Identification of Telmisartan as a Unique Angiotensin II Receptor Antagonist With Selective PPARγ–Modulating Activity

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Abstract—The metabolic syndrome is a common precursor of cardiovascular disease and type 2 diabetes that is characterized by the clustering of insulin resistance, dyslipidemia, and increased blood pressure. In humans, mutations in the peroxisome proliferator–activated receptor-γ (PPARγ) have been reported to cause the full-blown metabolic syndrome, and drugs that activate PPARγ have proven to be effective agents for the prevention and treatment of insulin resistance and type 2 diabetes. Here we report that telmisartan, a structurally unique angiotensin II receptor antagonist used for the treatment of hypertension, can function as a partial agonist of PPARγ; influence the expression of PPARγ target genes involved in carbohydrate and lipid metabolism; and reduce glucose, insulin, and triglyceride levels in rats fed a high-fat, high-carbohydrate diet. None of the other commercially available angiotensin II receptor antagonists appeared to activate PPARγ when tested at concentrations typically achieved in plasma with conventional oral dosing. In contrast to ordinary antihypertensive and antidiabetic agents, molecules that can simultaneously block the angiotensin II receptor and activate PPARγ have the potential to treat both hemodynamic and biochemical features of the metabolic syndrome and could provide unique opportunities for the prevention and treatment of diabetes and cardiovascular disease in high-risk populations. (Hypertension. 2004;43:993-1002.)

Key Words: receptors, angiotensin II ■ angiotensin II ■ renin-angiotensin system ■ insulin resistance ■ losartan

All currently available classes of antihypertensive drugs were developed before it was widely recognized that increased blood pressure is closely associated with insulin resistance and dyslipidemia and well before public health authorities established diagnostic criteria for the metabolic syndrome.1–3 Thus, the antihypertensive drugs in use today were designed primarily to affect cellular and biochemical mechanisms contributing to increased blood pressure and not to address the disordered carbohydrate and lipid metabolism that often accompany hypertension as part of the metabolic syndrome. Given the major impact of the metabolic syndrome on cardiovascular disease morbidity and mortality,4–6 the availability of antihypertensive agents that also improve insulin resistance and dyslipidemia could be of considerable clinical value.

Numerous studies have demonstrated that the peroxisome proliferator–activated receptor-γ (PPARγ) plays an important role in regulating carbohydrate and lipid metabolism and that ligands for PPARγ can improve insulin sensitivity, reduce triglyceride levels, and decrease the risk for atherosclerosis.7–15 PPARγ ligands also have modest antihypertensive effects reported at least in part to their ability to promote peripheral vasodilation.16–19 Several thiazolidinedione ligands for PPARγ have been approved for the treatment of type 2 diabetes; however, these agents have limited capacity to reduce blood pressure and can provoke fluid retention, weight gain, edema, and heart failure in a significant proportion of patients with diabetes.20–22 Such side effects can also occur with nonthiazolidinedione ligands of PPARγ and are unlikely to be related to the thiazolidinedione moiety per se.18,23,24 Thus, currently approved synthetic ligands for PPARγ cannot be used to effectively treat the abnormal hemodynamic features of the metabolic syndrome and are associated with adverse effects that are of particular concern for diabetic individuals predisposed to impaired cardiac function.

Recently, we observed an interesting structural resemblance between telmisartan, an angiotensin II (Ang II) type 1 receptor antagonist approved for the treatment of hypertension, and pioglitazone, a PPARγ ligand approved for the treatment of type 2 diabetes.25 This discovery supported the possibility that certain molecules might have the capacity not

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only to block the Ang II receptor, a key cell surface receptor involved in the regulation of blood pressure,26 but also to activate PPARγ, an intracellular nuclear hormone receptor involved in the regulation of carbohydrate and lipid metabolism. Theoretically, such bifunctional molecules could treat both the hemodynamic and biochemical features of the metabolic syndrome and have greater potential for preventing atherosclerotic cardiovascular disease than conventional antihypertensive agents. Moreover, given that blockade of the renin-angiotensin system can inhibit renal sodium reabsorption and attenuate the fluid retention and edema associated with peripheral vasodilators,27 such molecules could also lead to the development of new antidiabetic PPARγ ligands with improved safety profiles.

Herein we report that the biphenyl, nonetrazole Ang II receptor blocker (ARB) telmisartan can act as a partial agonist of PPARγ; influence the expression of PPARγ target genes involved in the regulation of carbohydrate and lipid metabolism; and reduce glucose, insulin, and triglyceride levels in an animal model of diet-induced insulin resistance. Molecular modeling studies suggest that telmisartan might influence PPARγ activity by interacting with regions of the ligand-binding domain (LBD) that are not typically engaged by full agonists of the receptor. In contrast, molecular modeling and receptor transactivation studies indicate that other ARBs lack telmisartan’s potential for receptor interaction and have relatively little or no effect on PPARγ activity. These findings (1) demonstrate that at least one of the ARBs currently in clinical use has the capacity to activate PPARγ and (2) suggest a number of interesting opportunities for developing improved therapeutic approaches to the metabolic syndrome as well as type 2 diabetes and other clinical disorders that might be influenced by activity of the renin-angiotensin system, PPARγ, or both.

Methods

Experimental Compounds

Ang II receptor antagonists and thiazolidinedione ligands of PPARγ were obtained from the pharmacy and purified by high-performance liquid chromatography. Candesartan cilexetil and olmesartan medoxomil were hydrolyzed to their active forms before high-performance liquid chromatography purification. Both losartan and its more active metabolite EXP 3174 were used in some studies.

PPAR Transactivation Assays

PPARγ activity was determined by transactivation assays in CV-1 cells (CCL-70 line from the American Type Culture Collection [ATCC], Manassas, Va) transfected by use of the GenePorter transfection reagent (Gene Therapy Systems) delivering 200 ng of the murine PPARγ expression plasmid pGAL4-mPPARγ-LBD, 1 μg luciferase reporter plasmid pUAS-tk-luc (both courteously provided by P. Tontonoz, Howard Hughes Medical Institute and Department of Pathology, University of California, Los Angeles), and 400 ng of pCMVSport β-gal (Gibco) as an internal control. Twenty-four hours after transfection, cells were treated with varying concentrations of the test compounds or DMSO vehicle control for 3 days. Additional studies were performed in adult human subcutaneous adipocytes (Cambrex, Walkersville, Md) to determine the effects of the test compounds on the expression of ACC2 in human muscle myotubes that had been derived by differentiation of the C2C12 myoblast cell line (CRL 1772 from the ATCC). Total RNA was isolated by standard methods, and cDNA was prepared and analyzed by real-time PCR testing with SYBR green reagents, as previously described.36 The cyclophilin (peptidylprollyl isomerase A) gene was used as an internal control, with results being determined in triplicate by the preferred method of Muller et al37 and displayed as the amount of mRNA in drug-treated samples relative to that in the vehicle-treated control, which was arbitrarily defined as 1. Primer specificity was confirmed by melting point analysis for each primer pair. Primer sequences are available on request.

PPARγ Target Gene Expression Assays

Expression levels of the PPARγ target genes AP2 and CD36 were determined by real-time polymerase chain reaction (PCR) of cDNA prepared from 3T3-L1 preadipocytes incubated with the test compounds or DMSO vehicle control for 3 days. Additional studies were performed in adult human subcutaneous adipocytes (Cambrex, Walkersville, Md) to determine the effects of the test compounds on expression of PCK1 that codes for phosphoenolpyruvate carboxykinase-1 (PEPCK-C). This gene was selected because PEPCK-C has been proposed to be a key mediator of the effects of PPARγ ligands on fatty acid metabolism and insulin sensitivity.33 Previous studies have also shown that acetyl coenzyme A carboxylase (ACC2) is a major regulator of muscle fatty acid metabolism.34,35 Therefore, we also tested the effects of rosiglitazone, telmisartan, irbesartan, and valsartan on the expression of ACC2 in murine muscle myotubes that had been derived by differentiation of the C2C12 myoblast cell line (CRL 1772 from the ATCC). Total RNA was isolated by standard methods, and cDNA was prepared and analyzed by real-time PCR testing with SYBR green reagents, as previously described.36 The cyclophilin (peptidylprollyl isomerase A) gene was used as an internal control, with results being determined in triplicate by the preferred method of Muller et al37 and displayed as the amount of mRNA in drug-treated samples relative to that in the vehicle-treated control, which was arbitrarily defined as 1. Primer specificity was confirmed by melting point analysis for each primer pair. Primer sequences are available on request.

Dietary Model of Insulin Resistance

Male Sprague-Dawley rats were placed on a high-fat, high-carbohydrate diet (Teklad Diet TD03203 containing 60% fructose, 10% lard, and 0.06% magnesium) at 6 weeks of age. Two days after starting the diet, the rats were randomized into 3 different groups...
Statistical Analysis

Statistical analysis was performed by Student t test or ANOVA followed by Dunnett multiple comparison test or the Student-Newman-Keuls test for comparisons across multiple groups. Statistical significance was defined as P<0.05. Data are expressed as mean±SEM.

Results

Telmisartan Is a Partial Agonist of PPARγ

We tested the ability of different ARBs to activate PPARγ in a heterologous transactivation assay that eliminates interference from endogenous nuclear receptors (Figure 1A). Thus, exogenously added drugs including rosiglitazone and telmisartan do not cause any activation of the reporter gene in the absence of the PPAR construct (data not shown). In the full assay system including the PPARγ construct, we found that telmisartan was the only ARB that caused substantial activation of PPARγ (Figure 1A). Although irbesartan appeared to cause slight activation of PPARγ (2- to 3-fold activation) when tested at 10 μmol/L, none of the other ARBs, including the active metabolite of losartan, increased PPARγ activity when tested at this concentration (Figure 1A). Moreover, telmisartan was the only ARB that activated PPARγ when tested at lower concentrations (1 to 5 μmol/L) that can be achieved in plasma with conventional oral dosing (data not shown).

Telmisartan concentrations of <10 μmol/L did not significantly affect the activity of PPARα or PPARδ, although 25 μmol/L telmisartan appeared to cause a modest activation (4-fold) of PPARα (data not shown).

Telmisartan functioned as a moderately potent (EC50=4.5 μmol/L), selective PPARγ partial agonist, activating the receptor to 25% to 30% of the maximum level achieved by the full agonists pioglitazone and rosiglitazone (Figure 1B and 1C). Corresponding EC50 values for rosiglitazone and pioglitazone were 0.066 and 1.5 μmol/L, respectively. As expected for a partial agonist, high concentrations of telmisartan (>10 to 20 μmol/L) in the presence of the full agonist rosiglitazone (1 μmol/L) attenuated the level of receptor activation otherwise achieved by the full agonist alone (Figure 1D). It should be noted that under normal circumstances, plasma concentrations of telmisartan do not reach 10 to 20 μmol/L. Thus, in patients with diabetes, administration of telmisartan is unlikely to interfere with the therapeutic effects of a simultaneously administered thiazolidinedione. We also tested telmisartan for its ability to activate full-length human PPARγ by using the full-length receptor and the luciferase reporter gene fused to the tandemly repeated PPARγ DNA response element. At 10 and 1 μmol/L, rosiglitazone achieved activation levels of 16-fold, whereas the values for telmisartan were 6-fold and 3-fold, respectively. Other sartans were inactive up to 40 μmol/L.
Molecular Modeling of Telmisartan in the PPARγ LBD

Docking studies of the molecular binding mode in PPARγ revealed that telmisartan is surrounded by helixes H3, H6, and H7 (Figure 2A). The region of the LBD occupied by telmisartan is similar to that occupied by other partial agonists of PPARγ, including GW0072 and nTZDpa (data not shown). The interaction of telmisartan with PPARγ might be explained by strong hydrophobic interactions with many of the residues forming the H3 and H7 helices. Additionally, the hydrogen bond between the 1'-benzimidazole nitrogen and the amide proton of Ser342 might also contribute toward stabilization of this interaction (Figure 2A), particularly given that the partial agonists GW0072 and nTZDpa also form hydrogen bonds with the same serine residue.

The superimposition of telmisartan bound to PPARγ on the cocrystal structure of rosiglitazone and PPARγ is shown in Figure 2B. In contrast to the binding of full agonists such as rosiglitazone, telmisartan, like other partial agonists including GW0072 and nTZDpa, does not appear to make direct contact with the activation function helix (AF-2) or with the adjacent histidine residues (Figure 2B). Interaction with the AF-2 helix has been responsible for receptor stabilization and activation by full agonists of PPARγ. The lack of interaction of telmisartan with the AF-2 helix likely explains its inability to fully activate the receptor.

To further understand the structural changes in the protein induced by binding of ligands with different biologic profiles (full agonists vs partial agonists), we analyzed the crystal structures of the PPARγ LBD in its native form (PDB code 1PRG), the PPARγ LBD bound to the full agonist rosiglitazone (PDB code 2PRG), and the PPARγ LBD bound to the partial agonist GW0072 (PDB code 4PRG). As previously noted, the crystallographically determined structure of PPARγ bound to a partial agonist (GW0072) was more similar to the native (apo) structure than the crystallographically determined conformation of the protein bound to the full agonist rosiglitazone. This was evidenced by the root mean square deviation (RMSD) of the backbone atoms that, for the partial agonist versus the apo protein, was 0.87, whereas this difference for the full agonist was 2.58. It is interesting to note that the shift in the structure of the native receptor induced by binding of GW0072 appears primarily to be a result of changes in the backbone conformation of helixes H3 and H7. The RMSD for the backbone atoms of helixes H3 and H7 in the 2 structures was found to be 0.79. Although binding of the full agonist resulted in a significant structural change in the native protein (RMSD 2.60 for the backbone atoms), the conformation of helixes H3 and H7 in this case did not differ significantly from the native protein (RMSD 0.44 for the backbone atoms). Based on these comparisons and the observation that protein-bound complexes of partial agonists like telmisartan, nTZDpa, and GW0072 are mainly stabilized by hydrophobic interactions with helixes H3 and H7, an alteration in the conformation of these helixes induced by partial agonists might contribute to the differences in receptor activation and target gene expression caused by such ligands compared with full agonists. In preliminary studies, high concentrations of telmisartan did not displace radiolabeled rosiglitazone from a recombinant form of the LBD (data not shown), further suggesting that telmisartan does not
interact with the receptor in the same fashion as conventional agonists of PPARγ.

Docking studies of several tetrazole-containing ARBs, including losartan, EXP 3174, irbesartan, olmesartan, and candesartan, in the PPARγ LBD revealed a different binding mode than for telmisartan. These tetrazole-containing ARBs made contacts with residues of helix H3 but not H7. Eprosartan, a non-tetrazole ARB, was seen to interact with residues of helix H7 but not H3. None of these ARBs revealed a hydrogen bond with the binding domain or any interaction with the AF-2 helix. Thus, insufficient interaction with the PPARγ LBD might explain the inability or poor ability of these other ARBs to activate the protein.

Telmisartan Induces Adipocyte Differentiation

PPARγ plays a critical role in adipogenesis and has been shown to be necessary and sufficient for fat cell differentiation in cultured cells and in mice. Therefore, we compared the ability of telmisartan and other ARBs to induce adipocyte differentiation of 3T3-L1 cells, a well-known characteristic of PPARγ ligands. Telmisartan but none of the other ARBs clearly induced adipogenesis when tested at a concentration of 1 to 5 μmol/L (Figure 3 and data not shown). Although a higher concentration of irbesartan (10 μmol/L) induced adipocyte differentiation, none of the other ARBs, including the active metabolite of losartan, induced adipogenesis even when tested at concentrations of up to 25 μmol/L. In other experiments (data not shown), we found that the level of adipogenesis induced by rosiglitazone was 2 to 3 times greater than that induced by telmisartan. The relatively modest effect of telmisartan on adipogenesis is not surprising, given that other partial agonists of PPARγ have also been found to be relatively weak stimulators, or even inhibitors, of adipogenesis compared with full agonists such as rosiglitazone.

Telmisartan Selectively Modulates the Expression of PPARγ Target Genes

PPARγ ligands influence the expression of multiple genes in differentiating preadipocytes and in mature adipocytes, such as AP2 (FABP4) and CD36. When tested at a concentration of 5 μmol/L, only telmisartan and irbesartan caused a substantial (>2-fold) increase in the expression of AP2 and CD36 in 3T3-L1 preadipocytes (Figure 4A and 4B). Whereas telmisartan and irbesartan both increased the expression of AP2 and CD36, they appeared to have different effects on the expression of 2 other important genes related to lipid metabolism. In differentiated human adipocytes, telmisartan was the only ARB at concentrations of 2.5 to 5.0 μmol/L that clearly increased the expression of the PCK1 gene encoding...
PEPCK-C (Figure 4C). This is noteworthy because the induction of PEPCK-C activity in adipocytes and the resultant increases in glyceroneogenesis and fatty acid reesterification have been proposed to play an essential role in the antidiabetic actions of PPARγ ligands. In murine muscle myotubes, 5.0 μmol/L telmisartan caused a 60% to 70% decrease in the expression of ACC2, whereas rosiglitazone, irbesartan, and valsartan had little or no effect on ACC2 expression (Figure 4D). ACC2 is a major regulator of muscle fatty acid metabolism, because this enzyme generates malonyl coenzyme A, a potent inhibitor of muscle carnitine palmitoyltransferase-1, which is a critical enzyme involved in fatty acid uptake in mitochondria. Accordingly, decreases in ACC2 expression might be expected to promote increased fatty acid oxidation in skeletal muscle.

Telmisartan Improves Glucose, Insulin, and Triglyceride Levels
Figure 5A shows body weights in control rats and in rats given either telmisartan or losartan. The 2-way ANOVA showed a significant effect of the drug (P<0.01), time (P<0.01), and a drug–time interaction (P<0.01) on body weight. Telmisartan administration caused a significant attenuation of weight gain compared with losartan and control groups (≈10%), whereas losartan appeared to have little or no effect on body weight compared with controls. Remarkably, the telmisartan-induced attenuation of weight gain could not be attributed to reduced energy intake, because daily food consumption was nearly identical in all groups as a result of the pair-feeding protocol (Figure 5B). Fluid intakes were similar among all 3 groups (data not shown).

Serum glucose levels measured after 5 weeks of treatment were significantly decreased in the telmisartan group compared with both the losartan group (P<0.01) and the control group (P<0.001) by ANOVA and Student-Newman-Keuls testing (Figure 6). Serum insulin levels also tended to be lower in the telmisartan-treated animals (Figure 6). Although overall ANOVA testing did not achieve statistical significance (P=0.09), the results of individual comparisons were consistent with lower insulin levels in the telmisartan group compared with the losartan group and the control group (both P=0.025 by 1-tailed t testing and P<0.01 by Student-Newman-Keuls testing; Figure 6B). Serum triglycerides were significantly decreased in the telmisartan treated group compared with both the losartan group (P<0.05) and the control group (P<0.01) by ANOVA and Student-Newman-Keuls testing; Figure 6).

During the oral glucose tolerance test, serum levels of glucose were similar among all 3 groups (Figure 7). However, serum insulin levels were significantly lower in the telmisartan group compared with both the losartan group and control group throughout the glucose tolerance test (Figure 7). The area under the curve for insulin in the telmisartan-treated rats, 3.7±0.3 ng·mL⁻¹·2 h⁻¹, was significantly lower than in the losartan-treated rats (6.0±0.7 ng·mL⁻¹·2 h⁻¹) and the control rats (5.3±0.7 ng·mL⁻¹·2 h⁻¹; P<0.05 by ANOVA and Student-Newman-Keuls testing). Compared with controls, losartan did not have significant effects on any of the parameters measured.

Discussion
On the basis of cellular assays of PPARγ activation, we have found that the Ang II receptor antagonist telmisartan is also a partial agonist of PPARγ, a well-known target of insulin-sensitizing drugs used to treat type 2 diabetes. In contrast, none of the other ARBs affected PPARγ activity with the possible exception of irbesartan, which appeared to cause a modest activation of the receptor when tested at a concentration of 10 μmol/L. In addition to activating PPARγ in cell-based transactivation assays, telmisartan increased the expression of known PPARγ target genes in both murine preadipocyte fibroblasts and human subcutaneous adipocytes and induced adipogenesis in 3T3-L1 preadipocyte fibroblasts, as expected for a PPARγ activator. Finally, in rats fed a high-fat, high-carbohydrate diet, orally administered telmisartan reduced glucose, insulin, and triglyceride levels, whereas losartan did not. In preliminary studies in obese Zucker rats that harbor mutant leptin receptors (data not shown), telmisartan did not appear to affect glucose, insulin, or triglyceride levels, suggesting that at least some of the beneficial metabolic effects of telmisartan might depend on the presence of an intact leptin signaling system.

The mechanism whereby telmisartan activates PPARγ remains to be determined; however, given the substantial chemical structural differences between telmisartan and all of the other commercially available ARBs, it is not surprising that telmisartan has unique biologic properties. There are a number of possible mechanisms that could theoretically mediate the effects of telmisartan on PPARγ activity, including but not limited to effects on the conformation or phos-
phosphorylation status of the receptor, effects on the activity of coactivators or corepressors that modulate the transcriptional effects of PPARγ, or even effects on endogenous ligands of PPARγ. For example, molecular modeling studies suggest that telmisartan might fit within the LBD of PPARγ in a complex that is stabilized by hydrophobic interactions with helices H3 and H7, as well as by a hydrogen bond with the amide proton of Ser342. This binding mode is similar to that observed for the partial agonist GW0072. In contrast, the other ARBs do not appear to have the same potential for interaction with the receptor like telmisartan. Although it remains to be determined whether the unusual ability of telmisartan to activate PPARγ is indeed related to the receptor interactions identified in the modeling studies, it is clear that substantial differences exist between the chemical structures of telmisartan and the other ARBs.

Although telmisartan was developed with the goal of selectively blocking the Ang II type 1 receptor to treat hypertension, the finding that this molecule can also activate PPARγ has potentially important therapeutic implications for pharmacological treatment of the metabolic syndrome, type 2 diabetes, and other clinical disorders that might be responsive to PPARγ activators. Because hypertension frequently occurs together with insulin resistance and dyslipidemia, the availability of multifunctional molecules that treat more than just increased blood pressure or the associated metabolic disturbances could be of considerable clinical value. It is widely believed that the currently available ARBs are metabolically neutral and have little or no impact on carbohydrate and lipid metabolism when administered in conventional doses used to treat hypertension. However, the current findings suggest that telmisartan might be an exception in this regard and provide insight into new strategies for developing molecules that could improve many if not all of the biochemical and blood pressure disturbances that compose the metabolic syndrome.

It should be emphasized that telmisartan is a partial agonist of PPARγ and appears to function as a selective PPAR modulator with different effects on gene expression than a full agonist of PPARγ like rosiglitazone. It is well known that partial agonists of PPARγ can exert different effects on gene expression patterns than do full agonists and that even full agonists might differ among themselves with respect to their precise effects on gene expression profiles. Thus, it is not surprising that telmisartan, but not rosiglitazone, affected the expression of ACC2, a key gene involved in the regulation of muscle fatty acid metabolism. In fact, intense interest exists in the development of selective PPARγ modulators that can exert beneficial effects on the expression of genes that regulate carbohydrate and lipid metabolism without causing changes in gene expression that promote weight gain, fluid retention, or the other adverse effects associated with administration of conventional PPARγ activators. In the current study, it is noteworthy that telmisartan attenuated weight gain despite the use of a pair-feeding protocol that ensured comparable food intakes among all of the experimental groups. Other partial agonists of PPARγ have also been shown to attenuate the weight gain ordinarily induced by a high-fat diet. The current observations raise the intriguing possibility that telmisartan might have the capacity to influence genes that regulate energy metabolism in vivo and should motivate future studies on the ability of telmisartan to attenuate weight gain in humans consuming high-fat, high-carbohydrate diets.

Figure 6. Telmisartan reduces serum levels of glucose, insulin, and triglycerides. Drug treatment groups and initial daily drug doses are listed below each bar graph. *P<0.001 by ANOVA, P<0.001 compared with control group, and P<0.01 compared with losartan group by Student-Newman-Keuls testing. **P=0.09 by ANOVA, P=0.025 compared with control group and losartan group by Student-Newman-Keuls testing. ***P<0.01 by ANOVA, P<0.01 compared with control group, and P<0.05 compared with losartan group by Student-Newman-Keuls testing.
closely related to their effects on kinin metabolism rather than insulin-sensitizing effects of ACE inhibitors might be more expected to lead to improvements in carbohydrate and lipid metabolism. However, recent studies have indicated that the insulin-sensitizing effects of ACE inhibitors might be more closely related to their effects on kinin metabolism rather than their effects on the renin-angiotensin system. Thus, based on the results of studies with ACE inhibitors, it could not have been predicted that any of the existing ARBs would activate PPARγ and improve the disturbances in carbohydrate and lipid metabolism that are characteristic of the metabolic syndrome.

Although it is generally believed that ARBs do not exert significant effects on carbohydrate metabolism, such views are based largely on the results of clinical trials and experimental studies that have been conducted with ARBs that are structurally quite different from telmisartan. For example, measurements of systemic insulin action with the euglycemic clamp technique have failed to reveal any consistent effect of losartan on insulin sensitivity. In the recent LIFE trial, the incidence of new-onset type 2 diabetes was reported to be significantly lower in hypertensive subjects treated with losartan than in those treated with atenolol, suggesting potential antidiabetic effects of angiotensin receptor blockade. However, given the known diabetogenic effects of β-adrenergic blockers, it is possible that the incidence of new-onset diabetes in the losartan arm of the trial was related to a prodiaetic effect of atenolol rather than an antidiabetic effect of ARB. In the CHARM Preserved trial, the incidence of new-onset type 2 diabetes was significantly lower in subjects given candesartan than in those given placebo. However, in other trials including CHARM Added, CHARM Alternative, and SCOPE, there was no significant difference in the incidence of new-onset diabetes in subjects given candesartan compared with controls. In the ALPINE and CROSS studies, candesartan appeared to have little or no effect on serum levels of insulin, glucose, or triglycerides. Although candesartan administration appeared to improve an indirect estimate of insulin action in the CROSS study, it failed to show any effect on the HOMA (homeostasis model assessment) index of insulin resistance in the ALPINE study. Finally, in the obese Zucker rat, Henriksen et al found that oral administration of an extremely high dose of irbesartan (50 mg/kg) improved insulin sensitivity but apparently failed to improve lipid levels. Thus, although the results of Henriksen et al are consistent with our finding of a weak effect of high concentrations of irbesartan on PPARγ activity, they do not imply that conventional doses of irbesartan or any other ARB could be used to activate PPARγ in vivo or treat the metabolic syndrome.

Recently, Janke and colleagues have reported that very high concentrations of Ang II can inhibit differentiation of human preadipocytes and that high concentrations of irbesartan can enhance adipogenesis. On the basis of these findings and recent evidence showing that a lack of adipose tissue can promote diabetes by causing excess storage of fat in muscle, liver, and pancreas, Sharma and colleagues have proposed that blockade of the renin-angiotensin system per se might prevent diabetes by promoting the recruitment and differentiation of adipocytes. In the current studies, we found that moderate concentrations of telmisartan and high concentrations of irbesartan could activate PPARγ and promote adipogenesis; however, other ARBs failed to show any effects on PPARγ activity or adipogenesis. PPARγ is known to play a pivotal role in adipogenesis, whereas the effect of the renin-angiotensin system on fat cell differentiation is much less clear, particularly given that very high concentrations of Ang II are typically required to observe effects on adipogenesis. If the adipogenic effects of telmisartan and irbesartan were related to blockade of the renin-angiotensin system in the cells we tested, one would have expected other ARBs also to induce adipogenesis. Moreover, the concentrations of telmisartan and irbesartan required to induce adipogenesis are far greater than the concentrations required to block the Ang II type 1 receptor, further suggesting that the in vitro effects of telmisartan or other ARBs on adipogenesis in the cells that we tested are unlikely to be related to Ang II receptor blockade. Thus, although our findings are consistent with the results of Sharma and colleagues, we believe that at least in the cell line we studied, the effects of telmisartan and...
irbesartan on adipocyte differentiation are more likely related to their ability to activate PPARγ rather than their ability to block Ang II receptors.

**Perspectives**

Aside from the potential use of telmisartan for the prevention and treatment of diabetes and the metabolic syndrome, the discovery that telmisartan can activate PPARγ has a number of implications for the development of next-generation molecules for treating clinical disorders that are influenced by activity of the renin-angiotensin system and PPARγ. Notwithstanding the results of the recent ALLHAT trial,53 drugs that interrupt the renin-angiotensin system are considered by many to be superior for preventing hypertension-related target-organ damage than are antihypertensive agents that were not designed to interrupt the renin-angiotensin system.58–70 The development of novel ARBs that ameliorate insulin resistance and dyslipidemia as well as hypertension could provide even more effective options for preventing target-organ damage and cardiovascular disease in patients with hypertension, diabetes, or both. Such agents, either alone or in combination with ACE inhibitors, could also be useful for the prevention of new-onset diabetes in patients with hypertension or in other high-risk populations. Finally, given the known inhibitory effects of Ang II receptor blockade on renal sodium reabsorption, the current findings could provide new opportunities for developing antidiabetic PPARγ ligands without the adverse effects of fluid retention, peripheral edema, and heart failure associated with conventional agonists of PPARγ, like rosiglitazone and pioglitazone.20–22

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