Angiotensin Type 1 Receptor Blockers Induce Peroxisome Proliferator–Activated Receptor-γ Activity

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Background—Angiotensin type 1 receptor (AT_1R) blockers (ARB) have been shown to reduce the incidence of type 2 diabetes mellitus by an unknown molecular mechanism. The peroxisome proliferator–activated receptor-γ (PPARγ) is the central regulator of insulin and glucose metabolism improving insulin sensitivity. We investigated the regulation of PPARγ function by ARBs.

Methods and Results—The ARBs irbesartan and telmisartan (10 μmol/L) potently enhanced PPARγ-dependent 3T3-L1 adipocyte differentiation associated with a significant increase in mRNA expression of the adipogenic marker gene adipose protein 2 (aP2), as measured by quantitative real-time polymerase chain reaction (irbesartan: 3.3±0.1-fold induction; telmisartan: 3.1±0.3-fold induction; both $P<0.01$). Telmisartan showed a more pronounced induction of aP2 expression in lower, pharmacologically relevant concentrations compared with the other ARBs. The ARB losartan enhanced aP2 expression only at high concentrations (losartan 100 μmol/L: 3.6±0.3-fold induction; $P<0.01$), whereas eprosartan up to 100 μmol/L had no significant effects. In transcription reporter assays, irbesartan and telmisartan (10 μmol/L) markedly induced transcriptional activity of PPARγ by 3.4±0.9-fold and 2.6±0.6-fold ($P<0.05$), respectively, compared with 5.2±1.1-fold stimulation by the PPARγ ligand pioglitazone (10 μmol/L). Irbesartan and telmisartan also induced PPARγ activity in an AT_1R-deficient cell model (PC12W), demonstrating that these ARBs stimulate PPARγ activity independent of their AT_1R blocking actions.

Conclusions—The present study demonstrates that a specific subset of ARBs induces PPARγ activity, thereby promoting PPARγ-dependent differentiation in adipocytes. The activation of PPARγ demonstrates new pleiotropic actions of certain ARBs, providing a potential mechanism for their insulin-sensitizing/antidiabetic effects. (***Circulation*. 2004;109: 2054-2057.**)

Key Words: diabetes mellitus ■ insulin ■ angiotensin ■ pharmacology

Angiotensin type 1 receptor (AT_1R) blockers (ARBs) are widely used in the treatment of hypertension and hypertension-related cardiovascular end-organ damage.[1] In addition to their important function as antihypertensive drugs, metabolic actions of ARBs have been described. Recent clinical trials have demonstrated that AT_1R antagonism substantially lowers the risk for type 2 diabetes compared with other antihypertensive therapies.[2] In addition, AT_1R blockade improves insulin sensitivity in animal models of insulin resistance.[3] The underlying mechanism of the insulin-sensitizing/antidiabetic effect of ARBs is widely unknown.

The nuclear hormone receptor peroxisome proliferator–activated receptor-γ (PPARγ) plays an important role in the regulation of insulin sensitivity.[4] Activated by its ligands such as prostaglandins or synthetic insulin-sensitizing thiazolidinediones/glitazones, PPARγ functions as a transcriptional regulator of multiple genes involved in glucose and lipid metabolism, thereby ameliorating type 2 diabetes.[4]

To elucidate the underlying mechanisms of the antidiabetic effect of ARBs, we investigated the effects of different ARBs on PPARγ function in 3T3-L1 cells, an established cell model to study PPARγ function.

Methods

Cell Culture

Mouse 3T3-L1 preadipocytes were cultured and differentiated as previously described by using a standard differentiation mixture (Mix: dexamethasone, 3-isobutyl-1-methylxanthine [IBMX], insulin, and 10% FBS).[5] Cells were treated with vehicle or the ARBs (eprosartan, irbesartan, losartan, telmisartan) until day 4 of differen-

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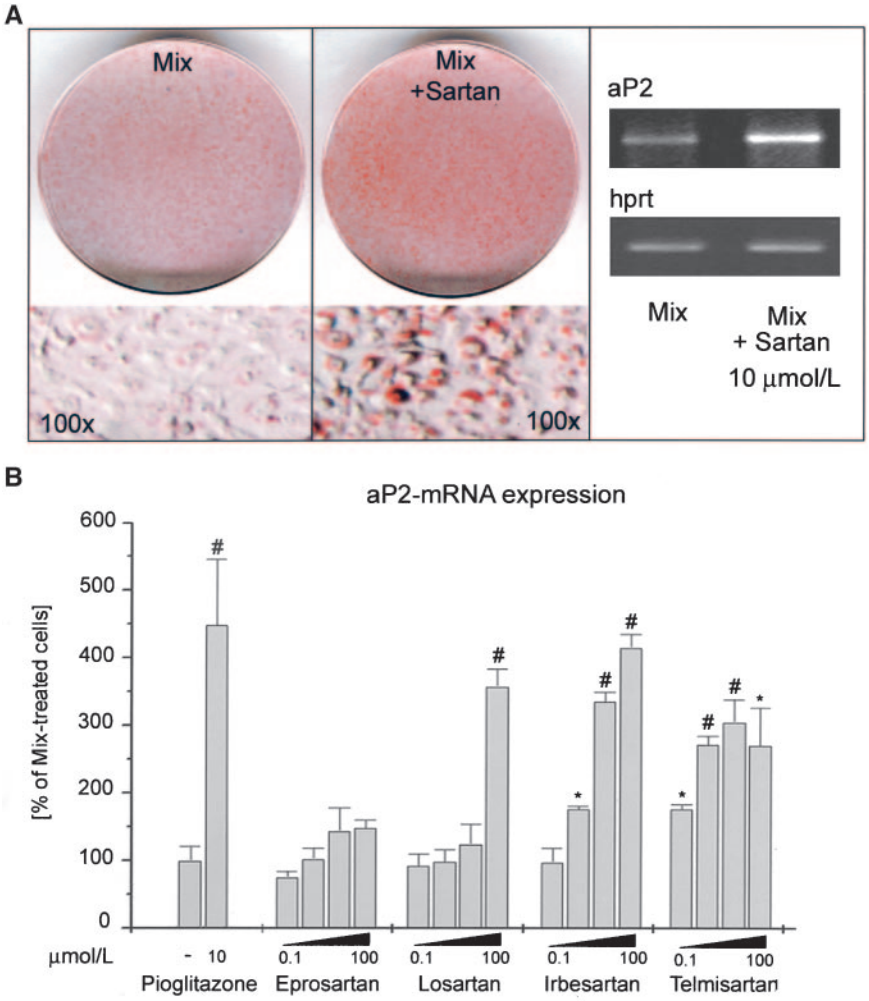

Figure 1. ARBs promote 3T3-L1 adipocyte differentiation. A, After 4 days of differentiation with Mix with or without irbesartan (Sartan, 10 μmol/L), 3T3-L1 cells were stained with Oil Red-O, and mRNA was isolated to measure aP2 expression by semiquantitative RT-PCR. Hypoxanthine guanine phosphoribosyl transferase (hprt) was used as a housekeeping gene. Representatives of 3 separate experiments are presented. B, Cells were treated as described in A with or without pioglitazone (10 μmol/L) and the ARBs (0.1, 1, 10, 100 μmol/L; eprosartan, losartan, irbesartan, telmisartan), and aP2 mRNA was quantified with the use of real-time PCR. Expression of aP2 was normalized to 18S expression. Experiments were repeated 3 times; results are presented as mean±SD. *$P<0.05$, #$P<0.01$ vs Mix alone.

tiation. Oil Red-O staining was performed as previously described.[5] PC12W cells were cultured as previously described.[1]

Semiquantitative RT-PCR and Quantitative Real-Time PCR

Real-time polymerase chain reaction (PCR) was performed as previously described with an ABI 7000 sequence detection system for real-time PCR.[6] Mouse 18S ribosomal RNA for real-time PCR and hypoxanthine guanine phosphoribosyl transferase or β-actin for semiquantitative reverse transcription (RT)–PCR were chosen as endogenous controls (housekeeping genes).

Transfection and Luciferase Assay

Transient transfection and luciferase assays were performed as previously described.[7] 3T3-L1 cells (day 4) and PC12W cells were transfected with the use of Lipofectamine 2000 (Invitrogen) with 1 μg (3T3-L1 cells) or 50 ng (PC12W cells) 3xAcyl-CoA oxidase PPAR response element (PPRE)–TK-luciferase reporter construct; PPARγ2 (10 ng) and RXRα (10 ng) expression vectors, provided by Dennis Bruemmer and Ronald Law (University of California at Los Angeles)[7]; pGal4-hPPARγDEF (hPPARγ ligand-binding domain [LBD] fused to Gal4 DBD) and pGal5-TK-pGL3, provided by Bart Staels (UR 545 INSERM, Institut Pasteur de Lille, Lille, France); and 10 ng pRL-CMV, a renilla luciferase control reporter vector. After 4 hours, transfection medium was replaced by 10% FBS DMEM plus the indicated ARBs, pioglitazone, or vehicle (dimethyl sulfoxide), and luciferase activity was measured after 24 hours.

Statistical Analysis

ANOVA and *t* test were performed for statistical analysis as appropriate. Statistical significance was designated at $P<0.05$. Values are expressed as mean±SD.

Results

ARBs Promote 3T3-L1 Adipocyte Differentiation

The ARBs irbesartan (10 μmol/L) and telmisartan (10 μmol/L; data not shown) potently enhanced lipid accumulation, as indicated by an increased Oil Red-O staining (Figure 1A), and markedly induced the expression of the adipogenic marker gene adipose protein 2 (aP2) in 3T3-L1 cells in a concentration-dependent manner (irbesartan 10 μmol/L plus Mix: 3.3±0.1-fold; telmisartan 10 μmol/L plus Mix: 3.1±0.3-fold induction; both versus Mix alone, $P<0.01$) (Figure 1A and 1B). Telmisartan showed a more pronounced induction of aP2 expression in low concentrations compared with the other ARBs (telmisartan EC_{50}: 0.13 μmol/L; irbesartan EC_{50}: 3.5 μmol/L) (Figure 1B). Losartan enhanced aP2 expression only at the highest concentration (100 μmol/L: 3.6±0.3-fold induction; $P<0.01$), whereas eprosartan had no significant effects (Figure 1B). The PPARγ ligand pioglitazone stimulated aP2 expression by 4.5±1-fold (10 μmol/L; $P<0.01$) compared with Mix alone (Figure 1B).

The PPARγ antagonist GW9662 (30 μmol/L) significantly blocked aP2 expression induced by pioglitazone (1 μmol/L), telmisartan (10 μmol/L), and irbesartan (100 μmol/L) to comparable extents (Data Supplement Figure, A).

ARBs Induce PPARγ Activity

Treatment of PPRE-transfected 3T3-L1 cells with irbesartan and telmisartan (10 μmol/L) markedly induced transcriptional activity of PPARγ 3.4±0.9-fold and 2.6±0.6-fold, respectively (both $P<0.05$ versus vehicle-treated cells), compared with 5.2±1.1-fold stimulation by pioglitazone at the same concentration ($P<0.01$ versus vehicle-treated cells) (Data Supplement Figure, B). Consistent with the effects on aP2 expression, losartan induced PPARγ-dependent transcription only at high concentrations (100 μmol/L: 2.2±0.46-fold; $P<0.05$ versus vehicle-treated cells), and eprosartan had no effect (Data Supplement Figure, B).

ARBs Activate the PPARγ LBD

To provide further insight into the mechanism whereby ARBs induce PPARγ activity, we assessed their ability to activate the chimeric Gal4-DBD-hPPARγ-LBD fusion protein on a Gal4-dependent luciferase reporter. In this system, the activation of the reporter is mediated solely through activation of the PPARγ LBD. Treatment with the ARBs led to a concentration-dependent activation of the reporter (losartan EC_{50}: >50 μmol/L; irbesartan EC_{50}: 26.97 μmol/L; telmisartan EC_{50}: 5.02 μmol/L; pioglitazone EC_{50}: 0.2 μmol/L) (Figure 2A).

Irbesartan and Telmisartan Induce PPARγ Activity Independent of the AT_1R

To study the role of the AT_1R blocking actions of ARBs during PPARγ activation, PPARγ2 and its heterodimeric partner RXRα were overexpressed in an AT_1R-deficient cell model (PC12W cells) (Figure 2B, box), and PPARγ-dependent transcription was measured with and without ARBs and pioglitazone. No regulation of PPARγ activity was observed in the absence of exogenous PPARγ2/RXRα (Figure 2B). After overexpression of the PPARγ2/RXRα heterodimer, irbesartan and telmisartan also induced PPARγ activity in AT_1R-deficient PC12W cells, clearly demonstrating that these compounds stimulate PPARγ activation independent of their AT_1R blocking actions (irbesartan: 2.1±0.3-fold; telmisartan: 1.9±0.4-fold; both $P<0.05$ versus vehicle-treated cells; pioglitazone: 4.2±1.4-fold; $P<0.01$ versus vehicle-treated cells).

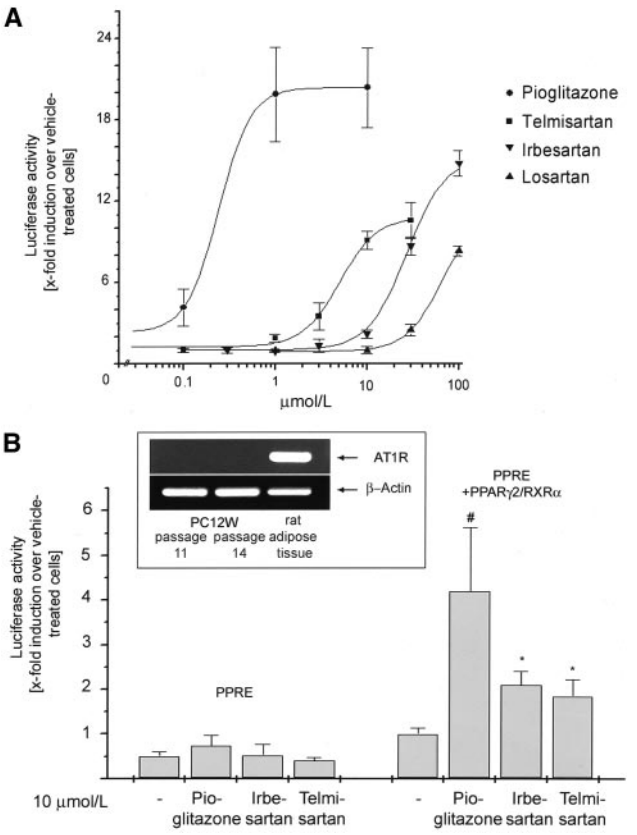

Figure 2. ARBs induce PPARγ activity. A, PC12W cells were transiently transfected with the pGal4-hPPARγDEF and pGal5-Tk-pGL3 reporter followed by stimulation with the ARBs as indicated and pioglitazone. B, Box, Semiquantitative RT-PCR for AT_1R was performed in PC12W cells (passages 11 to 14). β-Actin was used as a housekeeping gene. Cells from the same passages were transfected with PPRE-luciferase reporter construct with and without PPARγ2/RXRα expression vectors, followed by stimulation with pioglitazone (10 μmol/L), irbesartan (10 μmol/L), or telmisartan (10 μmol/L). Firefly luciferase activity was measured after 24 hours and normalized with activity of cotransfected renilla luciferase. Experiments were repeated 3 times; results are presented as mean±SD. *$P<0.05$, #$P<0.01$ vs vehicle-treated cells.

Discussion

The present study demonstrates that a subset of ARBs induces PPARγ activity by interaction with the PPARγ-LBD, thereby promoting PPARγ-dependent differentiation in 3T3-L1 adipocytes. Activation of PPARγ by these ARBs was also observed in the absence of AT_1Rs, demonstrating that the activation is independent of blocking the AT_1R. The induction of PPARγ activity demonstrates new pleiotropic actions of certain ARBs, providing a potential mechanism for their insulin-sensitizing/antidiabetic effects.

We identified ARBs with PPARγ-activating properties at low (telmisartan), medium (irbesartan), and very high concentrations (losartan) as well as a nonactivating ARB (eprosartan). Significant differences among the PPARγ-activating ARBs are likely caused by their physicochemical properties.[8] High lipophilicity is required to obtain sufficiently high penetration rates to bind to intracellular PPARγ. Telmisartan, the ARB with the highest lipophilicity, most potently induced PPARγ-dependent aP2 expression and PPARγ2 LBD activation at pharmacologically relevant concentrations.[8] These data imply that PPARγ-activating potency correlates with the degree of lipophilicity among the ARBs (telmisartan > irbesartan > losartan), whereas the maximal response may depend on other characteristics. When PPARγ-activating ARBs were compared with the PPARγ ligand pioglitazone, these compounds behaved like partial PPARγ agonists compared with the full agonism of glitazones. The detailed differences between PPARγ-activating ARBs and glitazones will be elucidated by understanding the molecular mechanism of PPARγ activation by lipophilic ARBs (eg, direct binding

to the LBD, coactivator recruitment), which remains to be determined in the future.

ARBs that activate PPARγ have also been demonstrated to stimulate the expression of major PPARγ target genes in in vivo animal models. The improvement of insulin sensitivity in obese Zucker rats by irbesartan was associated with a marked upregulation of the PPARγ target gene glucose transporter-4 (GLUT-4).[3,5] These data indicate that PPARγ activation by ARBs translates in the regulation of PPARγ target genes in vivo.

Despite the effects in animal models, clinical data in insulin-resistant or diabetic patients are still limited. Hypertensive patients receiving losartan had a 25% lower rate of new-onset diabetes than the atenolol-treated group, implicating an antidiabetic action of losartan.[2] In our study losartan failed to induce PPARγ activity in pharmacologically relevant concentrations, suggesting additional antidiabetic mechanisms regulated by losartan or its active metabolites. Angiotensin II has been recently shown to inhibit intracellular insulin signaling, which was restored by AT$_1$R antagonism.[9] Beneficial effects of ARBs on impaired insulin signaling may represent an additional molecular mechanism for insulin sensitization by these compounds. Telmisartan stimulated PPARγ at pharmacologically relevant concentrations. The concentrations needed for PPARγ activation are achieved in the plasma of hypertensive patients treated with telmisartan (280 ng/mL=0.54 μmol/L), which will likely result in additional positive effects on insulin sensitivity in the dosages used for antihypertensive treatment.[8] Beneficial effects of telmisartan not only on insulin sensitivity but also on plasma glucose lowering as well as an improved overall diabetic situation remain to be seen in suitable in vivo models and in clinical studies.

Angiotensin-converting enzyme (ACE) inhibitors have also been shown to prevent the onset of diabetes mellitus; however, the ACE inhibitor captopril was unable to induce PPARγ-mediated human adipocyte differentiation.[6] ACE inhibitors improve insulin sensitivity via the bradykinin pathway, which demonstrates an alternative antidiabetic mechanisms of these compounds.[10]

In conclusion, PPARγ activation by a specific subset of ARBs may provide new therapeutic options in the treatment of patients with the metabolic syndrome. In addition, the pharmacological characteristics of PPARγ-activating ARBs may serve as a starting point for the development of future substances combining dual functions (AT$_1$R antagonism and PPARγ activation) to treat hypertension and insulin resistance/type 2 diabetes.

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