Regulation of Endothelial Nitric Oxide Synthase by PPAR Agonists: Molecular and Clinical Perspectives

Gerald F. Watts, Bart Staels

Endothelial nitric oxide synthase (eNOS) is one of three NOS isoforms that catalyze the formation of nitric oxide (NO) and L-citrulline by the oxidation of the guanido-nitrogen group of L-arginine. The cardiovascular importance of this reaction relies on the formation of NO, a signaling molecule that regulates vascular tone, platelet aggregation, oxidative stress, leukocyte adherence, and smooth muscle cell mitogenesis. Peroxisome proliferator-activated receptors (PPARs) are a subfamily of the nuclear receptor family of transcription factors that control the expression of key genes involved in the regulation of metabolism, inflammation, and thrombosis. Transcriptional control involves ligand activation followed by either heterodimerization with a retinoid X receptor and binding to the promoter region of target genes, or a DNA-binding independent mechanism that interferes negatively with proinflammatory factor signaling pathways. Of the three PPAR isoforms (α, β/δ, and γ), PPAR-α is expressed chiefly in fatty acid–oxidizing tissues including liver, skeletal muscle, and heart, but also in endothelial and vascular smooth muscle cells and macrophages within the arterial wall. Despite a plethora of basic research demonstrating that PPAR-α activation by synthetic ligands (eg, fibrates) has favorable antiatherogenic properties, the corresponding effects on eNOS and the biology of NO has surprisingly not yet been explored.

See page 658

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Goya et al demonstrate for the first time that specific PPAR-α agonists, such as fenofibrate, regulate eNOS in cultured endothelial cells. Using classical molecular biology techniques and bovine aortic endothelial cells as a model, fenofibrate was shown to increase the mRNA expression, protein level, and enzyme activity of eNOS in a dose-dependent manner at concentrations within the range of its EC50 value for human PPARα. The authors found that the eNOS promoter sequence does not possess a PPAR response element and, accordingly, showed that fenofibrate did not enhance eNOS promoter activity. However, in mRNA stability assays, fenofibrate increased the half-life of eNOS mRNA. The observation that PPAR-α agonists stabilize mRNA levels is unusual and raises the question of whether these effects occur via PPAR-α. Further studies employing in vitro gene knock-down technology and/or in vivo analysis on PPAR-α–deficient mice are required to answer this important issue and to demonstrate the pathophysiological relevance of these findings. Goya et al also present limited data indicating that the effect of fenofibrate on eNOS was shared by bezafibrate, a less specific PPAR-α agonist, but not rosiglitazone, a highly specific PPAR-γ agonist.

From a molecular perspective, these findings have to be placed within the context of the other antiatherogenic mechanisms of action of PPAR-α agonists and the complexity of the regulation of eNOS. PPAR-α is classically involved in the systemic regulation of lipid and lipoprotein metabolism by, for example, regulating the expression of genes controlling fatty acid β-oxidation (eg, carnitine palmitoyl transferase-1), intravascular triglyceride lipolysis (eg, lipoprotein lipase, apo C-III and apo A-V), and high-density lipoprotein metabolism (eg, apo A-I, apo A-II and ABCA-1). PPAR-α activation can also have direct antiatherogenic effects on the different cell types of the vascular wall by, for example, decreasing the expression of adhesion molecules, tissue factor, interleukin-6 (IL-6), and endothelin-1. PPAR-α activation decreases cellular inflammation by inhibiting signaling pathways such as AP-1 and nuclear factor κB (NF-κB), which collectively decreases vascular and systemic oxidative stress. Interestingly, PPAR-α ligand activation also decreases the expression and activity of iNOS, providing another example of its antiinflammatory effects. Collectively, it appears that fibrates may reciprocally regulate two isoforms of NOS expressed in the arterial wall, providing a powerful antiatherogenic mechanism of action. Improvements in the aforementioned metabolic and signaling pathways are likely to be additional mechanisms by which fenofibrate can favorably regulate the bioavailability of NO in vivo. The quantitative contribution of the direct effect of fenofibrate on eNOS expression described by Goya et al in relation to the other antiatherogenic properties of this agent is at present unknown.

The molecular regulation of eNOS involves both genomic and nongenomic mechanisms. Hemodynamic shear stress, for example, increases the abundance of eNOS, while lipoproteins (eg, low-density lipoproteins), angiotensin-II, and cytokines (eg, tumor necrosis factor α) decrease eNOS message in part by decreasing mRNA stability. The eNOS promoter gene possesses consensus sequences that are potential binding sites
for transcription/nuclear factors such as AP-1, NF-κB, and IL-6.2,3 Hence, the suppression of inflammatory signaling pathways by PPAR-α activation provides an additional mechanism whereby fenofibrate could influence eNOS activity; this concurs with the finding in the present report that the eNOS gene does not possess a PPAR response element.3

Post-translational covalent modification of eNOS is also fundamentally important in the homeostasis of NO, chiefly by regulating the subcellular localization of the enzyme.4 Although phosphorylation may be important, acylation critically targets the localization of eNOS to plasmalemmal caveolae, a site where the enzyme activity is inhibited through association with caveolin. In response to activation (eg, by acetylcholine) of several G-protein coupled cell surface receptors, increase in cytosolic [Ca2+]i induces the allosteric binding of calmodulin to eNOS; the enzyme then dissociates from caveolin and starts to generate NO. This reaction is then acutely terminated by a decylation/reacylation cycle of eNOS and the reassociation of the enzyme with caveolin within caveolae. Whether PPAR-α agonists can directly influence covalent modifications of eNOS that acutely regulate its activity is unknown, and was not specifically investigated in the present study. The findings with rosiglitazone reported by the authors3 are, however, in line with previous reports showing that PPAR-γ agonists increase endothelial NO production without changing eNOS expression by modulating eNOS phosphorylation.6

Whether the molecular mechanisms discussed also apply to the effects of PPAR-α agonists in regulating NO in cardiomyocytes and other cell types requires further investigation. Preliminary data, however, suggest that fenofibrate exerts a protective effect against postischemic myocardial7 and cerebrovascular injury8 in mice by an antioxidant mechanism that protects the brain against oxidative insults. The findings with rosiglitazone investigated in the present study. The findings with rosiglitazone reported by the authors3 are, however, in line with previous reports showing that PPAR-γ agonists increase endothelial NO production without changing eNOS expression by modulating eNOS phosphorylation.6

In vivo, defective synthesis and release of NO from endothelial cells and impaired bioavailability of NO is referred to as “endothelial dysfunction.” Endothelial dysfunction is predictive of clinical cardiovascular events.9 Studies in patients with type 2 diabetes and with dyslipidemia have consistently demonstrated that fibrates can improve endothelial function in both conduit and resistance arteries.10–12 Correction of endothelial dysfunction may partly explain the benefit of fibrates in angiographic and clinical endpoint trials.13,14 In which favorable effects were only partially correlated with changes in plasma lipids and lipoproteins. But exactly how do fibrates improve the biology of NO? One plausible explanation relates to the inhibition of inflammatory pathways in the arterial wall that could cascade into a decrease in the oxidative catabolism of NO to peroxynitrite, improvement in the release of NO by several receptor G-protein signaling pathways, and enhancement of eNOS mRNA. Additional mechanisms involve reduction in the endothelial cell release of endothelin-115 due to a direct genomic effect or negative feedback from increased release of NO.16 Nongenomic mechanisms that increase endothelial NO production17 may explain emerging data from clinical studies indicating that PPAR-γ ligands improve endothelial function in human subjects.18

Finally, the mechanism of action of PPAR-α agonists described by Goya et al19 has to be considered in relation to other therapies that potentially regulate the bioactivity of eNOS. eNOS is a tightly coupled enzyme system that may be easily dysregulated by perturbations in availability of substrates (eg, L-arginine)19 and cofactors (eg, tetrahydrobiopterin),20 as well as by competitive inhibitors such as increased concentrations of asymmetrical dimethyl-arginine (ADMA).21 Uncoupling of eNOS also results in increased endothelial production of superoxide and the conversion of NO to peroxynitrite, a powerful pro-oxidant that antagonises expression and activity of eNOS. Recoupling of eNOS with L-arginine, tetrahydrobiopterin, and antioxidant supplements are potential approaches for augmenting the favorable effects of PPAR-α on eNOS. Accordingly, oral supplementation with coenzyme Q10 was recently shown to have a synergistic effect with fenofibrate in correcting forearm microcirculatory dysfunction in patients with type 2 diabetes22; the corresponding molecular mechanism requires investigation. Other pharmacotherapies, such as statins,23 ACE inhibitors,24 angiotensin-II receptor blockers,25 and calcium channel blockers26 could equally regulate eNOS activity by both genomic and nongenomic mechanisms. These complementary mechanisms of action on eNOS activity and the biology of NO provide a compelling rationale for testing the efficacy of fibrates combined with other treatments in decreasing cardiovascular events in clinical end-point trials.

References

7. Tabernero A, Schoonjans K, Juel S, Carpusca L, Auwerx J, Andriantsitohaina R. Activation of the peroxisome proliferator-activated receptor α antagonists de-


Regulation of Endothelial Nitric Oxide Synthase by PPAR Agonists: Molecular and Clinical Perspectives
Gerald F. Watts and Bart Staels

Arterioscler Thromb Vasc Biol. 2004;24:619-621
doi: 10.1161/01.ATV.0000125706.86492.69

Arteriosclerosis, Thrombosis, and Vascular Biology
is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/24/4/619

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/