Selective peroxisome proliferator–activated receptor (PPAR) γ modulation is a new pharmacological approach that, based on selective receptor-cofactor interactions and target gene regulation, should result in potent insulin sensitization in the absence of PPARγ-mediated adverse effects. Here, we characterize two angiotensin receptor blockers (ARBs), telmisartan and irbesartan, as new selective PPAR modulators (SPPARMs). Analysis of PPARγ protein–ligand interaction using protease protection showed that telmisartan directly interacts with the receptor, producing a distinct conformational change compared with a glitazone. Glutathione S-transferase pull-down and fluorescence resonance energy transfer assays revealed selective cofactor binding by the ARBs compared with glitazones with an attenuated release of the nuclear receptor corepressor and absence of transcriptional intermediary factor 2 recruitment by ARBs. Consistently, selective cofactor binding resulted in differential gene expression profiles in adipocytes (ARB versus glitazone treated) assessed by oligo microarray analysis. Finally, telmisartan improved insulin sensitivity in diet-induced obese mice in the absence of weight gain. The present study identifies two ARBs as new SPPARMs. SPPARM activity by ARBs could retain the metabolic efficacy of PPARγ activation with reduction in adverse effects exerting in parallel AT1 receptor blockade. This may provide a new therapeutic option for better cardiovascular risk management in metabolic diseases and may initiate the development of new classes of drugs combining potent antihypertensive and antidiabetic actions. *Diabetes* 54:3442–3452, 2005

The peroxisome proliferator–activated receptors (PPARs) are members of the nuclear receptor superfamily of ligand-activated transcription factors (1). A series of coactivators/corepressors are involved in ligand-induced transcription of genes allowing a tissue- and ligand-specific activation of these target genes by PPARs (2). The most abundant isoform in adipose tissue, PPARγ, plays an important role in the regulation of insulin sensitivity (3). Glitazones or thiazolidinediones (TZDs) are high-affinity ligands for this receptor and are currently used in the treatment of type 2 diabetes (4). Together with an improvement of insulin sensitivity and glucose tolerance, they improve lipid profiles (5). Given the side effects of glitazones, like weight gain, edema, and fluid retention, the characterization of new PPARγ ligands that retain metabolic efficacy without exerting adverse actions plays a central role in the development of new therapeutic strategies for insulin resistance and type 2 diabetes (6).

Angiotensin type 1 receptor (AT1R) blockers (ARBs) prevent the binding of angiotensin II to the AT1R and are widely used for the treatment of hypertension and related cardiovascular and organ damage (7). Recent clinical trials revealed a substantial lower risk in developing type 2 diabetes for patients treated with ARBs in comparison with other hypertensive treatment (8). In addition, the ARB telmisartan has been shown to improve metabolic parameters in rodents and type 2 diabetic patients (9–11). Recently, a subgroup of ARBs has been characterized as PPARγ ligands independent of their AT1R blocking actions (11–13). Although designed to bind to the AT1R, these ARBs have been shown to activate the PPARγ ligand binding domain (LBD) in the μmol/l range (11,13).

The underlying molecular mechanisms of PPARγ activation by ARBs are unknown. Here, we characterize the molecular interactions between PPARγ and two PPARγ-activating ARBs, telmisartan and irbesartan. Glutathione S-transferase (GST) pull-down and fluorescence resonance energy transfer (FRET) assays revealed selective...
Transfection and luciferase reporter assays. Transient transfection and luciferase assays were performed as previously described (16). COS-7 cells were plated in 24-well dishes and transfected using lipofectamine 2000 with 150 ng DR1–6 × PPRE-TkpGl basic, 20 ng hPPARγ-pSG5, 0, 50, 100, or 200 ng hPPARγpcDNA3 or the corresponding empty vectors. Firefly luciferase plasmids (30 ng and 3 ng pRL-CMV, a renilla luciferase reporter vector). After 3 h, the medium was replaced by 10% fetal bovine serum Dulbecco’s modified Eagle’s medium plus the indicated ARBs, pioglitazone or vehicle (DMSO), and luciferase activities were measured after 36 h using the Dual-Luciferase Reporter Assay System (Promega). Transfection experiments were performed in triplicate and repeated at least three times.

Gene expression analysis. After starvation of 3T3-L1 adipocytes for 16 h (Dulbecco’s modified Eagle’s medium with 0.5% fetal bovine serum), cells were incubated with the different ligands for 24 h. Isolated total RNA of three independent experiments was pooled. cRNA targets were amplified and fluorescently labeled with cyanine 3– or cyanine 5-CTP (Perkin Elmer/NEN Life Science) starting from 1 μg of total RNA in each reaction using a Low RNA Input Fluorescent Linear Amplification Kit (Agilent). Yields of cRNA were determined by ultraviolet spectrometry (NanoDrop ND1000). Aliquots (1.2 μg) of labeled cRNA were combined and hybridized to Agilent mouse oligo microarrays (G4121A; Agilent) following the manufacturer’s protocol. Four independent measurements were carried out for each treatment exchanged dye-labeled RNA probes (dye swap). After washing, the microarray slides were scanned using an Agilent microarray scanner (G2565BA), and data were performed with Feature Extraction software 7.1.1 (Agilent). The array data are available as the NCBI Gene Expression Omnibus series “GSE2486.”

Glucose uptake. Day 8 adipocytes were incubated for 72 h with the indicated ligands. After washing, cells were incubated for 30 min ± 10 μmol/l insulin followed by addition of deoxy-c-glucose (Sigma) and 0.5 μCi/ml deoxy-[^2,6]-H] glucose (Amersham) in a final concentration of 0.1 μmol/l for the last 6 min. The uptake was stopped by adding ice-cold PBS containing 20 μg/ml cytochalasin B (Sigma). After cell lysis, radioactivity was measured. The glucose transporter independent uptake was assessed by performing the same procedure, having 10 μg/ml cytochalasin B present as a background value.

Animals. Male C57BL/6 mice, aged 4–5 weeks, were purchased from Harlan Winkelmann (Borchen, Germany). All mice were housed in a temperature-controlled facility with a 12:12-h light/dark cycle and a diet of a 12% fat, 58% carbohydrate diet with a high-fat diet (60% kcal from fat) (17) for 16 weeks followed by randomization to either a vehicle-treated (0.5% Tween80/H2O), a pioglitazone-treated (10 mg · kg⁻¹ · day⁻¹), or a telmisartan-treated (3 mg · kg⁻¹ · day⁻¹) group. Animals were treated by oral gavage for 10 weeks. Body weight and food intake were determined throughout the experiment (twice a week). At the start and end of treatment, body composition was determined by nuclear magnetic resonance (Bruker’s Minispec MQ10). After 10 weeks’ treatment, blood samples were collected from overnight-fasted animals by retroorbital venous puncture under isoflurana anesthesia for analysis of serum insulin (mouse-insulin ELISA; Linco Research, St. Charles, MO) and glucose (colorimetric glucose test; Cypress Diagnostics, Langdorp, Belgium). An oral glucose tolerance test was performed using a dose of 2 g/kg body wt glucose. After 1 week, an insulin tolerance test (ITT) was performed by intraperitoneally injecting 0.5 units/kg insulin (Actrapid, Novo Nordisk). Tail vein blood was used for glucose quantification with a Glucometer (Precision Xtra; Abbott) during the oral glucose tolerance test and ITT. One week after the ITT, blood pressure was invasively measured in the abdominal aorta using a solid-state pressure transducer catheter (Micro-Tip 3F; Millar) and with a transducer catheter (Micro-Tip 3F; Millar Instruments) under isoflurana anesthesia for analysis of serum insulin (mouse-insulin ELISA; Linco Research, St. Charles, MO) and glucose (colorimetric glucose test; Cypress Diagnostics, Langdorp, Belgium). An oral glucose tolerance test was performed using a dose of 2 g/kg body wt glucose. After 1 week, an insulin tolerance test (ITT) was performed by intraperitoneally injecting 0.5 units/kg insulin (Actrapid, Novo Nordisk). Tail vein blood was used for glucose quantification with a Glucometer (Precision Xtra; Abbott) during the oral glucose tolerance test and ITT. One week after the ITT, blood pressure was invasively measured in the abdominal aorta using a solid-state pressure transducer catheter (Micro-Tip 3F; Millar Instruments) under isoflurana anesthesia. Afterward, animals were killed and organs were dissected. All animal procedures were in accordance with institutional guidelines and were approved.

Statistical analysis. Results from real-time PCR of cell lines, transfections, glucose uptakes, and animal experiments were analyzed by ANOVA followed by multiple comparison testing or with paired/unpaired t tests, as appropriate. Data are expressed as means ± SD or as indicated. Human data are presented as box and whisker plots and analyzed by an H test (Kruskal-Wallis) or U test (Mann-Whitney) as appropriate. Results were considered to be statistically significant at P < 0.05. For statistical analysis of microarray data, we used the significance analysis of microarrays (18).

RESULTS

PPARγ-activating ARBs negatively autoregulate PPARγ expression in murine and human adipocytes. A specific property of agonists for certain nuclear receptors is the downregulation of the receptor on mRNA and/or protein level upon ligand activation (19–21). We therefore tested the ability of ARBs to downregulate cofactor recruitment by ARBs. Oligo microarray analysis of RNA from 3T3-L1 adipocytes treated with the ARBs or the full agonist pioglitazone demonstrated qualitative differences in gene expression profiles. Finally, telmisartan improved insulin sensitivity in diet-induced obese mice in the absence of weight gain. Taken together, we have identified two ARBs, telmisartan and irbesartan, that are already widely in clinical use as new selective PPARγ modulators (SPPARMs).

RESEARCH DESIGN AND METHODS

3T3-L1 preadipocytes and COS-7 cells were purchased from the American Type Culture Collection. Cell culture reagents and lipofectamine 2000 were from Invitrogen. Trypsin and DMBSO were purchased from Sigma. [35S]-labeled methionine was purchased from Amersham. Irbesartan, telmisartan, eprosartan, pioglitazone, and rosiglitazone were kindly provided by the manufacturers. The plasmids DR1–6 × PPARγ response element (PPRE)–TkpGl basic, hPPARγ-pSG5, and murine transcriptional intermediary factor-2 (TIF2)-pSG5 were described elsewhere (14). Antibodies were purchased from Santa Cruz (PPARγ [E-8] sc7273) and Abcam (glyceraldehyde-3-phosphate dehydrogenase, ab4858).

Cell culture and differentiation. Mouse 3T3-L1 preadipocytes were cultured as described elsewhere (13). Adipocytes were serum deprived for 18 h and then treated with vehicle or various effectors as outlined. The isolation and differentiation of human preadipocytes is described elsewhere (15).

Quantitative real-time PCR. RNA isolation, reverse transcription, and quantitation of gene expression were performed as previously described using an ABI 7000 sequence detection system for real-time PCR (13). Human glyceraldehyde-3-phosphate dehydrogenase and mouse mitochondrial subunit 18S RNA were chosen for real-time PCR as endogenous controls (housekeeping genes). Sequences of primers and probes are shown in the supplemental data.

Protein analysis. Protein isolation was performed as previously described (16). Proteins were separated in 10% SDS polyacrylamide gels and transferred to polyvinylidene difluoride membrane (Amersham). After incubation with the primary antibody for PPARγ at 1:200 dilution, a secondary horseradish-conjugated antibody (Amersham) was added, and an enhanced chemiluminescent substrate kit (Amersham) was used for detection.

Assessment of receptor conformation by partial protease digestion. Two microliters of [35S]-methionine–labeled, full-length, human PPARγ was synthesized using the rabbit reticulocyte lysate and T7 RNA polymerase system (Promega) were preincubated with DMSO or the ligands for 60 min at 25°C in 40 μl digestion buffer (20 mmol/l KCl, 10 mmol/l Tris-HCl, pH 8.0, 1 mmol/l diithiothreitol, and 1 μg/ml cytochalasin B). Samples were removed before and 10, 30, and 60 min after adding trypsin to 75 μg/ml final concentration. The protease digestion was immediately terminated by the addition of denaturing gel loading buffer and boiling for 10 min. The products of digestion were separated by electrophoresis through a 10% SDS polyacrylamide gel. After drying, the gel was exposed to X-ray film. The GST pull-down. The [35S]-methionine–labeled cofactors TIF2 (AA 412–813), the coactivators vitamin D interacting protein (DRIP2, AA 250–700), and NCoR (AA 1990–2310) were synthesized using a Quick T7 TnT kit (Promega). After bacterial expression of GST-PPARγ LBD (AA 209–505) fusion protein and subsequent lysis, supernatants were incubated with glutathione-Sepharose 4B beads (Amersham), and proteins were quantified by sodium SDS gel electrophoresis and coomassie blue staining. A total of 15 μg GST-PPARγ fusion protein was incubated with 4 μl [35S]-methionine–labeled nuclear receptor interacting domain cofactors in the presence of increasing amounts of compound or vehicle for 2 h at room temperature in GST binding buffer. Bound proteins were washed four times and boiled and separated by SDS-PAGE. Bands were visualized by autoradiography and quantified by a FLA-9000 PhosphorImager (Fujiﬁlm).

FRET. The cofactor peptides were synthesized and bound to biotin. GST-PPARγ and cofactor peptides were labeled with allopregoxycyanin coupled to anti-GST antibody and R-phycocerythrin (RPE) coupled to streptavidine, respectively. Increasing amounts of compounds were incubated in the assay buffer with 35 nmol/l GST-PPARγ, 26.3 nmol/l allopregoxycyanin-labeled anti-GST antibody, 1.25 nmol/l RPE streptavidine, and 5–50 nmol/l of biotinylated cofactor peptide at 4°C for 4 h in 384-well plates. RPE was excited at 485 nm, and emission was measured at 565 nm (RPE emission) and at 670 nm (allopregoxycyanin emission). Fluorescence intensities were measured with a Genesis Freedom 200 (Tecan). Plots of fluorescence intensity (intensity at 670 nm/intensity at 635 nm) versus ligand concentration were calculated. Dose–response curves were done in triplicate using each cofactor.
FIG. 1. ARBs negatively autoregulate PPARγ expression. 3T3-L1 adipocytes (day 8) were serum deprived overnight and incubated with the ARBs or pioglitazone (all 10 μmol/l). A: mRNA expression of PPARγ was determined by real-time PCR after 24 h of incubation. B: mRNA expression of PPARγ after incubation of 10 μmol/l irbesartan or vehicle at different time points. C: PPARγ-protein expression after 24 h of incubation with the compounds at 10 μmol/l, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression is shown as a stable expressed protein. D: Human primary preadipocytes were isolated and differentiated to adipocytes. After incubation for 24 h with pioglitazone, irbesartan, and eprosartan at 10 μmol/l and telmisartan at 1 μmol/l, PPARγ mRNA expression was determined. Results are shown as box and whisker plots. *P < 0.05, #P < 0.01 vs. vehicle-treated cells.
PPARγ in 3T3-L1 and human adipocytes. Telmisartan and irbesartan downregulated PPARγ₁ and γ₂ mRNA levels in 3T3-L1 adipocytes (Fig. 1A and B). Western blotting experiments revealed modestly reduced PPARγ protein levels after telmisartan and irbesartan treatment (Fig. 1C). Eprosartan did not affect PPARγ mRNA or protein expression (Fig. 1A and C). Consistently, human adipocytes showed reduced levels of PPARγ mRNA levels after incubation with pioglitazone, telmisartan, and irbesartan (Fig. 1D). The non–PPARγ-activating ARB eprosartan had no effect (Fig. 1D). Together, these data demonstrate that PPARγ-activating ARBs moderately downregulate PPARγ expression, a typical characteristic of PPARγ receptor ligands.

**Telmisartan induces different PPARγ conformation changes compared with a TZD-full PPARγ agonist.**

**FIG. 2.** Digestion pattern of recombinant receptor protein in the presence of rosiglitazone and ARBs. [³⁵S]hPPARγ₂ was in vitro synthesized in a coupled transcription/translation system. After incubation with trypsin (75 μg/ml) for indicated times, digestion products were analyzed by SDS-PAGE followed by autoradiography. A: Labeled receptor was subsequently incubated with DMSO or 15 μmol/l rosiglitazone. B: Incubation under the same conditions with 150 μmol/l telmisartan and 150 μmol/l irbesartan. C: Different concentrations of telmisartan after 40 min of trypsin digestion.
Conformational changes of the receptor upon ligand-binding followed by a higher resistance to proteolytic degradation in protease digestion assays provides one line of evidence for direct ligand-receptor interactions. Although this in vitro assay usually requires high ligand concentrations, which do not correlate to concentrations required for receptor activation in transactivation assays, it has been shown that the full agonist rosiglitazone...
induces a conformational change in PPARγ, leading to a more compact structure of the receptor protecting the protein from trypsin digestion (22–24).

To test whether the activation of the PPARγ LBD by ARBs is mediated via a direct interaction of ARBs with the PPARγ protein, we compared the PPARγ2 digestion pattern of rosiglitazone with that of telmisartan and irbesartan. Due to two starting sites, the plasmid gave rise to a double band of labeled human PPARγ2. Rosiglitazone protected a 20- and 25-kDa band in a time-dependent digestion with trypsin compared with DMSO-incubated protein (Fig. 2A). In the presence of telmisartan, the resistant 25-kDa band was not protected but rather more susceptible to the protease. In contrast, incubation with irbesartan did not substantially influence the digestion pattern in the used concentration, which might result from the limited sensitivity of the digestion assay (Fig. 2B). Studying different concentrations of telmisartan, we observed that only concentrations >10 μmol/l showed significant effects in regard to stability of the 25-kDa band (Fig. 2C). The results of the protease digestion suggest a direct binding of telmisartan to PPARγ2, which induces a conformational change different to that elicited by rosiglitazone.

**PPARγ-activating ARBs change PPARγ cofactor specificity.** Conformational changes of PPARγ after ligand binding result in dissociation of corepressors and formation of coactivator complexes (25). We therefore tested the capacity of telmisartan and irbesartan to release the corepressor NCoR and to recruit the coactivators DRIP205 and TIF-2 to the PPARγ LBD in GST pull-down assays. With increasing concentrations of TZDs, the amount of PPARγ-bound NCoR decreased, and DRIP205 and TIF-2 were recruited (Fig. 3A). In contrast, ARBs exhibited only a slight effect on NCoR release and enhanced the interactions with DRIP205 with a lower potency than TZDs. Surprisingly, recruitment of TIF-2 was absent with ARB treatment in GST pull-down assays (Fig. 3A). To confirm these results, we performed FRET assays with peptides of the same cofactors. Rosiglitazone showed release of NCoR and strong recruitment of DRIP205 and TIF-2 (Fig. 3B). The slight release of NCoR by ARBs in the GST pull-down experiments was confirmed showing dissociation of NCoR from PPARγ starting at 3 μmol/l up to a maximum of ~50% of the rosiglitazone-induced effect at 100 μmol/l. Both compounds potently recruited DRIP205, reaching a plateau at 10 μmol/l. Consistent with the pull-down experiments, telmisartan was not able to induce recruitment of TIF-2, whereas irbesartan had a modest effect on TIF-2 recruitment at high concentrations (Fig. 3B). In line with a distinct conformational change of the receptor, characteristics of the ARBs in terms of cofactor recruitment differ from the TZD moiety with less efficiency to release NCoR and no, or rather slight, recruitment of TIF-2 to the PPARγ LBD. These results suggest that PPARγ-activating ARBs induce a conformation of PPARγ that is less permissive for dissociation of NCoR and configured for association with DRIP205, but not with TIF-2, characterizing these compounds as agonists inducing selective cofactor docking.

**Differential recruitment of TIF-2 is functionally relevant.** To prove functional relevance of the observed ligand specificity for TIF-2, COS-7 cells were transiently transfected with PPARγ2, a PPRE-dependent luciferase reporter vector, and increasing amounts of TIF-2 followed by stimulation with the ligands. In the absence of ectopic cofactor, pioglitazone (1 μmol/l), but also telmisartan and irbesartan (both 10 μmol/l), increased transcriptional activity of PPARγ (Fig. 4). Transfection of increasing amounts of TIF-2 led to an increase of basal ligand-independent activation, which was potentiated by pioglitazone and by irbesartan at the highest concentrations. Transcriptional activity induced by telmisartan did not benefit from ectopic cofactor expression and was equal to ligand-independent activation (Fig. 4). These data demonstrate the functional relevance of selective cofactor docking by ARBs.

**Divergent gene expression induced by pioglitazone and the ARBs in 3T3-L1 adipocytes.** Given the selective agonism with distinct cofactor docking by ARBs, we hypothesized that ARBs elicit a different gene expression profile compared with TZDs in 3T3-L1 adipocytes. Cells
were treated with pioglitazone, telmisartan, or irbesartan for 24 h, and pooled total RNA was hybridized on Agilent oligonucleotide microarrays. Genes with a false discovery rate of 1% (significance analysis of microarrays), when drug treatment and controls were compared, were considered as significantly deregulated. On the basis of functional relevance in regard to PPARγ and fat-cell physiology, a selection of these genes with a significant

![Figure 5](image)

*FIG. 5. Effect of ARBs and pioglitazone on the gene expression in 3T3-L1 adipocytes. A: Day 8 adipocytes were incubated for 24 h with 10 μmol/l of the compounds, and mRNA was isolated. After four hybridizations to Agilent oligo chips, PPARγ and adipocyte-related genes were clustered and divided in similarly and differentially regulated genes. B: Six randomly selected genes were confirmed by real-time PCR. *P < 0.05 vs. vehicle-treated cells.*
deregulation in at least one drug treatment was hierarchically clustered (26). Differential expression was confirmed by real-time PCR analysis for six different genes (Fig. 5B). Although in different magnitudes, most of the genes with identified PPREs in their promoters were similarly regulated by pioglitazone and the ARBs (Fig. 5A and B). Consistently with distinctive cofactor recruitment by ARBs, numerous genes involved in adipocyte function were differentially regulated by the compounds compared with the full agonist pioglitazone (Fig. 5A and B). Interestingly, there were also differences in the regulation of genes between irbesartan and telmisartan, suggesting additional differences in PPAR activation among these ARBs. These data implicate that selective cofactor binding induced by ARBs results in distinctive gene expression profiles identifying these compounds as selective PPAR ligands.

Alterations in deoxyglucose uptake due to specific gene expression profile. Next, we wanted to know whether selective PPAR activation by ARBs retains cellular metabolic functions of receptor activation. Therefore, we measured insulin-stimulated and insulin-independent incorporation of 3H-labeled deoxyglucose in 3T3-L1 adipocytes treated with the full agonist pioglitazone, the selective ARB ligands telmisartan and irbesartan, or the non–PPARγ-activating ARB eprosartan (Fig. 6). After 72 h of incubation, pioglitazone, telmisartan, and irbesartan significantly increased insulin-dependent and -independent deoxyglucose uptake, whereas eprosartan had no effect (Fig. 6).

Telmisartan improves insulin sensitivity in diet-induced obese mice without weight gain. To assess the effects of PPARγ-activating ARBs on insulin sensitivity, glucose tolerance, and body weight, we treated diet-induced obese C57BL/6J mice for 10 weeks with vehicle, telmisartan (3 mg·kg⁻¹·day⁻¹) or the full PPARγ agonist pioglitazone (10 mg·kg⁻¹·day⁻¹). Consistently with the PPARγ-modulating activity of telmisartan, animals treated with telmisartan gained significantly less weight compared with mice receiving vehicle or pioglitazone (Fig. 7A). Food intake was unaffected by telmisartan and pioglitazone (Fig. 7B). Mean arterial blood pressure was lower in the telmisartan group (vehicle: 89 ± 2.1 mmHg, telmisartan: 62.6 ± 2.5 mmHg [P < 0.05 vs. vehicle], pioglitazone: 90.8 ± 1.8 mmHg). Both treatment regimens significantly lowered fasting insulin and glucose levels compared with vehicle (insulin: vehicle: 4.2 ± 0.7 ng/ml, telmisartan: 2.5 ± 0.4 ng/ml [P < 0.05 vs. vehicle], pioglitazone: 1.3 ± 0.2 ng/ml [P < 0.01 vs. vehicle]; glucose: vehicle: 209 ± 18 mg/dl, telmisartan: 178 ± 5 mg/dl, pioglitazone: 154 ± 13 mg/dl [both P < 0.05 vs. vehicle]). Glucose tolerance and insulin sensitivity were significantly improved in the group treated with the ARB/SPARM telmisartan and in the pioglitazone-treated mice (Fig. 7C and D). Analysis of body composition by nuclear magnetic resonance revealed that differences in body weight are predominantly mediated by changes in body fat. Telmisartan decreased body fat content, whereas pioglitazone treatment augmented fat accumulation (Fig. 7E). Together, these data demonstrate that telmisartan improves insulin sensitivity in the absence of weight gain, providing in vivo evidence for its SPPARM activity.

DISCUSSION

Treatment of obese and insulin-resistant type 2 diabetic patients using PPARγ agonists such as rosiglitazone or pioglitazone leads to insulin sensitization and improved glycemic control. However, in parallel, exacerbation of weight gain and a number of adverse effects are frequently encountered. Data from hemizygous PPARγ-null mice, TZD-treated wild-type animals, and humans carrying PPARγ mutations have suggested that neither full agonism nor full antagonism of PPARγ provide the optimal approach to treat metabolic disorders (27–29). Therefore, the identification and development of improved PPARγ ligands with a moderate and more specific mode of activation remains an important objective.

A promising new group of such ligands are SPPARMs, which are compounds that, based on receptor-cofactor interactions, activate only a subset of the functions induced by cognate ligands or act in a cell-type selective manner. Recently, Berger et al. (23) described a non-TZD
partial agonist as a new SPPARM in preclinical studies. In accordance with the non-TZD partial agonist, we identified certain ARBs as partial agonists, which induce distinctive conformational receptor changes after binding. Irbesartan, in concentrations similar to telmisartan, failed to induce major changes of the PPAR\(_\gamma\)/H9253 protein digestion pattern, which might be a result of the limited sensitivity of the protease assay. However, irbesartan prominently regulated PPAR\(_\gamma\)/H9253 cofactor binding and induced selective gene expression, implicating that both ARBs, telmisartan and irbesartan, interact with PPAR\(_\gamma\) and exert SPPARM activity.

In line with the specific receptor conformation, ARBs selectively regulated adipocyte gene expression. Comparisons with gene expression patterns induced by the full agonist pioglitazone demonstrated that ARB-mediated regulation of genes involved in adipocyte lipid metabolism was moderate compared with that of the glitazone. Consistently with different effects of the ARBs on the conformational change, there was also distinct gene regulation between telmisartan and irbesartan. Some of these genes were chosen for further quantitative analysis. The prostacyclin receptor is an important inducer of adipogenesis.

**FIG. 7.** Telmisartan improves insulin sensitivity in vivo. C57BL/6J mice were fed a high-fat diet (60% kcal from fat) for 16 weeks followed by 10 weeks’ treatment with vehicle (n = 10), telmisartan (3 mg · kg\(^{-1} \cdot\) day\(^{-1}\), n = 8), or pioglitazone (10 mg · kg\(^{-1} \cdot\) day\(^{-1}\), n = 8). At the end of the treatment period, animals were metabolically analyzed. A and B: Body weight and food intake were determined frequently during treatment. C: Oral glucose tolerance tests with 2 g/kg body wt glucose and subsequent glucose analysis from the tail vein. D: ITT was performed by an intraperitoneal injection of 0.5 units/kg body wt insulin and glucose analysis. E: Total body fat content (in grams) was measured by nuclear magnetic resonance. Changes between pre- and posttreatment are shown. Results are presented as means ± SE. *P < 0.05, **P < 0.01 vs. vehicle; #P < 0.05, ##P < 0.01 vs. pioglitazone; n.s., nonsignificant.
Microarray and quantitative PCR analysis demonstrated that pioglitazone, but not the ARBs, potently enhanced expression of the prostacyclin receptor. Another important mediator of adipocytic lipid storage is glycerol kinase, which is potently stimulated by TZDs (32). Stimulation of glycerol kinase by ARBs was much weaker compared with that of pioglitazone. Together, we identified an important characteristic of the distinctive gene expression pattern induced by PPARγ-activating ARBs in adipocytes, which comprises a modest induction of adipogenic genes compared with the full glitazone agonists. This kind of selective gene regulation attenuates PPARγ-mediated adipogenesis, which may prevent weight gain during ligand therapy.

Selective cofactor dissociation/binding provides the molecular link between ligand-induced conformational changes of PPARγ and selective gene regulation, which then triggers specific metabolic in vitro/in vivo effects (23,33,34). The release of the corepressor NCOR at saturating concentrations was less pronounced with ARBs than with TZDs, which points toward their weaker adipogenic potential due to the inhibitory effect of NCOR in the differentiation process (35). Mice deficient for the PPARγ coactivator TIF-2 are protected against diet-induced obesity with an improvement of whole-body insulin sensitivity (36). Altered gene expression patterns as a result of TIF-2 deficiency were associated with a reduction of fatty acid storage in adipose tissue (36). These data demonstrate that TIF-2 is a PPARγ coactivator mainly involved in the adipogenic/lipogenic actions of the receptor and suggests that a TIF-2 independent activation of PPARγ has beneficial effects on body weight and metabolic parameters. Attenuated TIF-2 recruitment by ARBs may provide the molecular explanation for the modest induction of adipogenic genes in the gene expression analysis.

To prove the relevance of the SPPARM activity of ARBs in vivo, diet-induced obese mice were treated with telmisartan or pioglitazone. Both substances attenuated the obesity-mediated insulin resistance. However, only telmisartan was able to lower body weight in these animals, which was mainly mediated by a decrease in total body fat. These data are consistent with the study by Berger et al. (23), in which a newly identified SPPARM significantly improved parameters of insulin sensitivity without promotion of weight gain in diet-induced obese mice.

A number of clinical studies have already been conducted to provide clinical evidence for the beneficial metabolic effects of PPARγ-modulating ARBs in type 2 diabetic patients. Recently, Miura et al. (37) showed that by switching hypertensive type 2 diabetic patients from ARBs with no clinically relevant PPARγ activity to the PPARγ-modulating ARB telmisartan, a significant improvement of multiple metabolic parameters could be observed, including a decrease in fasting insulin and elevation of serum adiponectin levels. Body weight gain and edema were not detected in this study (37). In addition, in a double-blind, placebo-controlled trial in 119 diabetic patients with mild hypertension, only telmisartan significantly lowered plasma triglyceride levels, whereas the non–PPARγ-activating ARB eprosartan had no effect (10). However, in this study no change in glucose metabolism could be observed in the treatment groups (10). Together, these studies demonstrate that PPARγ-modulating ARBs are capable of retaining the beneficial metabolic PPARγ effects in vivo and in diabetic patients, which is associated with the circumvention of adverse effects such as weight gain and edema.

In conclusion, the present study characterizes two PPARγ-activating ARBs, telmisartan and irbesartan, as new SPPARMs. Selective PPARγ modulation by ARBs includes a distinct conformational change of the receptor associated with selective cofactor recruitment and a distinctive gene expression profile with a modest adipogenic pattern. Selective PPARγ modulation by ARBs may reduce the weight-promoting effects of PPARγ activation and in parallel retains PPARγ-mediated metabolic efficacy, providing a new therapeutic option for patients suffering from diabetes or the metabolic syndrome. Furthermore, the molecular characterization of these compounds will open new perspectives to develop future substances acting as SPPARM-ARBs.

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