Is Peroxisome Proliferator-Activated Receptor-γ a New “Pal” of Renin?

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that have a growing list of pleiotropic effects and have been implicated in inflammation, Alzheimer’s disease, cancer, diabetes, obesity, and cardiovascular diseases, including atherosclerosis and hypertension. The γ-subtype of the receptor (PPARγ) has been shown to regulate transcription of target genes by binding as a heterodimer with retinoid X receptor (RXR) to a hexameric direct repeat called the PPAR response element (PPRE). The consensus sequence of the PPRE is AGGTCAAGGTCA, where N is a single nucleotide separating the 2 receptor-binding half sites (italicized). For classic PPARγ response genes, PPARγ/RXRα heterodimers sit on the PPRE and recruit corepressors in the absence of ligand. On ligand binding, a conformational change in PPARγ is induced, resulting in a replacement of the corepressors with coactivators, thus achieving ligand-activated transcription of the target gene.

Thiazolidinediones (TZDs) are high-affinity synthetic ligands of PPARγ used clinically to improve insulin sensitivity in type-2 diabetes. Reports that TZDs also have cardioprotective effects, such as reducing blood pressure, suggest a potential link between PPARγ activity and the cardiovascular system. Compelling genetic evidence supporting this includes reports showing that patients with dominant-negative mutations in PPARγ exhibit severe early onset hypertension.1 These data also indirectly suggest that the beneficial effects of TZDs on blood pressure may not be derived solely from improved glycemic controls but perhaps also from direct effects of PPARγ in cardiovascular tissues, such as the blood vessel. Consequently, understanding the fundamental mechanisms controlling blood pressure by PPARγ is essential.

One of the major pathways in blood pressure regulation is the renin-angiotensin system. TZD treatment prevents the increase in blood pressure caused by infusion of angiotensin-II (Ang II) in rats2 and decreases blood pressure and improves endothelial function in double-transgenic mice exhibiting lifelong hypertension.3 PPARγ activation by TZDs causes a downregulation of Ang II type I receptor gene expression via a PPARγ-dependent mechanism in vascular smooth muscle cells.4 Therefore, PPARγ may play a role in the regulation of Ang II action.

Renin mediates the rate-limiting step in the synthesis of Ang II, and, as such, the transcriptional mechanisms controlling its synthesis have been the subject of extensive investigation. A transcriptional enhancer has been identified upstream of the promoter in the mouse, rat, and human renin genes,5 which has been reported to be required to control the baseline level of human renin expression in vivo.6 Among the transcription factor binding sites in the enhancer is the hormone response element (HRE). Several members of the nuclear hormone receptor superfamily, including retinoic acid receptor and RXR, have been shown to bind to the HRE and to regulate renin gene expression.7 In addition, vitamin D has been reported to negatively modulate renin gene expression through a vitamin D receptor–dependent mechanism, which may involve the HRE.7,8 Because the HRE is homologous to a PPRE, and PPARγ, retinoic acid receptor-α, RXRα, and vitamin D receptor are all members of the same subfamily of ligand-activated transcription factors, it should not be surprising that PPARγ may have to be included among those transcription factors thought to regulate renin expression.

In this issue of Hypertension, Todorov et al9 present data suggesting a role for PPARγ in the stimulation of renin expression. They show that pharmacological activators of PPARγ activate renin gene expression in a PPARγ-dependent manner in human renin-expressing Calu-6 cells. Although the effect was largest with the synthetic PPARγ ligand rosiglitazone, renin mRNA was also induced by the naturally occurring fatty acids linoleic acid and oleic acid. Calu-6 cells are renin-expressing cells derived from a pulmonary carcinoma that use a posttranscriptional pathway to regulate renin expression in response to cAMP.10 Importantly, the authors demonstrated that the PPARγ-mediated induction in Calu-6 cells was transcriptional and that both rosiglitazone and oleic acid increased renin mRNA expression in cultured native JG cells.

Perhaps the most intriguing finding from this report was that the PPARγ-mediated activation of the renin promoter did not map to the HRE in the renin enhancer but instead mapped to a sequence in the minimal promoter (−148 to −134) termed “Pal3” (Figure). Mutation of the Pal3 sequence in the context of a reporter construct containing only the minimal renin promoter fused to luciferase attenuated baseline transcription and abolished the induction by rosiglitazone. However, mutation of the Pal3 sequence when the renin enhancer was present in the reporter construct had only a minimal

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Graphical representation of position weight matrix for Pal3 sequences from Okuno et al.11 Each position in the sequence is displayed as a column of stacked symbols with the height of each nucleotide (A, C, T, or G) proportional to its frequency in alignments of Pal3 sequences. This figure was generated using the Web version of enoLOGOS (http://biodev.hgen.pitt.edu/cgi-bin/enologos/enologos.cgi). Under the matrix is the sequence of the Pal3 site identified by Todorov et al11 in the human (h) renin gene and it closest homology upstream of the rat (r) and mouse (m) renin genes.

effect on baseline promoter activity, and, interestingly, partial responsiveness to rosiglitazone was retained. Thus, the full induction by PPARγ may require sequences in both the enhancer and proximal promoter.

This is not the first time that a Pal3 sequence has been identified to interact with PPARγ. In a study by Okuno et al.,11 sequences binding either PPARγ/RXRα heterodimers or PPARγ/PPARδ homodimers were selected using a PCR-mediated random site selection assay. Although the classical PPRE with a spacer of 1 nucleotide (termed “DR1”) was identified when PPARγ and RXRα were added to the assay in equimolar amounts, the Pal3 sequence was identified when a 30-fold molar excess of PPARγ over RXRα was used (ie, favoring homodimer formation). The sequence of the Pal3 site as a position weight matrix is shown in the Figure, along with the closest homologies in the 5' flanking region of the human, rat, and mouse renin genes. By combining small interfering RNA and supershift analysis, Todorov et al.9 were able to confirm both PPARγ and RXRα binding to the human renin Pal3 sequence. Notably, 2 complexes were supershifted when PPARγ antisera was added to the gel shift reaction. One of these complexes was significantly reduced in the presence of PPARγ and RXRα small interfering RNA and consequently is likely to be a PPARγ/RXRα heterodimer. However, another supershift complex was retained and even increased in the presence of PPARγ and RXRα small interfering RNA. Because the PCR-mediated random site selection assay predicts the binding of PPARγ homodimers to the Pal3 site, the authors argue that knockdown of PPARγ could paradoxically increase the formation of PPARγ homodimers at the same time as its capacity to compete for RXRα is reduced. PPARγ homodimers are most likely to form when the molar ratio of PPARγ:RXRα is high, a situation opposite to what should be expected in the presence of PPARγ small interfering RNA. Therefore, their explanation appears counterintuitive. One aspect that must be considered in interpreting these results is the specificity of the PPARγ antisera. Accordingly, the identity of the proteins binding in this complex, whether they contain PPARγ homodimers, and whether other nuclear proteins bind to the Pal3 site require additional investigation and definitive proof.

Despite some differences in interpretation, the data in total support a role for PPARγ in the regulation of renin gene expression, at least in Calu-6 cells in culture. Clearly, then, the most important outstanding question from this research is: to what extent does PPARγ control renin expression in vivo? Unfortunately, the literature on the effect of TZDs on plasma renin activity is difficult to interpret, because some studies report an increase, whereas others report a decrease or no change. Tsai et al.12 used gene targeting to knock in and replace 1 allele of mouse PPARγ with a PPARγ gene carrying a dominant-negative mutation. These mice exhibited a modest increase in blood pressure, with a concomitant increase in angiotensinogen expression in inguinal adipose tissue and Ang II type 1 receptor expression in gonadal adipose. However, there was no alteration of renin mRNA in the kidney or adipose tissue in these mice.12 Similarly, there was no change in renal renin mRNA in the kidney of knockout mice lacking PPARγ that were rescued from embryonic lethality by expression of PPARγ in trophoblast cells.13 Although one may conclude that PPARγ does not regulate renin expression in vivo, other factors, such as feedback inhibition on renin expression, cannot be ruled out. Thus, the next obvious step is to generate a juxtaglomerular cell-specific knockout of PPARγ, and, indeed, tools exist to accomplish this. A flox/flox allele of PPARγ has been available for sometime,14 and a number of mouse models expressing crerecombinase under the control of the renin promoter, including a knock-in allele of cre-recombinase to the renin locus, have been reported.15 Such a study will ultimately reveal the extent to which PPARγ regulates renin gene expression and the physiological cues to which it responds.

PPARγ is an attractive molecule given its role as a fatty acid sensor and the increased incidence of hypertension in obese patients. It remains possible that PPARγ may represent a link among obesity, metabolic dysfunction, and activity of either the circulating or tissue renin-angiotensin system in hypertension. There is no doubt that the mechanisms regulating the renin-angiotensin system by PPARγ will prove to be quite complex given the positive stimulation on renin transcription reported in this issue, along with the negative impact on the Ang II type 1 receptor reported previously. That TZDs are generally thought to modestly lower blood pressure suggests that a delicate balance must exist between the effects of PPARγ on the renin-angiotensin system (renin versus Ang II type 1 receptor) and other vasoconstrictors, such as endothelin-1.

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