Peroxisome proliferator-activated Receptor δ Activators Induce IL-8 Expression in Nonstimulated Endothelial Cells in a Transcriptional and Posttranscriptional Manner

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that are implicated in the regulation of lipid and glucose homeostasis. PPAR agonists have been shown to control inflammatory processes, in part by inhibiting distinct proinflammatory genes (e.g. IL-1β and IFN-γ). IL-8 is a member of the proinflammatory chemokine family that is important for various functions, such as mediating the adhesion of eosinophilic granulocytes onto endothelial cells. The influence of PPARδ activators on the expression of IL-8 in noninduced quiescent endothelial cells is unclear. Therefore, we explored the influence of PPARδ activators on the expression of IL-8 in nonstimulated endothelial cells. PPARδ agonists induce IL-8 expression in human umbilical vein endothelial cells. This induction is demonstrated at the level of both protein and mRNA expression. Transcriptional activation studies using IL-8 reporter gene constructs and DNA binding assays revealed that PPARδ agonists mediated their effects via an NFκB binding site. It is well known that IL-8 is also regulated by mRNA stability. To provide further evidence for this concept, we performed mRNA stability assays and found that PPARδ agonists induce the mRNA stability of IL-8. In addition, we showed that PPARδ agonists induce the phosphorylation of ERK1/2 and p38, which are known to be involved in the increase of mRNA stability. The inhibition of these MAPK signaling pathways resulted in a significant suppression of the induced IL-8 expression and the reduced mRNA stability. Therefore, our data provide the first evidence that PPARδ induces IL-8 expression in nonstimulated endothelial cells via transcriptional as well as posttranscriptional mechanisms.

Peroxisome proliferator-activated receptors (PPARs)² are members of the nuclear receptor/ligand-activated transcription factors superfamily and consist of three different subtypes: PPARα, PPARδ, and PPARγ. The role of these receptors was originally thought to be restricted to lipid and lipoprotein metabolism, glucose homeostasis, and cellular differentiation (1, 2). PPARs are activated by natural ligands, such as eicosanoids and fatty acids. In addition, synthetic antidiabetic thiazolidinediones and lipid-lowering fibrates have been shown to act as activators of PPARγ and PPARα, respectively (3, 4). To date, PPARδ, which is expressed in almost all tissues, is the subtype that still remains an interesting target for new pharmaceutical drugs.

New evidence suggests that PPARδ plays a crucial role in the regulation of differentiation, cell growth, and the metabolism of lipids and glucose (5, 6). With respect to the development of novel drugs, the effects and side effects of PPARδ agonists are therefore of great importance.

In the last few years, knowledge concerning the impact of PPARδ in endothelial cell function has increased. During inflammation, proinflammatory stimuli such as LPS, TNFα, or IL-1β lead to a phenotypic change of the quiescent endothelium by induction of proinflammatory factors, such as IL-6, IL-8, MCP-1, or adhesion molecules, such as ICAM-1 or VCAM-1. Recently, Rival et al. demonstrated that the PPARδ activator L165041 suppresses TNFα-induced VCAM-1 and MCP-1 expression (7). Similar results were demonstrated by Fan et al., revealing that the PPARδ agonist GW501516 suppresses IL-1β-induced VCAM-1 and E-selectin expression in human umbilical vein endothelial cells (HUVECs) (8). Piqueras et al. demonstrated that PPARδ inhibits leukocyte recruitment and cell adhesion molecule expression (9). Recently, Liang et al. showed that the PPARδ agonist -165041 suppresses C-reactive protein-induced IL-6 expression (10). The expression profile of both cytokines during treatment with various PPARδ agonist concentrations in the endothelial quiescent status has yet to be analyzed. Nonetheless, these studies showed that PPARδ agonists could suppress the inflammatory processes in activated endothelial cells. The impact of PPAR agonists on the normally quiescent endothelium, however, remains to be elucidated. This could be of significant importance due to the possible broad range of applications of PPARδ agonists in various diseases, such as chronic inflammation, glucose metabolism, dyslipidemia, obesity, and cancer therapy, to mention only a small number of possible medical applications.

During the process of inflammation, proinflammatory cytokines are produced by various cell types. IL-8 is one of these important cytokines. It is secreted at very low levels from noninduced cells, although the secretion is increased rapidly by a
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A wide range of stimuli, such as TNF and IL-1 or bacterial products, such as LPS (11). There are two main methods of regulation: first, by transcriptional activation of the genes by NFκB, and second, by stabilization of the mRNA by the p38 MAPK pathway (12–16).

Besides the important proinflammatory action, IL-8 plays an important role in the tumor microenvironment. Secretion of IL-8 from cancer cells can aggravate the proliferation and survival of cancer cells, in part by autocrine signaling pathways (17). In addition, tumor-derived IL-8 can activate endothelial cells to promote angiogenesis. IL-8, however, is secreted not only by tumor cells, but also by endothelial cells, thereby enhancing endothelial cell survival, proliferation, and angiogenesis (18).

The present study investigated the influence of PPARδ activators on the production, secretion, and regulation of IL-8 in nonstimulated endothelial cells, revealing an induction of IL-8 expression conveyed by transcriptional, NFκB-dependent, and posttranscriptional mechanisms involving mRNA stability.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Recombinant human TNFα and IL-1β were purchased from R&D Systems (Minneapolis, MN). L165041, GW501546, PD98059, SB203580, LY294002, and actinomycin D were obtained from Sigma-Aldrich.

**Cell Culture**—HUVECs were purchased from PromoCell (Heidelberg, Germany) and were cultured until the fifth passage at 37 °C and 5% CO2 in endothelial cell growth medium (Cambrex, East Rutherford, NJ).

**Enzyme-linked Immunosorbent Assay (ELISA)**—The concentrations of IL-8 and IL-6 in cell culture supernatants were determined by ELISA. Commercially available ELISA kits (R&D Systems) for the quantification of IL-8 and IL-6 were used as described in the manufacturer’s instructions.

**Western Blot Analysis**—Whole cell protein and cytoplasmic and nuclear protein extracts were prepared as described previously (19). Membranes were incubated with the indicated primary antibodies. Antibodies were as follows: anti-p-p65, anti-IκB-α, anti-phospho-IκB-α anti-p65, anti-ERK1/2, anti-phospho-ERK1/2, anti-phospho-p38, anti-p38, anti-phospho-Akt (Ser473) anti-phospho-Akt (Thr308), anti-Akt, and anti-SP1 from Santa Cruz Biotechnology (Heidelberg, Germany) and anti-tubulin from LabVision (Fremont, CA). Primary antibody application was followed by incubation with horseradish peroxidase-conjugated secondary antibodies (anti-mouse and anti-rabbit IgG, Amersham Biosciences; anti-goat, Dako, Glostrup, Denmark). Blots were developed using an enhanced chemiluminescence detection system (ECL) (Amersham Biosciences), according to the manufacturer’s instructions.

**Cytotoxicity Assay**—The cytotoxic potential of L165041 was determined using the Cytotoxicity Detection kit (lactate dehydrogenase) from Roche, according to the manufacturer’s instructions. Twenty-four hours after seeding, cells were exposed to L165041 for 24 h at the indicated concentrations.

**RNA Extraction and RT-PCR**—RT-PCR analyses were performed using total RNA (150 ng) extracted from subconfluent cell cultures. Total cellular mRNA was isolated by the RNeasy Mini Procedure (Qiagen, Hilden, Germany) after DNase digestion. RT-PCR analyses for IL-6, IL-8, and GAPDH were performed using the One-Step RT-PCR kit (Qiagen). PCR products were resolved by 1–2% agarose gel electrophoresis, and ethidium bromide-stained bands were visualized with an ultraviolet transilluminator. A densitometric analysis was used to quantify band intensities using the public domain Java image-processing program ImageJ (v1.29s). Optical densities (ODs) of the IL-8 bands were corrected for loading differences based on the corresponding GAPDH bands. The primer sets for IL-8, IL-6, and GAPDH have been published previously (20).

**Transient Transfection and Analysis of Reporter Gene Expression**—HUVECs (1.0 × 105 cells/well in 12-well plates) were transfected with 0.5 μg of the appropriate firefly luciferase construct and 0.1 μg of phRG-TK vector (Promega, Madison, WI) using the SuperFect transfection reagent (Qiagen). Human IL-8 reporter gene constructs were generously provided by Dr. Naofumi Mukaida (Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, Japan). Twenty-four hours after transfection, cells were treated with vehicle (0.3% dimethyl sulfoxide) or with L165041 for 24 h. Luciferase activities were measured with the Dual-Luciferase Reporter Assay system (Promega).

**Preparation of Nuclear Extracts and Gel Mobility Shift Analysis**—HUVECs were treated with vehicle or incubated with L165041 for 60 min. Nuclear proteins were extracted as described previously (21). DNA binding reactions were performed with or without excess unlabeled NFκB consensus oligonucleotide (Promega) and p65 antibody (Santa Cruz Biotechnology). The electromobility shift analysis (EMSA) was performed as described previously (21).

**Immunolocalization of p65 NFκB**—For immunolocalization studies, HUVECs were plated onto 8-well chamber slides (Lab-Tek, Christchurch, New Zealand) at a density of 80%/well. The cells were grown in endothelial cell basal medium with 5% FCS for 24 h, fixed in 4% paraformaldehyde at room temperature for 10 min, and permeabilized with 0.1% Triton X-100 for 5 min at room temperature. Permeabilized cells were rinsed three times with PBS and incubated in blocking solution (1% bovine serum albumin/PBS) for 30 min at room temperature to remove non-specific binding of the antibody. All subsequent steps were carried out at room temperature, and cells were rinsed three times in 1% bovine serum albumin/PBS between each of the steps. RelA/p65 was detected using a rabbit polyclonal anti-RelA/p65 antibody (C-20; Santa Cruz Biotechnology) and an Alexa Fluor 594-conjugated goat anti-rabbit IgG secondary antibody. The slides were mounted in Vectashield immunofluorescence mounting medium (Vector Laboratories) and viewed with fluorescence microscopy. Cells were counterstained for nuclei with Hoechst staining.

**Statistical Analyses**—The data are expressed as means ± S.D. from at least three independent experiments. Statistical analyses were performed using Student’s t test.

**RESULTS**

**PPARδ Agonists Induce IL-8 Expression in Nonstimulated Endothelial Cells**—To evaluate the influence of the PPARδ agonists L165041 and GW501516 on quiescent nonstimulated endothelial cells, ELISA analyses were performed. Interest-
ingly, these experiments demonstrated a time- and concentra-
tion-dependent induction of IL-8 expression in nonstimulated
HUVECs treated with L165041 and GW501516, respectively
(Fig. 1). The results were comparable for both PPAR
agonists, indicating that the effects are specific to PPAR. To analyze
whether the concentrations used have any cytotoxic effects, we
performed cytotoxicity lactate dehydrogenase assays with our
model compound, L165041, and found no relevant cytotoxic
effects (supplemental Fig. 1). In further experiments we could
demonstrate comparable results analyzing the expression of
IL-6 in HUVEC treated with L165041 and GW501516, respec-
tively (supplemental Fig. 2).

**PPARδ Agonists Up-regulate the Steady State of IL-8 mRNA**—To
determine whether activation of PPARδ plays a role in the
steady state of IL-8 mRNA expression, we performed RT-PCR
analysis after treatment with the PPARδ agonist L165041. Con-
sistent with our protein expression data, application of the ago-
nist induced IL-8 mRNA expression in a concentration- and
time-dependent manner (Fig. 2). Comparable results could be
demonstrated for IL-6 (supplemental Fig. 3). The expression of
IL-8 can be regulated in a transcriptional and a posttranscrip-
tional manner. We therefore first examined a possible tran-
scriptional mechanism of control.

**PPARδ Agonists Up-regulate IL-8 Promoter Activity in an
NFκB-dependent Manner**—To analyze the underlying molecu-
lar mechanisms that mediate PPARδ-conveyed induction of
IL-8 mRNA expression, luciferase reporter assays were
employed. Luciferase reporter constructs containing the 5’-re-

gion of the IL-8 promoter and a series of deletion constructs
were transiently transfected into vehicle- and PPARδ agonist-
treated HUVECs.

Analysis of the expression of luciferase in vehicle and PPARδ
agonist-treated cells revealed an approximate 2.3-fold induc-
tion of basal luciferase activity with the wild type IL-8 construct
(Fig. 3a). Deletion of the AP1 site did not significantly influence
IL-8 promoter activity, whereas the deletion of NFκB led to a
complete loss (Fig. 3a). Recently, the IL-8 promoter was shown
to contain a PPRE site. To exclude the possibility that this site is
also an important point of action of PPARδ agonists, we ana-
alyzed an IL-8 promoter construct with a deletion of the PPRE
site. Interestingly, the deletion did not significantly influence
luciferase promoter activity during treatment with PPARδ ago-

![Figure 1](https://example.com/fig1.png)

**FIGURE 1.** Effects of GW501516 and L165041 on the IL-8 expression in supernatants of HUVEC. IL-8 protein content was assayed in culture supernatants by IL-8 ELISA (R&D Systems) according to the manufacturer’s instructions. a and b, HUVECs were left untreated (solvent only, 0.1% dimethyl sulfoxide) or were treated with L165041 (a) and GW501516 (b) for 24 h at the indicated concentrations. c, HUVECs were left untreated (solvent only, 0.1% dimethyl sulfoxide) or were treated with L165041 for the indicated times. Mean values from triplicate experiments are depicted ± S.E. (error bars). *p < 0.05 was considered significant.

![Figure 2](https://example.com/fig2.png)

**FIGURE 2.** IL-8 mRNA expression is induced by PPARδ agonists. RT-PCR analyses of total mRNA extracted from HUVECs that were treated with vehicle or L165041 for varying times (a) or varying concentrations (b) are shown. Results were confirmed in three independent sets of experiments.
nists, demonstrating that the inducing effect of IL-8 is conveyed only by the NFκB site (Fig. 3a).

NFκB-dependent Binding to the IL-8 Promoter Is Induced by PPARδ Agonist Treatment—To determine the nuclear factors that bind to the NFκB binding site, we performed EMSAs using nuclear extracts from HUVECs and a 32P-labeled NFκB oligonucleotide probe. In untreated HUVECs, a distinct complex was observed to bind and shift the migration pattern of the oligonucleotide (Fig. 3b, lane 1). A significant increase in DNA binding activity, however, was observed in the lysates of cells treated with L165041 (Fig. 3b, lane 2) and in cells treated with TNFα as a control (Fig. 3b, lane 6). A supershift was observed upon addition of the p65 antibody (Fig. 3b, lanes 4 and 5 and lanes 9 and 10), confirming that p65 binds to the IL-8 promoter. Competition assays using excess unlabeled oligonucleotides supported the assumption that nuclear proteins bind to the IL-8 promoter sequence in an NFκB-specific manner (Fig. 3b, lanes 3 and 8).

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FIGURE 3. PPARδ agonists induce IL-8 transcription in an NFκB-dependent manner. a, analyses of deletional IL-8 luciferase (Luc) reporter constructs in HUVECs. The Luc activities are expressed as x-fold activity compared with untreated controls (mean ± S.D. (error bars) of five independent triplicate assays). b, representative EMSAs using nuclear extracts of vehicle- and L165041-treated (50 μmol/liter, 1 h) HUVECs. Competition with unlabeled double-stranded NFκB consensus oligonucleotides is shown in lanes 3 and 8 (0.35 μmol/liter). A representative autoradiography from three independent experiments is presented. Supershift analyses were performed by addition of a p65-specific antibody (lanes 4 and 5 and lanes 9 and 10; 100 ng/μl). Arrows on the left indicate NFκB-containing transcription factor-binding complexes. Comparable results were obtained from three independent experiments.

FIGURE 4. PPARδ agonists induce p65 phosphorylation and nuclear translocation. Representative Western blot analysis of HUVECs that were treated with vehicle or L165041 (50 μM) for the indicated times is shown. Protein was separated by 8% SDS-PAGE, and proteins were detected by enhanced chemiluminescence. All comparable results were obtained from three independent experiments. a, total cellular protein showing the amount of phospho-p65 and p65. b, nuclear protein showing the amount of p65. Sp1 protein served as a control. c, cytoplasmic protein showing the amount of IκBα. Tubulin protein served as a control. d, representative immunofluorescent analysis of p65 in HUVECs that were treated with vehicle, L165041 (50 μM), or TNFα (20 ng/ml). Comparable results were obtained from three independent experiments.
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**Figure 5. IL-8 mRNA half-life is induced by PPARδ agonists.** HUVECs were incubated with vehicle or L165041 (50 nM) for 1 h, followed by incubation with fresh medium containing actinomycin D (10 μg/ml) for 0, 1, and 4 h. RT-PCR analyses for IL-8/GAPDH of total RNA extracted from subconfluent cell cultures were performed. The PCR products were separated by 2% agarose gel electrophoresis, and ethidium bromide-stained bands were visualized using an ultraviolet transilluminator. IL-8 bands were quantified by densitometric scanning, the results of which were normalized to amounts of GAPDH mRNA. Comparable results were obtained from three independent experiments.

**Discussion**

In the last decade, knowledge concerning PPAR activators has increased rapidly. The research community has primarily focused on PPARα and PPARγ agonists, which are already in clinical use. In the last 2 years, however, PPARδ agonists have raised significant interest, even though the research concerning this nuclear receptor and its ligands has been limited. Emerging evidence has demonstrated that PPARδ plays an important role in the regulation of differentiation, cell growth, and lipid and glucose metabolism (5, 22). Recently, the impact of PPARδ agonists in endothelial cell function and angiogenesis has been addressed (24). These results highlight the growing importance and the possible expanding area of employing PPARδ agonists for clinical use.

In the current study, we demonstrated that PPARδ agonists effectively induced IL-8 expression in nonstimulated quiescent endothelial cells. We identified two responsible mechanisms underlying this effect: a transcriptional mechanism conveyed by NFκB and a posttranscriptional mechanism mediated by a p38- and ERK1/2-dependent increase in mRNA stability. The phosphorylation of Akt seems to have no significant influence on IL-8 expression.

These results could also be bolstered by mRNA analysis. To analyze whether p38 and ERK1/2 influence PPARδ-induced IL-8 mRNA expression, we blocked the ERK1/2 and p38 signaling pathways using the specific inhibitors PD98059 and SB203580, respectively. These treatments led to partial neutralization of PPARδ agonist-induced IL-8 mRNA expression (Fig. 6c).

Inhibition of MAPK Signaling Pathways p38 and ERK1/2 Results in Decreased IL-8 mRNA Stability—To provide evidence that the MAPK signaling pathways influence PPARδ-induced IL-8 mRNA expression and stability, we performed mRNA stability assays while inhibiting the MAPK signaling pathways. We demonstrated that blockade of the ERK1/2 and p38 signaling pathways using PD98059 and SB203580, respectively, resulted in a reduced mRNA stability compared with the

PPARδ agonist only (Fig. 7).

PPARδ Agonists Induce IL-8 mRNA Stability—IL-8 expression is not only controlled on the transcriptional level, but can be also influenced by the stability of IL-8 mRNA. Therefore, we next analyzed IL-8 mRNA stability in solvent- and PPARδ agonist-treated endothelial cells. Surprisingly, a significant increase in IL-8 mRNA stability was found (Fig. 5), providing an additional mechanism for the induction of IL-8 expression.

PPARδ Agonists Induce ERK1/2, p38, and Akt Phosphorylation—It is known that the p38 and ERK1/2 MAPK signaling pathways are involved in the regulation of the mRNA stability of IL-8. We therefore analyzed the phosphorylation status of MAPK ERK1/2 and p38 as well as Akt by Western blot analysis. Interestingly, the MAPK ERK1/2 and p38 were quickly phosphorylated after treatment with PPARδ agonists, comparable results could be demonstrated for Akt (Fig. 6a). To analyze whether these signaling pathways influence PPARδ-induced IL-8 protein expression, we blocked the ERK1/2, p38, and Akt signaling pathways using the specific inhibitors PD98059, SB203580, and LY294002, respectively. Except for Akt signaling, these treatments led to partial and significant neutralization of PPARδ agonist-induced IL-8 expression, demonstrating the importance of the p38 and ERK1/2 MAPK signaling pathways in PPARδ-mediated IL-8 expression (Fig. 6b). The phosphorylation of Akt seems to have no significant influence on IL-8 expression.

Immunochemistry staining of NFκB p65 in HUVECs treated with PPARδ agonists (Fig. 4d). The increase in p65 staining was comparable with the influence of TNFα.

**Figure 6. PPARδ agonist-induced IL-8 protein expression**—(A) Western blot analysis of PPARδ agonist-treated endothelial cells. Surprisingly, a significant increase in NFκB p65 staining was observed in HUVECs treated with PPARδ agonists. (B) The phosphorylation of MAPK ERK1/2 and p38 as well as Akt by Western blot analysis. Interestingly, the MAPK ERK1/2 and p38 were quickly phosphorylated after treatment with PPARδ agonists, comparable results could be demonstrated for Akt (Fig. 6a). To analyze whether these signaling pathways influence PPARδ-induced IL-8 protein expression, we blocked the ERK1/2, p38, and Akt signaling pathways using the specific inhibitors PD98059, SB203580, and LY294002, respectively. Except for Akt signaling, these treatments led to partial and significant neutralization of PPARδ agonist-induced IL-8 expression, demonstrating the importance of the p38 and ERK1/2 MAPK signaling pathways in PPARδ-mediated IL-8 expression (Fig. 6b). The phosphorylation of Akt seems to have no significant influence on IL-8 expression.

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Important proinflammatory cytokines, such as TNFα or IL-1β, as well as LPS are known to induce IL-8 expression via NFκB-containing transcription factor complexes targeting the IL-8 promoter (25–27). To our knowledge, this is the first study to demonstrate that PPARδ agonists lead to a concentration-dependent phosphorylation and nuclear translocation of p65 in nonstimulated endothelial cells, thereby increasing NFκB DNA binding. In addition, we found that the PPRE element at the IL-8 promoter did not significantly influence IL-8 promoter activity. These data are contrary to those obtained for stimulated endothelial cells. Liang et al. convincingly demonstrated that C-reactive protein in stimulated endothelial cells suppresses NFκB phosphorylation when the cells are treated with the PPAR agonist GW501516, thereby resulting in reduced IL-6 and IL-8 expression (10). A similar result was demonstrated by Rodriguez-Calvo et al. for LPS-stimulated adipocytes, in which reduced IL-6 expression and NFκB DNA binding activity were detected after the previously LPS-stimulated adipocytes were treated with the PPARδ agonist GW501516 (28). The same group also showed an increase of IL-6 mRNA and protein in the control group treated only with GW501516 at very low PPARδ concentrations (28). These results were bolstered by our analysis concerning the expression of IL-6 in endothelial cells. In addition, Rival et al. demonstrated that the PPARδ activator L165041 inhibits TNFα-induced VCAM-1 and MCP1 expression at concentrations up to 100 μM (7). The important difference between those studies and the current work is that nonstimulated quiescent endothelial cells were used in the present study, revealing the possibility of a dual function of the PPARδ agonist in endothelial cells that is dependent on the activation status of the cell. This novel information may be important, because this would implicate a differentiated use of these compounds regarding their application in the treatment of various diseases. It is possible that this dual action also explains the different results concerning the influence of PPARδ activators in the treatment and development of various tumor entities. Recently, Bility et al. demonstrated that PPARδ agonists inhibit chemically induced skin carcinogenesis, whereas Kim et al. demonstrated that chemically induced skin carcinogenesis is not influenced by PPARδ (29, 30). Han et al. demonstrated that PPARδ agonists induce human lung cancer cell proliferation, whereas He et al. found that they do not influence lung cancer cell proliferation (31, 32). It is quite possible that the activation status of the cancer cells or the cancer micro-

![FIGURE 6. PPARδ agonists activate MAPK signaling pathways.](image-url)

**a**, representative Western blot analysis of HUVECs that were treated with vehicle or L165041 (50 μM) for the indicated times up to 60 min. Total cellular protein was separated by 8% SDS-PAGE. ERK1/2, phospho-ERK1/2, p38, phospho-p38, Akt and phospho-Akt (Thr308 and Ser473) protein were detected by enhanced chemiluminescence. Comparable results were obtained from three independent experiments. **b**, effects of MAPK signaling pathway inhibition on L165041-induced IL-8 expression in supernatants of HUVEC. HUVECs were pretreated for 30 min with 30 μM PD98059, 1 μM SB203580, and 10 μM LY294002 and then exposed to 50 μM L165041 for 6 h. The amount of IL-8 released by HUVECs into the culture medium was assessed by ELISA. Values are expressed as mean ± S.E. (error bars) (n = 4). *, p < 0.05 was considered significant. **c**, effects of MAPK signaling pathway inhibition on L165041-induced IL-8 mRNA expression in HUVECs. HUVECs were pretreated 30 min with 30 μM PD98059 and 1 μM SB203580 and then exposed to 50 μM L165041 for 6 h. The amount of IL-8 mRNA expression by HUVEC was assessed by RT-PCR. Results were confirmed in three independent sets of experiments.
environment, including macrophages, fibroblasts, endothelial cells, and others, influences the way PPARα exerts its effects.

It was recently demonstrated that PPARα activation induces endothelial cell proliferation, VEGF production, and angiogenesis (33). These results are consistent with those of our study; however, we employed quiescent cells instead of cytokine-induced endothelial cells. We showed that PPARα activation led to a proangiogenic and therefore a proinflammatory effect and that PPARα activation led to the induction of VEGF expression. The induction of the proinflammatory and proangiogenic cytokine IL-8 by PPARα agonists supports these results, as Li et al. demonstrated that IL-8 enhances endothelial cell survival and proliferation, regulates the production of matrix metalloproteinases, and can induce angiogenesis (18).

Aside from the mechanism involving the conveying of NFκB, we demonstrated an influence of PPARα on the mRNA stability of IL-8. The regulation of IL-8 mRNA stability is a well accepted and important mechanism of IL-8 mRNA expression in various cell types and conditions (11, 34, 35). Many authors have demonstrated that the p38 MAPK signaling pathway, and to a minor extent ERK1/2, control IL-8 mRNA stability (13, 35–37). Here, we also demonstrated that the induction of p38 and ERK1/2 by PPARα agonists resulted in an increase in IL-8 mRNA stability and therefore IL-8 production that could be partially abolished by inhibiting the MAPK signaling pathway. A comparable mechanism has already been described for a PPARα agonist. Ren et al. demonstrated that the activation of PPARα led to reduced mRNA stability and therefore a decrease of nephrin expression in kidney epithelial cells (23). Interestingly, PPARα agonists activated Akt but failed to influence IL-8 expression by this signaling pathway.

Taken together, we demonstrated that in nonstimulated quiescent HUVECs, treatment with PPARα agonists resulted in an NFκB-dependent transcriptional and posttranscriptional MAPK signaling pathway-dependent induction of IL-8 expression. These novel findings reveal that the effects of PPARα agonists may depend on the activation status of endothelial cells and that this status is responsible for a more pro- or antiinflammatory action. These findings may influence the development of PPARα agonists for clinical use.
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