Modulation of COX-2 Expression by Statins in Human Aortic Smooth Muscle Cells

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Cyclooxygenase (COX)-2 and COX-1 play an important role in prostacyclin production in vessels and participate in maintaining vascular homeostasis. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, which is crucial in cholesterol biosynthesis. Recently, cholesterol-independent effects of statins have been described. In this study, we evaluated the effect of two inhibitors of HMG CoA reductase, mevastatin and lovastatin, on the production of prostacyclin and the expression of COX in human aortic smooth muscle cells. Treatment of cells with 25 μM mevastatin or lovastatin resulted in the induction of COX-2 and increase in prostacyclin production. Mevalonate, the direct metabolite of HMG CoA reductase, and geranylgeranyl-pyrophosphate reversed this effect. GGTI-286, a selective inhibitor of geranylgeranyltransferases, increased COX-2 expression and prostacyclin formation, thus indicating the involvement of geranylgeranylated proteins in the down-regulation of COX-2. Furthermore, Clostridium difficile toxin B, an inhibitor of the Rho GTP-binding protein family, the Rho selective inhibitor C3 transferase, and Y-27632, a selective inhibitor of the Rho-associated kinases, targets of Rho A, increased COX-2 expression whereas the activator of the Rho GTPase, the cytotoxic necrotizing factor 1, blocked interlukinin-1α-dependent COX-2 induction. These results demonstrate that statins up-regulate COX-2 expression and subsequent prostacyclin formation in human aortic smooth muscle cells in part through inhibition of Rho.

The competitive inhibitors of 3-hydroxymethylglutaryl coenzyme A (HMG CoA) reductase, also called statins, inhibit the rate-limiting step in the synthesis of cholesterol by blocking the conversion of HMG CoA to mevalonate (1). In this way statins are clinically useful for primary and secondary prevention of atherosclerosis (2, 3). However, some of their beneficial effects in therapy seem unrelated to the decrease in low density lipoprotein-cholesterol.

By modulating the initial part of the cholesterol synthesis pathway, statins decrease the level of numerous important intermediate compounds including isoprenoids that contain geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP). Isoprenoids are lipid attachments involved in posttranslational modification of some proteins such as the γ subunit of the heterotrimeric G proteins, the small G proteins Ras, and Ras-like proteins such as Rho, Rap, Rab, or Ral (4, 5). Statins can thus modulate various biological or physiological mechanisms.

Cyclooxygenases are involved in the metabolism of arachidonic acid to prostaglandins (PGs) and thromboxane (TX) A2 (6). In vascular biology, the two major products of COX are TXA2, which is mainly formed by the constitutive form of COX, COX-1 in activated platelets, and prostacyclin or PGI2, which is mainly produced in vascular cells by COX-1 and the inducible form of COX, COX-2 (7, 8). TXA2 participates in platelet aggregation and vascular contraction, whereas PGI2 acts as an anti-aggregant for platelets and a vasodilator. PGI2 plays an important role in vascular physiology as illustrated by the therapeutic effect of stable analogs of PGI2 such as iloprost (9). Platelets from patients suffering from hypercholesterolemia are characterized by hypersensitivity to various aggregating agents. Notarbartolo et al. (10) have shown that simvastatin decreased platelet aggregation in hypercholesterolemic subjects and supported a decrease in the thromboxane platelet production, although the underlying mechanism of the statin effect on platelet function remains unclear.

In this study, we demonstrated in human aortic smooth muscle cells (hASMC) that two different statins, mevastatin and lovastatin, increased COX-2 expression and PGI2 formation. We further demonstrated using selective inhibitors of geranylgeranyltransferases and modulators of Rho GTPases that geranylgeranylated proteins such as Rho seem to be responsible for COX-2 down-regulation, which is prevented by statins.

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1 The abbreviations used are: HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; GGPP, geranylgeranyl pyrophosphate; FPP, farnesyl pyrophosphate; PG, prostaglandin; TX, thromboxane; COX, cyclooxygenase; PGI2, prostacyclin; hASMC, human aortic smooth muscle cells; IL, interleukin; HIV, human immunodeficiency virus; MOPS, 4-morpholinepropanesulfonic acid; CNF, cytotoxic necrotizing factor; NOS, nitric oxide synthase.
Experimental Procedures

Materials—hASMCS and the corresponding culture media (SmGM, SmBM) were from Clonetics (Biowhittaker Europe, Verviers, Belgium). Mevastatin, lovastatin, mevalonate, squalene, farnesyl-pyrophosphate, geranylgeranylated-prophosphate, arachidonic acid, and actinomycin D were from Sigma-Aldrich. Statins in lactone form were dissolved in 0.1 M NaOH to generate the active form and the pH adjusted to 7.4 by adding 0.1 M HCl as described previously (11, 12). Prenyltransferase inhibitors (farnesyltransferase inhibitor FTI-277 and geranylgeranyltransferases inhibitor GGTI-286) were from Calbiochem (La Jolla, CA). Recombinant human IL-1α was from R&D (Minneapolis, MN). The selective inhibitor of Rho-associated kinase, Y-27632, was from TOCRIS (Bristol, UK). Reduced glutathione (GSH) was purchased from Sigma. Donkey anti-mouse IgGs conjugated-C extra) and enhanced chemiluminescence (ECL) from Amersham Pharmacia Biotech (Les Ulis, France). TRITC-conjugated-C3 transferase was used as a fusion protein with the HIV TAT protein (Clontech, CA). Anti-rabbit phosphorylated ERK antibody was from Cell Signaling Technology (Beverly, MA). Anti-rabbit-α-tubulin antibody was from Sigma. Anti-human-α-smooth muscle actin antibody was from Sigma. The secondary antibody used was goat anti-rabbit fluorescent conjugate from Life Technologies (Gaithersburg, MD). All other reagents were obtained from standard commercial sources.

Cell Culture and Incubation—hASMCS were grown in SmGM culture medium supplemented with 5% fetal bovine serum, 5 mg/l insulin, 2 μg/ml gentamycin, and 50 μg/ml amphotericin B according to Clonetics. hASMCS were used at passage 9. Cells were subcultured in 12-well plates or 60-mm dishes and cultured until subconfluence was reached. The medium was then replaced by a serum-free culture medium containing 0.5% Albumax™ for 48 h prior to the addition of statins or the other reagents. For statins, cells were further co-incubated in the same medium with or without the different isoprenoids in the absence or presence of IL-1α for 48 h. Shorter incubation periods were used for GGTI-286, FTI-277, and toxins as indicated. The concentration of ethanol or Me2SO did not exceed 0.3% and did not alter the growth of cells.

Western Blot Analysis—After incubation, hASMCS were washed twice in phosphate-buffered saline, lysed in 200 l of lysis buffer (20 mM Tris/HCl buffer, pH 7, 1 M NaCl, 1% SDS, 5% dextran sulfate, 0.1% pyrophosphate, and 100 μg/ml salmon sperm DNA. Membranes were washed twice for 10 min with 2 M sodium phosphate buffer, pH 9 for 25 M mevastatin, lovastatin, and farnesyl, respectively, compared with 35.6 ± 1.2 and 9.8 ± 0.3 units/ml for 25 M mevalonate, farnesyl-pyrophosphate, respectively. Actinomycin D was added to the cells for 24 h with IL-1α in the presence or absence of mevastatin. 5 μM actinomycin D was then added for various periods of time. Total RNA were extracted with Trizol® according to the manufacturer’s instruction. Northern blot analysis was performed as described previously (19). 10 μg of total RNA was fractionated on a formaldehyde/MOPS/EDTA/1% agarose gel and stained with ethidium bromide. RNA was transferred to nitrocellulose membranes and cross-linked by UV irradiation (Stratalinker® UV cross-linker, Stratagene). The cDNA probes used were a 2.1 kilobases of human COX-2 cDNA fragment (20) and β-actin cDNA fragment (CLONTECH Laboratories Inc, Palo Alto, CA). cDNA was labeled using a Ready to Go kit (Amerham Pharmacia Biotech) and [α-32P]dCTP (PerkinElmer Life Sciences). Membranes were first prehybridized for 4 h and then hybridized overnight at 42 °C with the COX-2 probe (106 cpm/ml) in 50 mM Tris/HCl buffer, pH 7, containing 50% formamide, 10× Derhardt’s solution, 1 M NaCl, 1% SDS, 5% dextran sulfate, 0.1% pyrophosphate, and 100 μg/ml salmon sperm DNA. Membranes were washed twice for 10 min with 2× SSC/0.1 SDS at room temperature, twice in 1× SSC/0.1 SDS at 60 °C, and once in 0.1× SSC/0.1 SDS at 60 °C. For β-actin detection, membranes were hybridized with 0.5 106 cpm/ml. Signals were quantified using a Fuji bioimaging analyzer (Fuji, Tokyo, Japan), and the ratio of COX-2/β-actin was determined.

Statistics—Results are shown as average mean ± S.E. of n different experiments. Data were analyzed by Student’s paired t test. A p value <0.05 was accepted as significant.

Results

Effects of Statins on Prostacyclin Release and COX-2 Expression—Exposure of human aortic smooth muscle cells to 25 μM mevastatin or lovastatin for 48 h led to a statistically significant increase in prostacyclin production compared with basal conditions. Cells secreted 43.2 ± 6.8 and 46.2 ± 6.7 ng/ml of 6-keto-PGF1α (n = 9) when incubated with 25 μM mevastatin andLovastatin, respectively, compared with 35.6 ± 5.4 in untreated cells (p < 0.01). An increase in prostacyclin production was also observed in the presence of 0.5 ng/ml IL-1α (405 ± 61 and 412 ± 59 ng/ml of 6-keto-PGF1α, n = 9 for 25 μM mevastatin andLovastatin, respectively, compared with 299 ± 43, n = 9, for IL-1α alone, p < 0.01).

Western blot analysis of these cells using a selective antibody for COX-2 showed an increase in COX-2 expression in cells treated with 25 μM mevastatin orLovastatin compared with untreated cells (Fig. 1A). Under these conditions, neitherLovastatin nor mevastatin induced apoptosis, as assessed by examination of Hoechst 33342-stained cells (data not shown). Treatment of the cells with increasing concentrations of statins along with 0.5 ng/ml IL-1α caused an increased induction in COX-2 expression at 25 μM statins as detected at the protein level (Fig. 1B). Under these conditions, no modification of COX-1 expression by statins alone or in the presence of IL-1α was observed (Fig. 2).
Effect of Mevalonate on Statin-induced COX-2—To determine the mechanism of COX-2 protein induction by statins, cells were first co-incubated with mevastatin or lovastatin in the presence of different compounds of the cholesterol biosynthesis pathway. We tested the effect of mevalonate, the direct HMG CoA reductase metabolite, to check whether the effect of statins is due to direct inhibition of this enzyme. We incubated cells with 25 \muM mevastatin or lovastatin together with 100 \muM \( \Delta^7 \)-mevalonate. Induction of COX-2 by statins, both in the absence or presence of IL-1\( \alpha \), was reversed by \( \Delta^7 \)-mevalonate (Fig. 3). Mevalonate alone did not modulate in a statistically significant manner the IL-1\( \alpha \)-dependent COX-2 induction (18% increase in cells treated by IL-1\( \alpha \) + mevalonate compared with IL-1\( \alpha \) alone, unpaired \( t \) test, \( n = 4 \)). Up-regulation of COX-2 by mevastatin or lovastatin was not modified after treatment with 10 \muM squalene, the late metabolite in the cholesterol synthesis pathway (data not shown), suggesting that regulation of cellular cholesterol level is not involved in this effect.

Involvement of Isoprenoids in the Regulation of COX-2—The implication of the isoprenoid compounds in the modulation of COX-2 expression both under basal conditions or after incubation with IL-1\( \alpha \) was further confirmed by testing the importance of the isoprenoids intermediates, FPP and GGPP. As shown in Fig. 4, co-treatment of cells with 10 \muM GGPP completely reversed the induction of COX-2 by mevastatin or lovastatin in the presence or absence of IL-1\( \alpha \). In contrast, 10 \muM FPP did not significantly modify the effect of statins or of IL-1\( \alpha \) (Fig. 4; 7% increase in cells treated by IL-1\( \alpha \) + FPP compared with IL-1\( \alpha \) alone, unpaired \( t \) test, \( n = 3 \)). These findings suggested to us that geranylgeranylated proteins negatively regulate COX-2 expression. To test this hypothesis further, we used GGTI-286, a recently described selective inhibitor of geranylgeranylationtransferase (21). Induction of COX-2 by GGTI-286 alone was detected at 10 \muM after 24-hour of incubation. In the presence of IL-1\( \alpha \), the increase in COX-2 expression was clear at 5 and 10 \muM (Fig. 5). 10 \muM GGTI-286 also increased PGI\(_2\) production in a statistically significant manner (50.6 \pm 10.9 compared with 12.3 \pm 2.8 ng/ml of 6-keto-PGF\(_1\alpha\), for untreated cells, \( n = 4, p < 0.03 \)). We further used a selective inhibitor of farnesyltransferases, FTI-277, to check whether farnesylation was involved in COX-2 expression (22). Since it has been reported that FTI-277 had low IC\(_{50}\) for inhibiting farnesyltransferases (IC\(_{50}\) = 20 nM compared with the IC\(_{50}\) for GGTI-286 = 3 \muM), we first checked the effect of low concentrations of FTI-277 (0.3–3 \muM) and showed no modification of COX-2 expression nor prostacyclin formation. In separate experiments, we verified as well that further treatment of cells with 10 \muM FTI-277 did not alter COX-2 expression (Fig. 5).

Treatment of Cells with Mevastatin or GGTI-286 Increases COX-2 mRNA Level—We next performed Northern blot analysis of COX-2 mRNA to demonstrate that modulation of COX-2 protein by statins or GGTI-286 was also obtained at the RNA level. Northern blot analysis was carried out with 25 \muM mevastatin, the concentration required to induce COX-2 protein. Cells were incubated in 60-mm plates for 24 and 36 h with IL-1\( \alpha \) in the absence or presence of mevastatin. Incubation of cells with mevastatin in the presence of IL-1\( \alpha \) resulted in a 3.2- and 5.8-fold increase in COX-2 mRNA level at 24- and 36-h incubation times, respectively, compared with cells treated with IL-1\( \alpha \) alone (Fig. 6A).

We next compared the stability of the COX-2 mRNA of IL-1\( \alpha \)-treated cells in the presence or absence of mevastatin. Cells were treated with 0.5 \muM IL-1\( \alpha \) in the presence or absence of 25 \muM mevastatin for 24 h. 5 \muM actinomycin D was then added to block transcription, and total RNA was extracted after an incubation period of 0.5, 1, 2, 4, or 8 h. Fig. 6B reveals little difference in the mRNA stability of COX-2 IL-1\( \alpha \) and IL-1\( \alpha \) + mevastatin-treated cells with half-lives of 4.7 and 5.5 h, respectively.

We finally tested the effect of GGTI-286 on COX-2 mRNA. Cells were incubated with 10 \muM GGTI-286 for 8, 12, and 18 h. Fig. 7 shows an increase in COX-2 mRNA levels by GGTI-286
at 8 and 12 h followed by a decrease at 18 h.

Effect of Modulators of Rho GTPases on Prostacyclin Formation and COX-2 Expression—The Rho family of GTP-binding proteins contains many geranylgeranylated proteins that play an important role in cell adhesion, actin dynamics, or regulation of gene transcription and includes Rho, Rac, and Cdc42 proteins (23, 24). To determine whether the inhibition of these proteins mediates the effects observed by statins, we incubated the cells in the presence or absence of IL-1α/H9251 or C. difficile toxin B (toxin B), an inhibitor of the different Rho GTPases (25). Treatment of hASMC for 6 h with 2 nM of toxin B induced COX-2 as shown in Fig. 8A. Toxin B also increased COX-2 mRNA (Fig. 8B). In parallel, we tested the effect of E. coli cytotoxic necrotizing factor 1 (CNF1), a toxin reported to activate Rho GTPase proteins by preventing Rho GTP hydrolysis (26, 27). Treatment of cells with 30 nM CNF1 inhibited induction of COX-2 by IL-1α (Fig. 8C). Both toxins modulated PGI2. Toxin B increased PGI2, whereas CNF1 inhibited IL-1α-dependent formation (Table I).

We further tested the effect of C. botulinum C3 transferase, a selective inhibitor of Rho A and C proteins, on COX-2 expression and prostacyclin formation. We used a fusion protein with the TAT protein of HIV with the C. botulinum C3 transferase to allow rapid introduction of the protein into cells (14). Treatment of the cells with 20 μg/ml of TAT-C3 transferase resulted in an induction of COX-2 with a maximal expression at 6 h (Fig. 9A). In the same samples, PGI2 was statistically increased (Table I). Finally, since the serine/threonine kinases ROCK are among the identified targets of Rho, we tested the effect of a selective inhibitor of the ROCK I and II, Y-27632, on COX-2 expression (28). Cells were incubated with 10 μM Y-27632 for 3, 6, and 24 h. This treatment resulted in the increase in COX-2 expression at 6 and 24 h as shown in Fig. 9B.

DISCUSSION

We have shown for the first time that inhibitors of HMG CoA reductase, lovastatin and mevastatin, increase the expression.
Negative Regulation of COX-2 by Geranylgeranylated Proteins and Rho

TABLE I
Prostacyclin formation in response to inhibitors or activators of Rho GTPases

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-1αa</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>10.1 ± 1 (n = 4)</td>
<td>38 ± 2.2 (n = 4)</td>
<td>34 ± 1.6 (n = 3)</td>
<td>20.7 ± 1.3 (n = 4)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td></td>
</tr>
<tr>
<td>Toxin B (2 nM)</td>
<td>31.4 ± 4.6 (n = 4)</td>
<td>44.3 ± 4.5 (n = 4)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>20.7 ± 1.3 (n = 4)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td></td>
</tr>
<tr>
<td>CNF1 (30 nM)</td>
<td>5.3 ± 0.8 (n = 3)</td>
<td>20.7 ± 1.3 (n = 4)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>20.7 ± 1.3 (n = 4)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>7.4 ± 1 (n = 3)</td>
<td>20.7 ± 1.3 (n = 4)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>20.7 ± 1.3 (n = 4)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>13.1 ± 1.6 (n = 3)</td>
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</tr>
<tr>
<td>TAT-C3 transferase (20 μg/ml)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>20.7 ± 1.3 (n = 4)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>20.7 ± 1.3 (n = 4)</td>
<td>13.1 ± 1.6 (n = 3)</td>
<td>13.1 ± 1.6 (n = 3)</td>
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a Values indicate the amount of 6-keto-PGF1α.

b p < 0.02 versus cells with no treatment.

c p < 0.01 versus IL-1α-stimulated with no treatment.

d p < 0.05, versus cells with no treatment.

of COX-2 in human aortic smooth muscle cells. We confirmed the implication of the mevalonate pathway and isoprenoids in the negative modulation of the expression of COX-2 by demonstrating that the direct metabolite of HMG CoA reductase, mevalonate and the isoprenoid, GGPP, reversed the induction of COX-2 by statins and that GGTI-286, the inhibitor of geranylgeranylated proteins, induced COX-2 expression in human aortic smooth muscle cells. Rho GTPases are geranylgeranylated proteins important in cell migration, contraction, cell shape, adhesion, and gene expression (23, 24). It has been shown that one of these proteins, Rho, is linked to the activation, contraction, or proliferation of vascular cells (47). Moreover, Rho (A or C) controls the expression of different proteins in vessels including NOS-II (48), NOS-III (45), TGFβ (49), and pre-pro-endothelin-1 (ET-1) expression (50, 51). In our system, C. difficile toxin B and CNF1, selective inhibitor and activator of all Rho GTPases, respectively, affected COX-2 expression either at the basal level or after activation by IL-1α. This suggests that Rho GTPases participate in COX-2 regulation. The further demonstration that both C. botulinum C3 transferase and Y-27632, the selective inhibitors of Rho and ROCK, respectively, induced COX-2 expression stressed the role of these proteins in the negative regulation of COX-2 expression and PGI2 formation and that these geranylgeranylated small G proteins are one of the targets of statins.

Since it has been described that induction of COX-2 in some cells could participate in the apoptosis process, we tested whether statins induced apoptosis in HASMC. Although it has been described that some statins could induce apoptosis or sensitize hASMC to death receptor-induced apoptosis (52, 53), in vessels were considered as beneficial in participating for instance in physiological functions or anti-inflammatory processes. Although COX-2 expression is detected in atherosclerotic plaque where it is distributed in the intima and media (35) and urinary prostacyclin derivatives increased in patients with atherosclerotic plaques (36), the consequence of endothelial and smooth muscle cell increase in COX-2 expression is still a matter of debate. An increase in urinary prostacyclin derivatives has been shown recently in two murine models of atherosclerosis, ApoE-deficient mice and low-density lipoprotein receptor-deficient mice on a high fat diet (37, 38). Selective inhibition of COX-2 failed to decrease the extent of atherosclerosis in these models suggesting that COX-2, although expressed in the atherosclerotic lesions, does not participate in its progression (38). Little is known about the roles of PGI2 and PGE2 on vessels. PGI2 and PGE2 inhibit vascular proliferation and cell-cell interaction (39, 40). In normal volunteers, COX-2 contributes to the formation of PGI2 in vivo since selective inhibitors of COX-2 decreased its systemic formation (8). Laminar shear forces have also been demonstrated to increase COX-2 expression in cultured endothelial cells (41). As suggested recently by FitzGerald and Patrano, PGI2 may be part of a homeostatic defense mechanism limiting the consequences of platelet activation in vivo (42). The further demonstration that both C. difficile toxin B and CNF1, selective inhibitor and activator of all Rho GTPases, respectively, affected COX-2 expression either at the basal level or after activation by IL-1α. This suggests that Rho GTPases participate in COX-2 regulation. The further demonstration that both C. botulinum C3 transferase and Y-27632, the selective inhibitors of Rho and ROCK, respectively, induced COX-2 expression stressed the role of these proteins in the negative regulation of COX-2 expression and PGI2 formation and that these geranylgeranylated small G proteins are one of the targets of statins.

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careful examination of Hoechst 33342-stained cells showed no evidence of cell death under our conditions. Observation under phase contrast, however, evidenced some morphological changes with a slight increase in rounded cells that appear to become less adherent.

The effect of statins on COX-2 expression was also noted in the presence of IL-1α, a cytokine important in the atherosclerotic vascular wall and largely described to activate COX-2 (17, 20, 54). We treated cells with IL-1α first to verify the normal induction of COX-2 in hASMC as reported previously (55, 56) and to further check whether statins could modify COX-2 induction. We showed that these two statins increased COX-2 expression in the presence of IL-1α.

The mechanism by which inhibition of Rho increases COX-2 expression is not clear. Rac1 has been shown to down-regulate Rho activation (57). Signaling through IL-1 receptors implicates activation of Rac, which in turn is involved in the activation p38 MAP kinases and NF-κB, both of which are important in the regulation of COX-2 expression (23, 58, 59). The balance between these two GTPases might be important in determining gene expression, i.e. COX-2. Up-regulation of COX-2 is also induced by protein kinase A activation in response to prostanoids for example in different cell types including macrophages, vascular cells, and hepatic stellate cells (60–62). In view of the results presented here, it may be of interest that protein kinase A is known to phosphorylate and inactivate Rho A (63) and induce COX-2 (61, 62). Further investigation is required to understand whether or not these mechanisms are involved in the regulation of COX-2 expression by statins and if they are similar in untreated and IL-1α-stimulated cells.

In the present study, statin did not modify the half-life of the mRNA of COX-2 in IL-1α-activated cells indicating that transcriptional regulation is essentially implicated in the induction of COX-2 by statins. Our results are similar to those reported for NOS-II where regulation at the transcriptional level has been demonstrated in response to Toxin B, C3 transferases (48), and Y-27632 (64) but different from those indicating that statins and Rho GTPase inhibitors could increase the stability of the NOS-III mRNA (45, 46). Recently, Slice et al. have demonstrated in NIH 3T3 cells that Goαi is able to increase COX-2 promoter activity through activation of Rho A (65, 66). These data contrast with ours, which show an inhibition of COX-2 promoter activity through activation of Rho A (65, 66). Signaling through IL-1 receptors implicates activation of Rac1, p38 MAP kinases and NF-κB (57). Signaling through IL-1 receptors implies activation of Rac1, p38 MAP kinases and NF-κB (57).

In conclusion, the present study shows that statins and Rho GTPase inhibitors could increase the expression of COX-2 in human vascular smooth muscle cells. Inhibition of Rho activity in vessels may be important to restore vascular function and could account for the cholesterol-unrelated effects of statins.

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