0013-7227/96/$03.00/0
Endocrinology

Vol. 137, No. 10
Printed in U.S.A.

Thiazolidinediones Produce a Conformational Change in Peroxisomal Proliferator-Activated Receptor-γ: Binding and Activation Correlate with Antidiabetic Actions in db/db Mice

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ABSTRACT

The thiazolidinediones are novel insulin sensitizers that serve as orally active antidiabetic agents in rodents, nonhuman primates, and man. We have examined the effects of 4-week oral administration of three thiazolidinediones (AD-5075, BRL 49653, and CS-045) on plasma glucose and triglyceride concentrations in obese hyperglycemic db/db mice. All three agents lower plasma glucose and triglyceride concentrations. Normal levels of glucose are achieved after treatment with AD-5075 (>1.7 mg/kg) or BRL 49653 (≥30 mg/kg), whereas CS-045 (100 or 300 mg/kg) produces only modest reductions in either parameter. Although the thiazolidinediones have demonstrated insulin-sensitizing activities both *in vivo* and *in vitro*, their primary molecular target has been unclear. We have compared the *in vivo* antidiabetic actions described above with the *in vitro* activities on peroxisomal proliferator-activated receptor-γ (PPARγ). Hamster PPARγ1 was transiently expressed in COS-1 cells to study the binding of [^{3}H]AD-5075. The concentrations of compounds needed to displace radiolabeled AD-5075 from PPARγ correlate with their *in vivo* potency; the K_i values for displacement by cold AD-5075, BRL 49653, and CS-045 are 22, 68, and 1600 nM, respectively. To examine activation of the receptor, it was transiently cotransfected into COS-1 cells with a reporter plasmid containing two copies of a peroxisome proliferator response element. The EC_{50} values for activation are 2, 6, and 140 nM for AD-5075, BRL 49653, and CS-045, respectively. We have also analyzed limited proteolytic digests of *in vitro* translated hamster PPARγ. The thiazolidinediones produce a conformational change in PPARγ analogous to those produced by agonists of other nuclear hormone receptors. In the presence of saturating concentrations of either AD-5075 or BRL 49653, a receptor fragment of 27 kDa is protected from proteolysis by trypsin. These data support the conclusion that the antidiabetic actions of the thiazolidinediones are directly mediated through binding to PPARγ and the resulting active conformation of the receptor. Therefore, binding and transactivation assays using PPARγ should serve to identify other novel therapeutic agents with potential antidiabetic activities. (*Endocrinology* **137:** 4189–4195, 1996)

THE THIAZOLIDINEDIONES are a novel class of antidiabetic agents that produce potent insulin-sensitizing activities *in vivo* (1). In animal models of insulin resistance and noninsulin-dependent diabetes mellitus (NIDDM), such sensitization results in significant reductions in both blood glucose and lipid levels (2–4). Recently, in clinical trials with human NIDDM patients, the thiazolidinedione CS-045 (troglitazone) proved efficacious in the treatment of insulin resistance, hyperglycemia, and hyperlipidemia. This agent is expected to be approved for treatment of NIDDM in Japan.

It has been demonstrated that the thiazolidinediones can serve as agonists of peroxisomal proliferator-activated receptor-γ (PPARγ) (5). In mammals, the PPAR family of nuclear hormone receptors consists of three receptor subtypes encoded by separate genes: PPARα, PPARδ, and PPARγ. PPARs regulate the expression of genes by binding to peroxisome proliferator response elements (PPREs) in enhancer regions of regulated genes (6). This binding regulates the cellular transcription apparatus. The receptors bind to PPREs as heterodimers with a retinoid X receptor (RXR) (7). PPARα is expressed at high levels in liver (8, 9) and regulates the expression of genes involved in the β-oxidation of fatty acids as well as other aspects of lipid metabolism (10, 11). Peroxisomal proliferators, including hypolipidemic agents such as clofibrate, are PPARα agonists (12, 13). The pleiotropic effects of these compounds appear to be mediated through their activation of PPARα and the subsequent altered expression of a constellation of hepatic genes. For example, recent observations suggest that the hypolipidemic action of fibrates in humans is the result of a PPARα-mediated decrease in apolipoprotein C III transcription (14, 15). PPARδ is ubiquitously expressed in a broad range of mammalian tissues (8, 16–18). Neither the function nor the array of genes regulated by this orphan receptor is presently known. Two isoforms of PPARγ, γ1 and γ2, are present in both mice (17, 19, 20) and humans (21, 21a). Both isoforms result from alternative promoter use and differential RNA splicing of a single gene (22).

PPARγ is expressed at high levels in adipose tissue (23); furthermore, forced expression of PPARγ in pluripotent cells results in their differentiation into adipocytes (19). Interestingly, a number of thiazolidinediones have also demonstrated marked adipogenic activity when applied to preadipocytes *in vitro* (24–26). In addition, they antagonize the

Received May 8, 1996.
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antiadipogenic effects of the cytokine TNFα (27) and have been shown to possess insulin-sensitizing activity *in vitro*, potentiating insulin activation of glycogen synthase in cultured adipose tissue (28). These data and the direct demonstration of PPARγ activation by the compounds suggest that the antidiabetic activity of the thiazolidinediones may be mediated through the activation of PPARγ in adipose tissue.

To further define the molecular basis for the antidiabetic actions of the thiazolidinediones, we compared the *in vivo* efficacy and the *in vitro* activity of three compounds, AD-5075, BRL 49653, and CS-045. When administered to db/db mice, the order of potency for the hypoglycemic effect of the thiazolidinediones was AD-5075 > BRL 49653 ≫ CS-045. In binding assays using PPARγ prepared from transiently transfected COS-1 cells, the K_i values for displacement of [^{3}H]AD-5075 by AD-5075, BRL 49653, and CS-045 followed the same rank order as did the EC_{50} values for activation of the receptor in transient transactivation assays performed in COS-1 cells. Thus, the potency of thiazolidinediones as PPARγ agonists correlates with their antidiabetic efficacy. Using protease protection assays, we demonstrate that binding of the thiazolidinediones to PPARγ produced an altered conformation of the receptor such that a fragment of 27 kDa was resistant to digestion. Such conformational changes have previously been observed with agonists of other nuclear hormone receptors (29, 30). Apparently, this agonist-induced conformation of the receptor activates the cell's transcriptional machinery. Our data support the conclusion that PPARγ is the primary site of action for this novel class of antidiabetic agents.

Materials and Methods

In vivo studies

Male db/db mice (10–11 weeks old; C57Bl/KFJ; Jackson Laboratories, Bar Harbor, ME) were housed five per cage and allowed *ad libitum* access to ground Purina rodent chow (Ralston Purina, St. Louis, MO) and water. The animals and their food were weighed every 2 days and were dosed daily by gavage with vehicle (0.5% carboxymethylcellulose) with or without the test compound at the indicated dose. Drug suspensions were prepared daily. Plasma glucose and triglyceride concentrations were determined from blood obtained by tail bleeds at 3- to 5-day intervals during the 30- to 32-day study period. Glucose and triglyceride determinations were performed on either an Alpkem RFA/2 320 Micro-Continuous Flow Analyzer (Astoria-Pacific International, Clackamas, OR) or a Boehringer Mannheim Hitachi 911 automatic analyzer (Boehringer Mannheim, Indianapolis, IN) using heparinized plasma diluted 1:6 (vol/vol) with normal saline. Lean animals were age-matched heterozygous mice maintained in the same manner.

Plasmids

The pSG5-haPPARγ1 expression construct was generated by inserting the hamster PPARγ complementary DNA (cDNA) (31) into the *Eco*RI site of the pSG5 expression vector (Stratagene, La Jolla, CA) and was kindly provided by Dr. Johan Auwerx (Institut Pasteur, Lille, France). The reporter construct, pPPRE-chloramphenicol acetyltransferase (CAT), contained two copies of the peroxisomal-proliferator response element from the enhancer region of the murine acyl coenzyme A (CoA)-oxidase gene adjacent to the glutathione-*S*-transferase minimal promoter and the CAT gene. It was kindly provided by Dr. Tom Rushmore (Merck Research Laboratories, West Point, PA).

Cell culture and transfections

COS-1 cells were seeded at 2.1×10^5 cells/dish in 35-mm dishes (for transactivation assays) or 3×10^6 cells/dish in 150-mm dishes (for binding assays) in DMEM (high glucose) containing 10% charcoal-stripped FCS, nonessential amino acids, 100 U/ml penicillin G, and 100 μg/ml streptomycin sulfate at 37 C in a humidified atmosphere of 10% CO_2. After 24 h, transfections were performed with Lipofectamine (Life Technologies, Gaithersburg, MD) according to the instructions of the manufacturer. In general, for transactivation experiments, transfection mixes contained 1 μg receptor expression vector, 1 μg reporter vector, and 1 μg pCH110 (Pharmacia, Piscataway, NJ) as an internal control and, for binding studies, 20 μg receptor expression vector.

Binding assay

Transfected cells were grown for 48 h after transfection with the receptor expression vector. Receptor preparation and binding methods were based on earlier reports (32). Cell lysates containing receptor were prepared in TEGM (10 mM Tris-HCl, pH 7.2; 1 mM EDTA; 10% glycerol; 7 μl/100 ml β-Mercaptoethanol; 10 mM Na molybdate; 1 mM dithiothreitol; 5 μg/ml aprotinin; 2 μg/ml leupeptin; 2 μg/ml benzamide; and 0.5 mM phenylmethylsulfonylfluoride). Plates were placed on ice, rinsed with TEG (10 mM Tris-HCl, pH 7.2; 50 mM EDTA; and 10% glycerol), and scraped into 0.5 ml TEGM. The material was pooled, frozen in liquid nitrogen to lyse the cells, and thawed on ice. The lysate was centrifuged at 228,000 × *g* for 20 min at 4 C to remove debris and stored frozen (−80 C) until use. For each assay, an aliquot of receptor-containing lysate (0.1–0.25 mg protein) was incubated with 10 nM ditritiated AD-5075 (21 Ci/mmol) with or without test compound for ~16 h at 4 C in TEGM (300 μl final volume). Unbound ligand was removed by incubation on ice for ~10 min after the addition of 200 μl dextran/gelatin-coated charcoal. After centrifugation at 3000 rpm for 10 min at 4 C, 200-μl aliquots of supernate were counted in a liquid scintillation counter.

Transactivation assay

After transfection, cells were incubated for 48 h in culture medium with or without increasing concentrations of AD-5075, BRL 49653, or CS-045. Cells lysates were produced using reporter lysis buffer (Promega Corp., Madison, WI) according to the manufacturer's instructions. CAT activity was determined using radiolabeled butyryl CoA as substrate in a diffusion-based assay according to the manufacturer's protocol (New England Nuclear-DuPont, Wilmington, DE). β-Galactosidase activity was determined as previously described (33).

Protease digestion assay

The protease digestion assay was performed by the method of Allen *et al.* (29) with minor modifications. The plasmid pSG5-haPPARγ1 was used to synthesize ^{35}S-radiolabeled PPARγ1 in a coupled transcription/translation system according to the protocol of the manufacturer (Promega). The transcription/translation reactions were subsequently aliquoted into 22.5-μl volumes, and 2.5 μl PBS with or without a thiazolidinedione were added. These mixtures were incubated for 20 min at 25 C, separated into 4.5-μl aliquots, and 0.5 μl distilled water (dH_2O) or dH_2O-solubilized trypsin were added. The protease digestions were allowed to proceed for 10 min at 25 C, then terminated by the addition of 20 μl denaturing gel loading buffer and boiling for 5 min. The products of the digestion were separated by electrophoresis through a 1.5-mm 12% polyacrylamide-SDS gel. After electrophoresis, the gels were fixed in 10% acetic acid (vol/vol)-40% methanol (vol/vol) for 30 min, treated in EN^{3}HANCE for an additional 30 min, and dried under vacuum for 2 h at 80 C. Autoradiography was then performed to visualize the radiolabeled digestion products.

Materials

DMEM and other cell culture reagents were obtained from Life Technologies (Grand Island, NY). [1-^{14}C]Butyryl CoA, [^{35}S]methionine, and EN^{3}HANCE were purchased from DuPont-New England Nuclear. All other reagent grade chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). AD-5075 (5-[4-[2-(5-methyl-2-phenyl-4-oxazolyl)-2-hydroxyethoxy]benzyl]-2,4-thiazolidinedione), BRL 49653 (5-(4-[2-[methyl-(2-pyridyl)amino]ethoxy]benzyl)thiazolidine-2,4-dione) and CS-045 (5-[4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl-methoxy)benzyl]-2,4-thiazolidinedione) were kindly provided by Dr. Gerard Kiec-

zykowski, Dr. Philip Eskola, Dr. Conrad Santini, Mr. Joseph F. Leone, and Mr. Peter A. Cicala (Merck Research Laboratories, Rahway, NJ). As the thiazolidinediones were solubilized in dimethylsulfoxide, control cells were incubated with equivalent dimethylsulfoxide concentrations of 0.1% or less, a concentration that demonstrated no effect on PPARγ activity. [^{3}H] AD-5075 was kindly provided by Drs. Allan Jones, Avery Rosegay, and Frank Tang (Merck Research Laboratories, Rahway, NJ)

Results

Male db/db mice are severely insulin resistant, hyperglycemic, and hypertriglyceridemic. Treatment of such animals with any of the thiazolidinediones shown in Fig. 1 by daily oral gavage reduced both plasma glucose and triglyceride concentrations. Treatment with either AD-5075 (1.7 or 5 mg/kg) or BRL 49653 (30 mg/kg) produced normal concentrations of glucose, whereas CS-045 treatment (100 or 300 mg/kg) resulted in only modest reductions (Fig. 2). All three compounds reduced triglyceride concentrations more than glucose. Both AD-5075 and BRL 49653 reduced triglycerides to normal levels or lower, whereas CS-045-treated mice achieved levels found in lean animals (Fig. 3). Taken together, these data demonstrate that the *in vivo* rank order of potency for these three compounds is AD-5075 > BRL 49653 ≫ CS-045.

Having ranked the antidiabetic efficacy of these thiazolidinediones in the insulin-resistant db/db mouse, we then characterized their interactions with PPARγ1 to determine an *in vitro* ranking for comparison. Binding of ditritiated AD-5075 ([^{3}H]AD-5075) to the hamster PPARγ1 was characterized using receptor transiently expressed in COS-1 cells. The ligand bound saturably to a single class of sites with an affinity of 6 nM (Fig. 4). The three unlabeled thiazolidinediones were used to generate competition curves with 10 nM [^{3}H]AD-5075 (Fig. 5a). The K_i values for displacement by cold AD-5075, BRL 49653, or CS-045 were 22, 68, and 1600 nM, respectively. This order is consistent with the *in vivo* activity of the compounds.

To determine the *in vitro* potency of the three thiazolidinediones as agonists of PPARγ, COS-1 cells were cotransfected with an expression vector containing hamster PPARγ1 and a reporter construct containing two copies of the acyl CoA oxidase PPRE consensus sequence adjacent to the glutathionine-*S*-transferase minimal promoter and the CAT gene. After transfection, the cells were incubated for 48 h in medium with or without increasing concentrations of AD-5075, BRL 49653, or CS-045. All three compounds activated the receptor in a dose-dependent manner (Fig. 5b). Paralleling the results of the above binding assays, the compounds demonstrated EC_{50} values of 2, 6, and 140 nM, respectively. These data clearly demonstrate that the thiazolidinediones are agonists of PPARγ and that structural diversity in the compounds alters both their affinity for the receptor and their potency as agonists. Additional experiments using the transactivation assay, demonstrated that none of the three

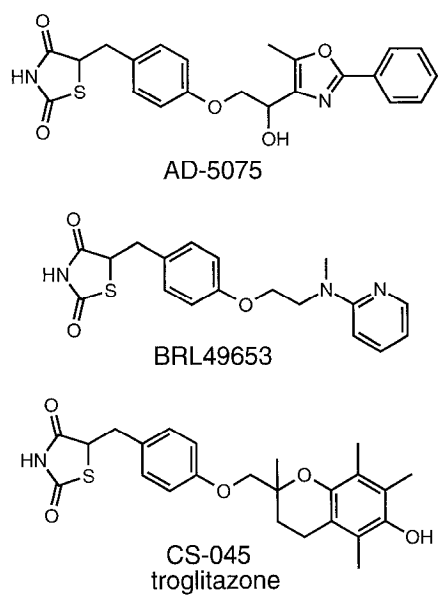

FIG. 1. Structures of thiazolidinediones.

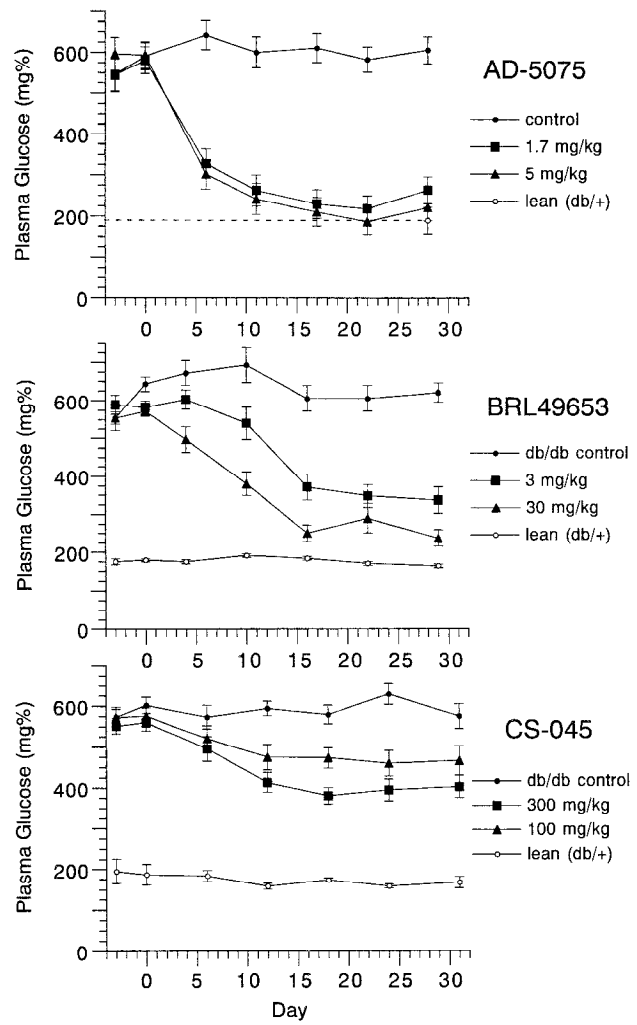

FIG. 2. Thiazolidinediones lower plasma glucose concentrations. Daily oral treatment of db/db mice with AD-5075, BRL 49653, or CS-045 at the indicated doses lowered plasma glucose concentrations. Doses of AD-5075 of 1.7 mg/kg or more lowered glucose to levels that were not significantly different from those in age-matched heterozygous lean mice. To achieve a similar effect required doses of BRL 49653 of 30 mg/kg or more, whereas even at doses as great as 300 mg/kg, CS-045 produced only modest reductions in glucose concentrations.

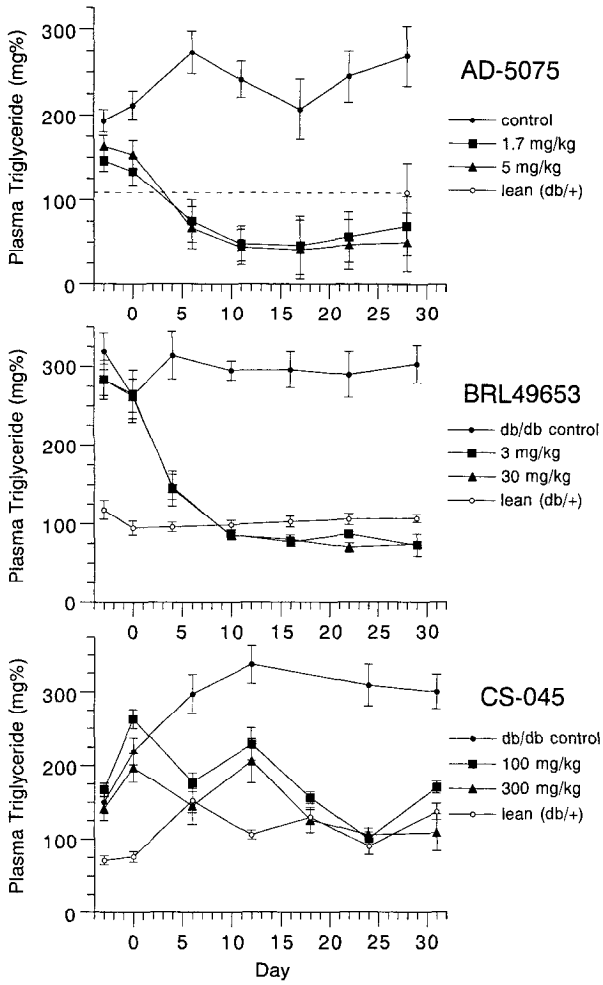

FIG. 3. Thiazolidinediones lower plasma triglyceride concentrations. Daily oral treatment of db/db mice with AD-5075, BRL 49653, or CS-045 at the indicated doses lowered plasma triglyceride concentrations to the level of age-matched heterozygous lean mice or below.

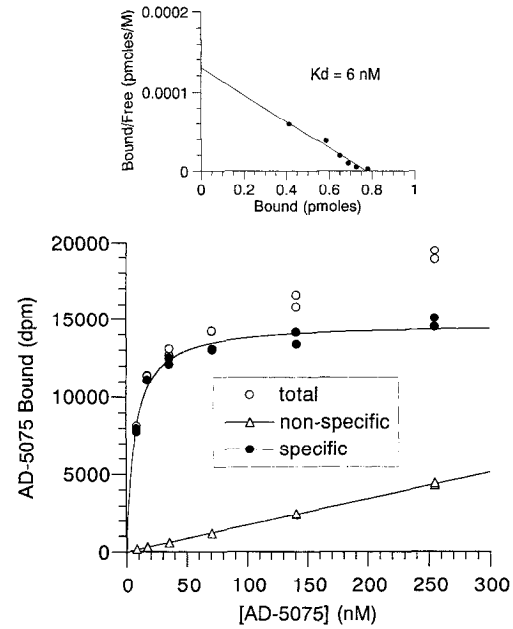

FIG. 4. [^{3}H]AD-5075 binds specifically to PPARγ1. Hamster PPARγ1 produced by transient transfection of COS-1 cells was incubated with increasing concentrations of [^{3}H]AD-5075. Saturation analysis demonstrates a single class of specific binding sites with a K_d of 6 nM.

thiazolidinediones tested transactivated transcription via either PPARα or PPARδ (data not shown).

Proteolytic analysis has previously been used to demonstrate that high affinity ligands of the steroid, retinoid, and thyroid receptors can, upon binding, specifically alter the conformation of the receptors (29, 30). It has been proposed that such conformational changes are critical to the activity of the receptors. To determine whether similar alterations in conformation occur when the thiazolidinediones interact with PPARγ, a limited protease digestion assay was performed (29). Radiolabeled PPARγ was synthesized by incubating the expression vector containing the hamster PPARγ1 cDNA in a coupled, *in vitro* transcription/translation system. The newly synthesized receptor was preincubated alone or in the presence of a saturating concentration of thiazolidinedione and then treated with increasing concentrations of trypsin. The digestion mixture was separated by SDS-PAGE and visualized by autoradiography. As demonstrated in Fig. 6, incubation of the receptor (M_r, ~54 kDa) with increasing concentrations of trypsin in the absence of ligand leads to the complete digestion of the receptor. In contrast, when PPARγ was preincubated with 10 μM AD-5075 or BRL 49653, a major protease-resistant fragment of 27 kDa was observed (Fig. 6). Similar results were observed with 1 μM AD-5075 or 100 μM CS-045 (data not shown). This conformational change suggests that the active conformation of PPARγ, like that of the steroid, thyroid, and retinoic acid receptors, assumes a more compact structure after the binding of agonist.

Discussion

The results presented here demonstrate that thiazolidinediones are orally active antidiabetic agents in db/db mice. These compounds significantly lowered plasma glucose and triglyceride concentrations in the obese, insulin-resistant db/db mouse. Glucose levels were normalized with AD-5075 and BRL 49653 at doses of 1.7 and 30 mg/kg, respectively. In contrast, CS-045 only partially normalized blood glucose levels at doses up to 300 mg/kg. The compounds are more efficacious as hypotriglyceridemic agents, because at the doses studied, all three normalized plasma triglyceride levels. We conclude that the rank order of the *in vivo* efficacy of the thiazolidinediones studied is AD-5075 > BRL 49653 ≫ CS-045.

It has recently been demonstrated that thiazolidinediones are ligands of the nuclear hormone receptor PPARγ (5). To correlate the role of PPARγ with the antidiabetic action of these agents, the compounds were tested for their ability to bind to and activate the receptor *in vitro*. In binding assays using PPARγ from transiently transfected cells, the compounds competed for binding of [^{3}H]AD-5075 with K_i values that paralleled their *in vivo* efficacy. Similarly, in transient transactivation assays, the order of potency of the com-

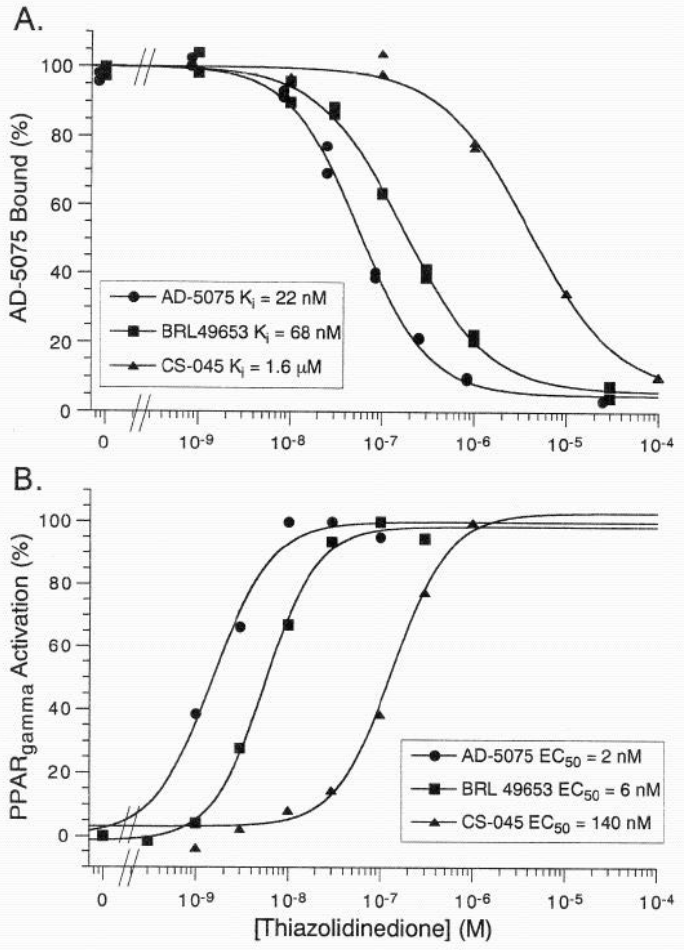

FIG. 5. The binding and activation of PPARγ1 by the thiazolidinediones parallels their *in vivo* efficacy. A, Competition curves generated by incubation of 10 nM [^{3}H]AD-5075 with hamster PPARγ1 produced by transient transfection of COS-1. The percentage of ligand bound after incubation in the presence of the indicated concentration of each unlabeled compound for ~16 h is plotted. Similar results were obtained in at least two independent experiments performed in duplicate. B, Activation of PPARγ in COS-1 cells transiently cotransfected with pSG5-hamPPARγ1 and pPPRE-CAT. The figure plots normalized CAT activity after incubation in the presence of the indicated concentrations of each compound for 48 h. Similar results were obtained in two independent experiments performed in triplicate.

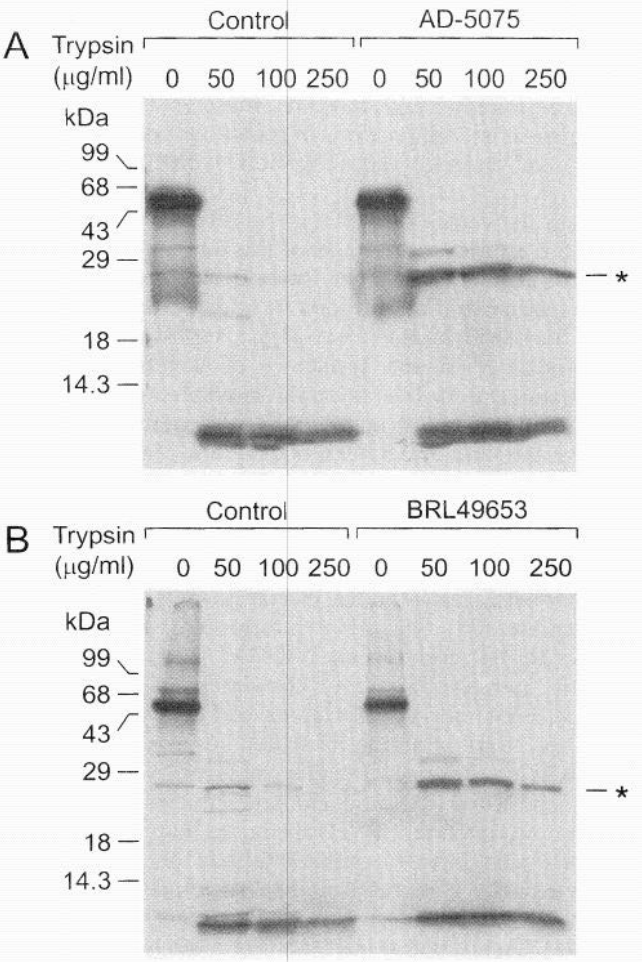

FIG. 6. Thiazolidinediones produce a partially protease-resistant conformation of PPARγ. [^{35}S]PPARγ was synthesized *in vitro* in a coupled transcription/translation system. It was subsequently preincubated with 0.1% dimethylsulfoxide (Control), 10 μM AD-5075, or 10 μM BRL 49653, then incubated with dH_2O or increasing concentrations of trypsin. Digestion products were analyzed by SDS-PAGE followed by autoradiography. An *asterisk* indicates the 27-kDa protease-resistant fragment of PPARγ.

pounds was AD-5075 > BRL 49653 ≫ CS-045. In protease protection assays, saturating concentrations of either AD-5075 or BRL 49653 altered the conformation of PPARγ such that a 27-kDa fragment became resistant to digestion. Thus, saturating concentrations of thiazolidinediones produce conformational changes in PPARγ that impart resistance to protease digestion in a manner analogous to those produced by agonists of other nuclear hormone receptors (29, 30). These conformational changes presumably reflect activation of the receptors. Therefore, our results confirm and extend the observation (5) that thiazolidinediones are a novel class of PPARγ agonist. Furthermore, the data presented here strongly support the conclusion that the antidiabetic actions of thiazolidinediones are directly mediated through their ability to serve as agonists of PPARγ.

PPARγ has been shown to be expressed at high levels in mammalian adipose tissue. It has become apparent that the receptor plays an important regulatory role in adipocyte differentiation and metabolism. Forced overexpression of PPARγ in fibroblasts was shown to be sufficient to cause increased expression of adipocyte-specific genes and differentiation into adipocytes (23). Furthermore, ectopic expression of PPARγ2 and C/EBPα in cultured myoblasts caused a change from myogenesis to adipogenesis (34). It has been demonstrated that the transcriptional activity of the adipocyte-specific fatty acid binding protein, aP2, and phosphoenolpyruvate carboxykinase adipocyte gene promoters are both up-regulated by PPARγ (19, 35). In light of these findings, it is not surprising that the thiazolidinediones have demonstrated several direct actions on adipocytes *in vitro*. They have been shown both to serve as potent adipogenic agents, augmenting differentiation of 3T3-L1 preadipocytes (25), and to antagonize the antiadipogenic actions of TNFα (27). Recently, these antidiabetic agents were found to augment insulin activation of glycogen synthase in cultured

adipose tissue (28). They have also been shown to antagonize isoproterenol-dependent inhibition of insulin-induced activation of phospatidylinositol 3-kinase in 3T3-L1 adipocytes (36). Taken together with our data, these results suggest that the insulin-sensitizing actions of the thiazolidinediones may be mediated through the activation of PPARγ in adipose tissue.

Beyond activation of PPARγ, the mechanism(s) that underlies the antidiabetic effects of the thiazolidinediones remains unresolved. However, several observations support the suggestion that aberrations in adipose tissue gene regulation and lipid homeostasis play a significant role in the pathogenesis of insulin resistance of skeletal muscle, the major tissue responsible for insulin-mediated glucose uptake and utilization. First, it has long been recognized that there is a close association between obesity and the development of insulin resistance and NIDDM (37). In fact, obesity, which is found in more than 80% of NIDDM patients, appears to be the greatest risk factor for this disease. It has been demonstrated that weight loss, *i.e.* diminutions in fat tissue, results in significant improvements in insulin sensitivity and glucose disposal (38). Second, it is important to note that in addition to hyperglycemia, NIDDM is characterized by markedly increased levels of circulating triglycerides and FFA (38). Elevated concentrations of FFAs potentiate FFA uptake and lipid oxidation by skeletal muscle, resulting in a decrease in insulin-mediated glucose disposal (39); FFAs also promote increased hepatic gluconeogenesis. Third, it has been demonstrated that TNFα abrogates insulin signal transduction, particularly in skeletal muscle (40, 41), and that adipocyte-specific expression of the cytokine is elevated in both animal models of insulin resistance (42) and obese humans (43). Treatment of such animal models with the thiazolidinedione pioglitazone partially normalized expression of TNFα in parallel with its insulin-sensitizing actions (44). Thus, PPARγ-mediated alterations in expression of a set of adipose tissue genes may lead to reduced release of FFAs, TNFα, or other factors. These effects could, in turn, indirectly ameliorate muscle insulin resistance or increased hepatic glucose output, resulting in a substantial decrease in glucose. Ongoing efforts to embellish our knowledge of the roles played by both PPARγ and adipose tissue in regulating insulin's metabolic actions should further our understanding of the pathogenesis of NIDDM and uncover novel treatment strategies.

In summary, we have demonstrated that the *in vivo* efficacy of thiazolidinediones as antidiabetic agents correlates with their potency as PPARγ agonists *in vitro*. Protease protection assays demonstrated that the activated state, after binding of these insulin-sensitizing PPARγ agonists, exists in an altered physical conformation that is resistant to digestion by trypsin. Thus, it is apparent that PPARγ is a primary site of action of the antidiabetic thiazolidinediones.

Acknowledgments

The authors thank Dr. Gerard Kieczykowski, Dr. Philip Eskola, Dr. Conrad Santini, Mr. Joseph F. Leone, and Mr. Peter A. Cicala of the Merck Medicinal Chemistry Laboratories for providing AD-5075, BRL 49653, and CS-045. We appreciate the preparation of [^{3}H]AD-5075 by Drs. Allan Jones, Avery Rosegay, and Frank Tang of the Merck Research Laboratories. We are indebted to Dr. Johan Auwerx (Institut Pasteur, Lille, France) and Dr. Tom Rushmore (Merck Research Laboratories, West Point, PA) for generously providing vectors pSG5-haPPARγ and pPPRECAT, respectively. The assistance of Mr. Edward Brady, Ms. Michele Mariano, Mr. Joseph McDonnell, Mr. Roger Meurer, Dr. Vernon Moore, Ms. Julie Olszewski, and Mr. Peter Zafian in performance of the animal studies is also gratefully acknowledged. Dr. Jeffrey Toney generously assisted in the initiation of the binding assays.

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