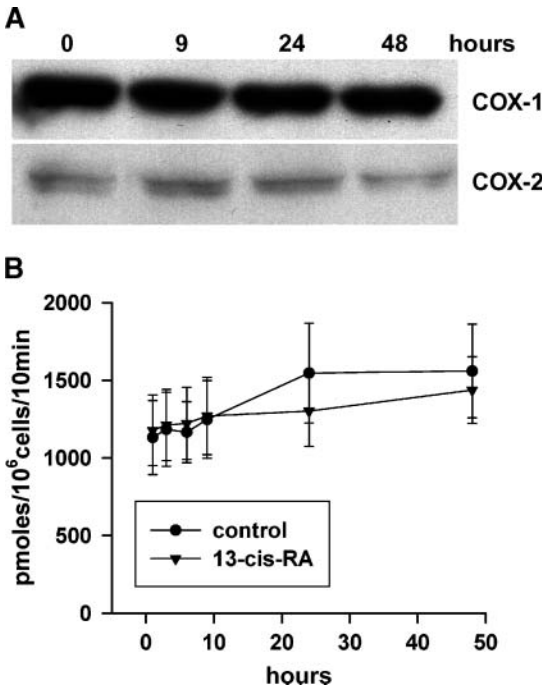

Fig. 4. Cyclooxygenase (COX) isoenzyme expression and COX activity as a function of time of exposure of HUVECs to 13-*cis*-RA. HUVECs were treated with 1 μmol/l of 13-*cis*-RA for the indicated periods of time. Afterwards, COX isoenzyme expression (A) and COX activity (B) were determined as described in Materials and Methods. A: A representative, out of two with similar results, immunoblotting analysis of COX-1 and COX-2 proteins. The COX activity was evaluated as the sum of all HPLC peaks corresponding to eicosanoids formed through the COX pathway; values are mean ± SEM (n = 4).

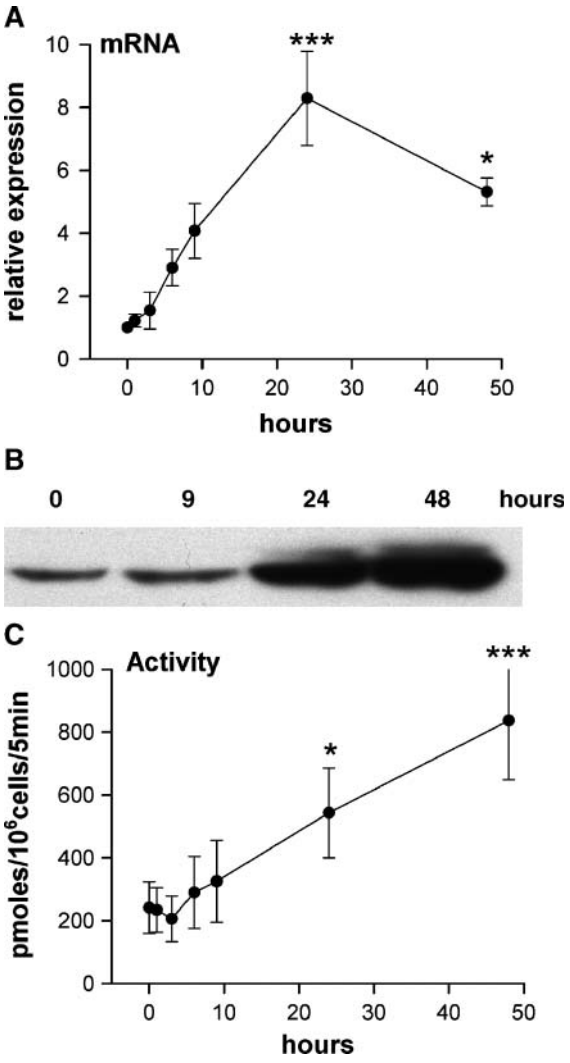

Fig. 5. PGI synthase (PGIS) expression and PGIS activity as a function of time of exposure of HUVECs to 13-*cis*-RA. HUVECs were treated with 1 μmol/l of 13-*cis*-RA for the indicated periods of time. Afterwards, PGIS mRNA levels (A), protein (B), and activity (C) were determined as described in Materials and Methods. The amount of RNA was normalized to β-actin as endogenous control, and RNA of untreated cells was used as a calibrator sample. B: A representative, out of three with similar results, immunoblotting analysis of PGIS protein. Numerical values are mean ± SEM (n = 5). *$P < 0.05$, and *** $P < 0.001$ compared with control cells (time 0).

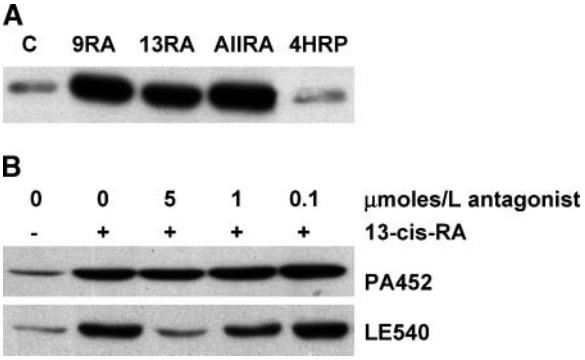

Fig. 6. Effect of different RA species, RA receptor (RAR), and retinoid X receptor (RXR) antagonists on PGIS expression. A: Cells were treated with 1 μmol/l of 13-*cis*-RA (13RA), 9-*cis*-RA (9RA), all-*trans*-RA (allRA), or RA-*p*-hydroxyanilide (4HRP) for 24 h in the presence or absence of the drugs. Afterwards, PGIS protein levels were determined as described in Materials and Methods. A representative, out of two with similar results, immunoblotting analysis is shown. B: HUVECs were treated or not with 1 μmol/l of 13-*cis*-RA for 24 h in the presence or absence of the indicated antagonist concentration. A representative, out of three with similar results, immunoblotting analysis is shown.

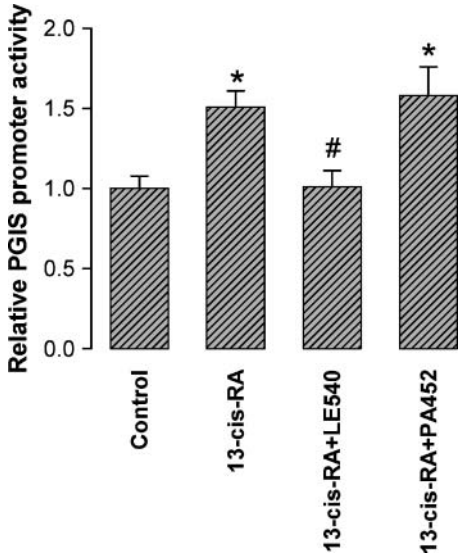

Fig. 8. Effect of 13-*cis*-RA on PGIS transcriptional activity. HUVECs were transfected with a plasmid containing 1,963 bp of the PGIS promoter (pGL3/PGIS-1963). Twenty-four hours after transfection, cells were treated with 1 μmol/l 13-*cis*-RA for 18 h in the presence or absence of 5 μmol/l PA452 or 5 μmol/l LE540, and cells were harvested for measurement of luciferase activity. Results are the mean ± SEM of two independent experiments performed by quadruple. * $P < 0.05$ vs. control cells; # $P < 0.05$ vs. cells treated with 13-*cis*-RA alone.

PGIS protein expression, suggesting that RA increases PGIS in ECs through a transcriptional mechanism. In addition, in transient transfection assays using a construct containing approximately 2 kbp of PGIS promoter, 13-*cis*-RA significantly increased PGIS promoter activity. This activity was inhibited by LE540 but not by PA452 (**Fig. 8**).

DISCUSSION

RA plays an important role in the embryonic development of the cardiovascular system (43) and influences angiogenesis (44). It also has several biological activities that promote the synthesis of vasorelaxant molecules (17) and inhibit the action of vasoconstrictor agents (19–21). In addition, it exhibits antithrombotic and antiatherogenic potential (18, 22–31). In view of all these properties, natural and synthetic retinoids are used extensively as chemopreventive, chemotherapeutic, and anti-inflammatory drugs, particularly 13-*cis*-RA. The present study shows that RA specifically increases the ability of ECs to synthesize PGI_2. The fact that 13-*cis*-RA increased the amount of 6-oxo-$PGF_{1\alpha}$ accumulated in the culture medium and in response to thrombin without altering COX isoenzyme expression and activity indicates that it exerts a specific action on the PGI_2 biosynthetic pathway. In addition, in HUVECs treated with IL-1β, which induced COX-2 but not PGIS expression (not shown), 13-*cis*-RA dramatically increased the ability of HUVECs to synthesize PGI_2. These results are in agreement with our previous reports showing that PGIS could be the limiting enzyme for PGI_2 biosynthesis, especially under inflammatory conditions, where COX activity is substantially increased due to high COX-2 expression (8, 9). Therefore, RA promotes PGIS expression, thereby increasing PGI_2 biosynthesis.

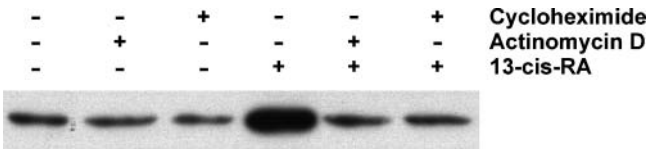

Fig. 7. Effect of actinomycin D and cycloheximide on 13-*cis*-RA-induced expression of PGIS. HUVECs were treated or not with 1 μmol/l of 13-*cis*-RA for 24 h in the presence or absence of the drugs. Afterwards, PGIS protein was determined as described in Materials and Methods. A representative, out of two with similar results, immunoblotting analysis is shown.

These findings were consistent with the fact that ASA reversed the ability of HUVECs treated with RA to inhibit platelet aggregation in both IL-1β-treated and -untreated conditions. In contrast, ASA was unable to reverse the effect of HUVECs on platelet aggregation when ECs were preincubated with IL-1β alone. These results suggest that mediators other than prostanoids were involved in the HUVEC-mediated inhibition of platelet aggregation, especially when HUVECs where pretreated with IL-1β. This fact was consistent with our previous finding that IL-1β treatment induces impaired vasoconstriction in rat aortas by several mechanisms, with a particularly relevant role of endothelial nitric oxide stores, and with a scarce implication of prostanoid biosynthesis in this effect (45). In view of the present results, it becomes apparent that the effect of RA on HUVEC-mediated inhibition of

platelet aggregation was strongly dependent on prostanoid biosynthesis with both resting and IL-1β-treated ECs, although other mechanisms cannot be ruled out.

Our present findings are consistent with a previous report showing an increase of PGI_2 in ECs treated with 13-*cis*-RA and all-*trans*-RA but not with etretinate (32), and also with a report describing an increase in PGI_2 particularly in ECs stimulated with IL-1β caused by all-*trans*-RA through a mechanism independent of COX expression (33). In the present work, we found for the first time that RA induces PGIS expression in terms of mRNA and protein, which correlated with an increase in PGIS enzyme activity.

The notion that the action of 13-*cis*-RA on PGIS was at the transcriptional level was supported by the fact that both actinomycin D (inhibits transcription) and cycloheximide (inhibits translation) totally suppressed the effect of 13-*cis*-RA on PGIS protein expression. This concept is reinforced by the results of transfection assays.

The fact that natural retinoids 9-*cis*-RA and all-*trans*-RA, which bind to RAR and RXR, stimulated PGIS expression but not 4HRP, which activates neither RAR nor RXR, supports the idea that the RA action was mediated by receptors of RA. 13-*cis*-RA binds RAR with low affinity, suggesting intracellular conversion to 9-*cis*-RA and all-*trans*-RA. Implication of RARs was demonstrated by the fact that PA452 (an RXR pan-antagonist) did not modify the effect of 13-*cis*-RA on PGIS expression, whereas LE540 (an RAR pan-antagonist) concentration-dependently inhibited the expression of PGIS elicited by 13-*cis*-RA. Implication of RAR was also confirmed by transfection experiments, in which 13-cis-RA significantly increased PGIS transcriptional activity, although to a lesser extent than mRNA levels as usually occurs in transient transfection reporter systems (46), and most importantly that it was exclusively inhibited by LE540. Our results demonstrate that RA induces PGIS expression through a transcriptional mechanism mediated by RAR. However, no putative RAR response elements (RARE) were identified in the PGIS promoter by in silico analysis. Multiple studies have highlighted the complexity of cellular responses elicited by RA and RAR. RA can exert a direct activation of nuclear receptors such as peroxisome proliferator-activated receptor δ PPARδ (47), and the RAR-dependent modulation of several transcription factors and signaling pathways involved in dissimilar cell functions has recently been described (48–51). Furthermore, because RA-responsive elements other than canonical RARE have been associated with RA-dependent effects (52), RAR-sensitive transcription factors or unidentified RA-responsive elements could be involved in the induction of PGIS by RA. Further experiments are needed to elucidate the specific factors involved in the regulation of PGIS by RA.

Prostanoids have a wide range of biological activities that are often opposed. PGI_2 is a relevant mediator that plays an important role in vascular biology, preventing thrombosis, acting as a potent vasorelaxant agent (1). It also has an inhibitory effect in carcinogenesis (53). Modulation of final specific PG synthases could be a useful approach for selective treatments. We have previously shown that the relaxing and constricting prostanoid ratio released by ECs depends on the PGIS/COX activity ratio (8, 9, 11). This study shows that RA selectively induces PGIS expression and activity, which results in an increase in the PGIS/COX activity ratio that leads to a net increment in PGI_2 biosynthesis.

Our results add further support to the concept that RA could be beneficial for vascular response, particularly in patients with cardiovascular risk, and in cancer therapy.

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