Specific Activation of the Nuclear Receptors PPARγ and RORA by the Antidiabetic Thiazolidinedione BRL 49653 and the Antiarthritic Thiazolidinedione Derivative CGP 52608

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ABSTRACT
The thiazolidinedione BRL 49653 and the thiazolidinedione derivative CGP 52608 are lead compounds of two pharmacologically different classes of compounds. BRL 49653 is a high affinity ligand of peroxisome proliferator-activated receptor γ (PPARγ) and a prototype of novel antidiabetic agents, whereas CGP 52608 activates retinoic acid receptor-related orphan receptor α (RORA) and exhibits potent antiarthritic activity. Both receptors belong to the superfamily of nuclear receptors and are structurally related transcription factors. We tested BRL 49653 and CGP 52608 for receptor specificity on PPARγ, RORA, and retinoic acid receptor α, a closely related receptor to RORA, and compared their pharmacological properties in in vitro and in vivo models in which these compounds have shown typical effects. BRL 49653 specifically induced PPARγ-mediated gene activation, whereas CGP 52608 specifically activated RORA in transiently transfected cells. Both compounds were active in nanomolar concentrations. Leptin production in differentiated adipocytes was inhibited by nanomolar concentrations of BRL 49653 but not by CGP 52608. BRL 49653 antagonized weight loss, elevated blood glucose levels, and elevated plasma triglyceride levels in an in vivo model of glucocorticoid-induced insulin resistance in rats, whereas CGP 52608 exhibited steroid-like effects on triglyceride levels and body weight in this model. In contrast, potent antiarthritic activity in rat adjuvant arthritis was shown for CGP 52608, whereas BRL 49653 was nearly inactive. Our results support the concept that transcriptional control mechanisms via the nuclear receptors PPARγ and RORA are responsible at least in part for the different pharmacological properties of BRL 49653 and CGP 52608. Both compounds are prototypes of interesting novel therapeutic agents for the treatment of non-insulin-dependent diabetes mellitus and rheumatoid arthritis.

BRL 49653 \((\pm)-5-(4-[(2-methyl-2-pyridylamino)ethoxy](phenyl)methyl]2,4-thiazolidinedione)\) is a novel hypoglycemic and hypolipemic agent used in animal models of NIDDM (Oakes et al., 1994). BRL 49653 and structurally related thiazolidinediones such as ciglitazone, pioglitazone, and troglitazone improve insulin resistance by enhancing insulin action in skeletal muscle, liver, and adipose tissue. Although the precise mechanism of action remains unknown, it has been recently shown that these thiazolidinediones, as well as the prostaglandin J3 metabolite 15d-PGJ2, are ligands of the PPARγ (Forman et al., 1995; Lehmann et al., 1995; Lambe and Tugwood, 1996). Thiazolidinedione-induced activation of PPARγ correlates with the antidiabetic actions in vivo (Berger et al., 1996). PPARγ is a member of the nuclear receptor superfamily of transcription factors (for a review, see Schoonjans et al., 1996). The three known PPAR subtypes (α, β, and γ) exhibit typical tissue distribution in adult animals and during development (Braissant et al., 1996). The expression of PPARγ is one of the earliest events during the differentiation of fibroblasts to adipocytes (Tontonoz et al., 1994a), and ectopic expression of PPARγ promotes this conversion (Tontonoz et al., 1994b). PPARγ regulates the transcription of several adipocyte-specific genes, including phosphoenolpyruvate carboxykinase, adipocyte fatty acid binding protein aP2, and leptin (Schoonjans et al., 1996). Antidiabetic thiazolidinediones and 15d-PGJ2 promote adipocyte differentiation and suppress leptin gene expression in concentrations similar to their \(K_d\) values for binding to PPARγ (De Vos et al., 1996; Kallen and Lazar, 1996). These findings suggest a pivotal role for PPARγ and its ligands in controlling adipocyte development and glucose homeostasis.

ABBREVIATIONS: PPAR, peroxisome proliferator-activated receptor; ROR, retinoic acid receptor-related orphan receptor; RORA, retinoic acid receptor-related orphan receptor α; RAR, retinoic acid receptor; RXR, retinoid X receptor; RA, all-trans retinoic acid; GR, glucocorticoid receptor; CAT, chloramphenicol acetyltransferase; FCS, fetal calf serum; NIDDM, non-insulin-dependent diabetes mellitus; PGD2, prostaglandin D2; 15d-PGJ2, 15-deoxy-\(12,14\)-prostaglandin J2.
CGP 52608 [1-[3-allyl-4-oxo-thiazolidine-2-ylidene]-4-methyl-thiosemicarbazone] is the lead compound of a structurally different class of thiazolidinedione derivatives with potent therapeutic effects in experimental arthritis models (Missbach et al., 1996). This compound has been shown to specifically activate RORA, another member of the nuclear receptor superfamily (Wiesenberg et al., 1995). RORA (RORα or RZRα) is one of three known subtypes (α, β, and γ) of the ROR. Each subtype shows a characteristic tissue expression pattern (for a review, see Carlborg and Wiesenberg, 1995). In searching for a natural ligand, the pineal gland hormone melatonin was found to specifically activate RORA and to compete with CGP 52608 for binding (Wiesenberg et al., 1995). Structure-activity relationship studies with CGP 52608 analogues revealed a striking correlation between activation of RORA and inhibition of rat adjuvant arthritis, suggesting a key role of this receptor in mediating the antiarthritic effects of these compounds (Missbach et al., 1996).

The identification of orphan receptor ligands is always an important step toward a better understanding of their regulatory functions during development and homeostasis. In the case of PPARs, structurally diverse compounds such as peroxisome proliferators, anti diabetic thiazolidinediones, fatty acids, prostaglandin and leukotriene derivatives, and the endogenous steroid dehydroepiandrosterone have been shown to activate PPARs, either directly as ligands or indirectly by as-yet-unknown mechanisms (Devchand et al., 1996; Peters et al., 1996; Forman et al., 1997; Kliewer et al., 1997). So far, known activators of RORs are the pineal gland hormone melatonin (Becker-Andre et al., 1994; Wiesenberg et al., 1995) and the antiarthritic thiazolidinedione derivatives (Missbach et al., 1996).

Given the diversity of compounds capable of activating PPARs, the aim of this study was to investigate the specificity of PPARγ and RORA activation by the anti diabetic thiazolidinedione BRL 49653 and the antiarthritic thiazolidinedione derivative CGP 52608 and to compare their effects in functional assays in which either compound had shown typical effects. The models used were leptin producing 2T3-CL1 cells (2 x 10^6 cells per well in a six-well plate) grown overnight in Schneider's medium (Life Technologies, Grand Island, NY) without FCS. Liposomes were formed by incubating 2 μg of the reporter plasmid, 1 μg of receptor expression vector, and 1 μg of the reference plasmid pCH110 (Pharmacia, Piscataway, NJ) with 15 μg of N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (Boehringer-Mannheim, Mannheim, Germany) for 15 min at room temperature in a total volume of 100 μl. After dilution with 0.9 ml of Schneider's medium, the liposomes were added to the cells. Eight hours after transfection, 500 μl of Schneider's medium supplemented with the indicated ligand was added. After an additional 16 hr, the cells were harvested, and CAT assays were performed. The CAT activities were normalized to β-galactosidase activity, and induction factors were calculated as the ratio of CAT activity of ligand-stimulated cells to that of mock-induced controls. Each condition was analyzed in triplicate, and data are shown as mean values with standard deviation.

**Materials and Methods**

**Compounds.** All thiazolidinediones and derivatives were synthesized in the Department of Chemical Research (Novartis Pharma AG, Basel, Switzerland). The structures of CGP 52608 and BRL 49653 are given in Fig. 1. CGP 52608, CGP 55707, and CGP 55066 were synthesized as described by Missbach et al. (1996), and BRL 49653 (racemate) was synthesized as described by Cantello et al. (1994). Melatonin and PGD2 were obtained from Fluka (Buchs, Switzerland), and 15d-PGJ2 was from Cayman Chemical (Ann Arbor, MI). Thiazolidinedione derivatives and melatonin were dissolved in dimethylsulfoxide, and prostaglandins were dissolved in ethanol at 10 mM; dilutions were made in cell culture medium before use.

**Preparative separation of the enantiomers of BRL 49653.** The two enantiomers of BRL 49653 were separated from the racemate by chiral high performance liquid chromatography (Abbott et al., 1994). A 500 x 50-mm column loaded with Chiracel absorbance was used. The eluent was 15% ethanol in n-heptane, and the flow rate was 150 ml/min. The enantiomers were detected by UV (254 nm). Retention times were 14.6 min for the R(+)-enantiomer and 37.6 min for the S(-)-enantiomer, yielding 99.6% pure R(+)-enantiomer and 77% pure S(-)-enantiomer.

**DNA constructs, transfection, and CAT assays.** Materials and methods were described in detail by Wiesenberg et al. (1995). Briefly, we used the pBLCAT2-derived CAT reporter constructs, containing, in the XbaI site, natural response elements for ROR (CAAAAAAGGTCTCA), identified in the human 5-lipoxygenase gene promoter (Steinhilber et al., 1995); for PPAR (AATGCTAGTATAGTTCAATAGTCTCA), found in the mouse bifunctional enzyme gene promoter (Bardot et al., 1993); or for RAR (AGGTTCCAGCAGAGTCAGCCAGTCAGCTCA), from the human RARβ gene promoter (De The et al., 1990). The cDNAs of human RORA (Becker-Andre et al., 1993), Xenopus laevis PPARγ (Dreyer et al., 1992), human RXRα, and human RARα have been subcloned into the expression vector pSG5 (Stratagen, La Jolla, CA).

**Drosophila SL-3 cells (2 x 10^6 cells per well in a six-well plate) were grown overnight in Schneider's medium (Life Technologies, Grand Island, NY) without FCS. Liposomes were formed by incubating 2 μg of the reporter plasmid, 1 μg of receptor expression vector, and 1 μg of the reference plasmid pCH110 (Pharmacia, Piscataway, NJ) with 15 μg of N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (Boehringer-Mannheim, Mannheim, Germany) for 15 min at room temperature in a total volume of 100 μl. After dilution with 0.9 ml of Schneider's medium, the liposomes were added to the cells. Eight hours after transfection, 500 μl of Schneider's medium supplemented with the indicated ligand was added. After an additional 16 hr, the cells were harvested, and CAT assays were performed. The CAT activities were normalized to β-galactosidase activity, and induction factors were calculated as the ratio of CAT activity of ligand-stimulated cells to that of mock-induced controls. Each condition was analyzed in triplicate, and data are shown as mean values with standard deviation.

**Secretion of leptin by fully differentiated 3T3-F442A adipocytes.** The clonal cell line 3T3-F442A (Green and Kehinde, 1976) was obtained through the courtesy of Dr. B. Fève (INSERM Créteil, France). Differentiation was carried out as described previously (Dani et al., 1989). Cells were plated onto 12-well tissue culture plates (Falcon) at a density of 1.5 x 10^3 cells/cm^2 in Dulbecco's...
modified Eagle’s medium supplemented with 8% FCS, 200 units/ml penicillin, 50 μg/ml streptomycin, 33 μM biotin, and 17 μM pantethe- 
nate. Differentiation was initiated after the cells reached confluency by 
adding to the standard medium 2 mM triiodothyronine, 17 nM 
insulin, 100 nM dexamethasone, and 100 μM isobutylmethylxanthine ( 
differentiation medium). After 3 days, the medium was replaced by 
differentiation medium without dexamethasone and isobutylmethyl-
Xanthine and changed thereafter every second day for 2 weeks until 
differentiation to adipocytes was complete.

For measurement of leptin secretion, fully differentiated adipocy-
tes were incubated for 72 hr in fresh medium containing test 
compounds or the vehicle. Immunoactive leptin was quantified in 
the supernatants by radioimmunoassay using rabbit polyclonal an-
tibodies against recombinant mouse leptin (Rentsch et al., 1995). 
Typically, 50-μl standards or samples were incubated for 18 hr at 4°C 
with 50 μl of radiolabeled [125I]leptin, 50 μl of antiserum (diluted 
1:4000), and 50 μl of phosphate-buffered saline in the presence of 
0.1% Tween-20 and 0.1% bovine serum albumin (final concentra-
tions). Bound and free leptin were separated by centrifugation (30 
min at 3000 × g at 4°C) after the addition of 600 μl of 20% polyeth-
ylene glycol and 50 μl of γ-globulin (10 mg/ml). 50 μl of radiolabeled 
leptin were incubated for 72 hr in fresh medium containing test 
compounds or the vehicle. Immunoactive leptin was quantified in 
the supernatants by radioimmunoassay using rabbit polyclonal an-
tibodies against recombinant mouse leptin (Rentsch et al., 1995). 
Briefly, male Lewis rats (LEW/TIF; specific pathogen free, 250–270 g body weight, five animals/group; Ciba Animal Farm, Sisseln, Switzerland) received 
either dexamethasone alone (0.15 mg/kg p.o.) or a combination of 
dexamethasone (0.15 mg/kg p.o.) and CGP 52608 or BRL 49653 
(0.01–10 mg/kg p.o.) for 9 days. Test compounds were dissolved in 
0.5 ml of DMSO plus 4.5 ml of 0.75% methylcellulose and were admin-
istered by oral gavage on days 4 and 9. Blood glucose and plasma triglyceride levels were estimated using 
commercially available test kits (Boehringer-Mannheim). Differences 
tween groups were statistically evaluated by Student’s t test.

Rat adiponectin arthritis. Adiponectin arthritis was induced as de-
scribed previously (Wiesenbarg et al., 1989). Briefly, maleLewis rats 
(LEW/TIF; SPF, 180–200 g body weight) were immunized by an 
intradermal injection of Freund’s complete adjuvant (0.2 mg of heat-

denatured Mycobacterium butyricum (Difco, Detroit, MI) in 0.05 ml of 
phosphate-buffered saline in the presence of 
0.1% Tween-20 and 0.1% bovine serum albumin (final concentra-
tions). Bound and free leptin were separated by centrifugation (30 
min at 3000 × g at 4°C) after the addition of 600 μl of 20% polyeth-
ylene glycol and 50 μl of γ-globulin (10 mg/ml). Pellets were counted 
in a gamma counter. The detection limit was 0.048 ng of leptin/ml. 
Experiments were performed in triplicate, and the results are given 
as mean values with standard deviation.

Glucocorticoid-induced insulin resistance. Male Lewis rats 
(LEW/TIF; specific pathogen free, 250–270 g body weight, five animals/group; Ciba Animal Farm, Sisseln, Switzerland) received 
either dexamethasone alone (0.15 mg/kg p.o.) or a combination of 
dexamethasone (0.15 mg/kg p.o.) and CGP 52608 or BRL 49653 
(0.01–10 mg/kg p.o.) for 9 days. Test compounds were dissolved in 
0.5 ml of DMSO plus 4.5 ml of 0.75% methylcellulose and were admin-
istered by oral gavage on days 4 and 9. Blood glucose and plasma triglyceride levels were estimated using 
commercially available test kits (Boehringer-Mannheim). Differences 
tween groups were statistically evaluated by Student’s t test.

Results

Specific activation of PPARγ and RORA. PPARγ- 
and RORA-mediated transcriptional activation was investigated in 
transiently transfected Drosophila SL-3 cells under se-
rum-free conditions to overcome the high constitutive activity 
of RORA in the presence of serum (Wiesenbarg et al., 1995; Missbach et al., 1996). SL-3 cells were transfected with the expression vectors for X. laevis PPARγ and human RXRα or with human RORA and a thymidine kinase-CAT reporter 
construct containing either the PPAR response element 
found in the promoter of the mouse bifunctional enzyme 
(Bardot et al., 1993) or the ROR response element of the 
human 5-lipoxygenase gene (Steinhilber et al., 1995). To fur-
ther evaluate receptor specificity, all compounds were also 
tested on RARα/RXRα heterodimers on the RA response 
element of the human RARα gene promoter. The structures of 
the lead compounds BRL 49653 and CGP 52608 are shown in 
Fig. 1, and the results of receptor-mediated gene activation 
are shown in Fig. 2.

The antidiabetic thiazolidinedione BRL 49653 (0.01–1 μM) 
specifically stimulated PPARγ-mediated gene activity. Sur-
prisingly, only minor differences in potency were seen be-
tween the two enantiomers of BRL 49653. The racemate 
and both enantiomers induced a 2–7-fold gene induction in con-
centration ranges of 0.01 and 1 μM. The putative natural 
PPARγ ligand 15d-PGJ2 was distinctly less potent and in-
duced only a 1.4–2.6-fold gene induction in the same concen-
tration range. PGD2, a natural precursor of 15d-PGJ2 (10 
μM), as well as the antiarthritic thiazolidinedione derivative 
CGP 52608, two structural analogues (CGP 55066, CGP 
55707), and the pineal gland hormone melatonin did not 
induce PPARγ-mediated gene activation at 10 μM.

RORα-mediated gene activation was shown only for the 
antiarthritic thiazolidinedione derivative CGP 52608 (0.01–1 
μM, 3.1–4.9-fold) and the putative natural ROR ligand mel-
atonin (0.01–1 μM, 2.4–4.6-fold) (Missbach et al., 1996). The RAR was selected for specificity control (Carlberg et al., 1994).

These results demonstrate PPARγ specificity for the 
teradiabetic thiazolidinedione BRL 49653 and 15d-PGJ2 
and RORA specificity for the antiarthritic thiazolidinedione der-
ivative CGP 52608 and the pineal gland hormone melato-

Influence on leptin secretion by differentiated 3T3-
F422A adipocytes. PPARγ regulates the activity of several 
adipocyte-specific genes, including leptin (Tontonoz et al., 1994b; Schoonjans et al., 1996), and antiarthritic thiazo-
lidinediones suppress leptin gene expression in differenti-
ated adipocytes (De Vos et al., 1996; Kalten and Lazar, 1996). 

The effects of BRL 49653 and CGP 52608 on leptin produc-
tion were investigated in fully differentiated 3T3-F422A adipocytes 
were transfected with the expression vectors for X. laevis PPARγ and human RXRα or with human RORA and a thymidine kinase-CAT reporter

Construct containing either the PPAR response element

found in the promoter of the mouse bifunctional enzyme

(Bardot et al., 1993) or the ROR response element

of the human 5-lipoxygenase gene (Steinhilber et al., 1995). To fur-

one derivative CGP 52608 and both analogues (10 μM), as well as melatonin (0.1–10 μM), were inactive.

Our data confirm published results for antidiabetic thiazolidinediones (Kallen and Lazar, 1996) and show that PPARγ ligands, but not RORA-activating compounds, inhibit leptin production in differentiated adipocytes.

**Effects on glucocorticoid-induced insulin resistance.** Glucocorticosteroids up-regulate leptin secretion in adipocyte cultures (Sliker et al., 1996), and pharmacological doses induce leptin gene expression within 24 hr in rat adipose tissue (De Vos et al., 1995). Glucocorticoids induce insulin resistance in rats (Inoue et al., 1996), whereas antidiabetic thiazolidinediones improve insulin responsiveness in animals and humans (Turner, 1996). To compare the in vivo profile of BRL 49653 and CGP 52608 in a model responding to PPARγ ligands, both compounds were tested in rats with glucocorticoid-induced insulin resistance.

Male rats received pharmacological doses of dexamethasone alone (0.15 mg/kg p.o.) or in combination with BRL 49653 or CGP 52608 (0.01–10 mg/kg p.o.) for 9 days, and changes in blood glucose, plasma triglycerides, and body weight were monitored on days 4 and 9 after overnight fasting. Additional test groups received BRL 49653 or CGP 52608 (0.01–10 mg/kg p.o.) without dexamethasone. The effects of BRL 49653 and CGP 52608 on dexamethasone-induced insulin resistance are shown in Fig. 3.

Blood glucose levels were significantly enhanced by dexamethasone after 4 and 9 days of treatment. BRL 49653 (0.01–10 mg/kg p.o.) dose-dependently reduced the elevated glucose levels after 4 days, but this reduction was transient and no longer seen after 9 days. CGP 52608 (0.01–10 mg/kg p.o.) did not lower the corticosteroid-induced elevated glucose levels during 9 days of treatment.

Plasma triglyceride levels were slightly but not signifi-
cantly enhanced by dexamethasone. However, the combined treatment of dexamethasone and BRL 49653 resulted in significant triglyceride-lowering effects after 4 and 9 days. In contrast, combined treatment with CGP 52608 had no effect after 4 days, but the highest dose of CGP 52608 significantly enhanced triglyceride levels after 9 days of treatment.

A progressive decrease in body weight was seen after 4 and 9 days of treatment with dexamethasone, indicating that catabolic doses of the steroid were used. Combined treatment with BRL 49653 improved the body weight loss under dexamethasone, whereas CGP 52608 caused an additional weight loss.

BRL 49653 and CGP 52608 (0.01–10 mg/kg p.o.) had only minor effects in normal rats (data not shown). BRL 49653 did not change blood glucose and triglyceride levels but slightly enhanced body weight gain in comparison with controls. CGP 52608 had no effects on plasma triglyceride levels, but the highest dose slightly but significantly lowered blood glucose and delayed body weight gain.

Taken together, although the effects of BRL 49653 on blood glucose and triglyceride levels in this model of induced insulin resistance were not as pronounced as those in models of genetic insulin resistance (Oakes et al., 1994; Lehmann et al., 1995), the results are qualitatively similar in that BRL 49653 improved dexamethasone-induced insulin resistance and weight loss. CGP 52608 exhibited a clearly different profile. Additional weight loss and enhanced triglyceride levels were seen during combined treatment with dexamethasone.

**Antiarthritic activity in rat adjuvant arthritis.** Rat adjuvant arthritis is a chronic T cell-dependent autoimmune disease with many similarities to rheumatoid arthritis, such as chronic inflammation, progressive joint destruction, enhanced T cell responses, and pronounced cytokine-mediated acute-phase reactions. CGP 52608 and structural analogues exhibit potent inhibitory effects in this experimental autoimmune model (Missbach et al., 1996).

To further compare the *in vivo* pharmacological profiles of

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Leptin secretion</th>
<th>% of Control</th>
</tr>
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<tbody>
<tr>
<td>CGP 52608</td>
<td>10</td>
<td>103 ± 8</td>
<td></td>
</tr>
<tr>
<td>CGP 55066</td>
<td>10</td>
<td>88 ± 3</td>
<td></td>
</tr>
<tr>
<td>CGP 55707</td>
<td>10</td>
<td>90 ± 4</td>
<td></td>
</tr>
<tr>
<td>Melatonin</td>
<td>10</td>
<td>114 ± 14</td>
<td></td>
</tr>
<tr>
<td>BRL 49653 (racemate)</td>
<td>0.1</td>
<td>132 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>128 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>117 ± 16</td>
<td></td>
</tr>
<tr>
<td>BRL 49653 R(+)</td>
<td>0.2</td>
<td>19 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>25 ± 12</td>
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<tr>
<td></td>
<td>0.01</td>
<td>31 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>117 ± 16</td>
<td></td>
</tr>
<tr>
<td>BRL 49653 S(-)</td>
<td>0.2</td>
<td>13 ± 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>27 ± 14</td>
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<tr>
<td></td>
<td>0.01</td>
<td>33 ± 4</td>
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<tr>
<td></td>
<td>0.002</td>
<td>102 ± 11</td>
<td></td>
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<tr>
<td>15d-PGJ2</td>
<td>10</td>
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<td></td>
<td>1</td>
<td>85 ± 34</td>
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<tr>
<td>PGD2</td>
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<td>135 ± 27</td>
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<tr>
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<td>0.1</td>
<td>115 ± 18</td>
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**Fig. 3.** Effects of BRL 49653 and CGP 52608 on glucocorticoid-induced insulin resistance in rats. Male Lewis rats received either dexamethasone alone (D) (0.15 mg/kg p.o.) or a combination of dexamethasone and BRL 49653 (D + BRL) or CGP 52608 (D + CGP) (0.01–10 mg/kg p.o.) for 9 days. Control animals received the vehicle (C). Blood glucose and plasma triglyceride levels and body weight were measured on days 4 and 9 after overnight fasting. Differences between groups were statistically evaluated by Student’s *t* test. *, *p < 0.05; **, *p < 0.01; ***, *p < 0.001.
BRL 49653 and CGP 52608, we tested both compounds in rat adjuvant arthritis.

Daily oral treatment was started on the day of arthritis induction (day 0) and continued up to day 30. Groups of five arthritic rats received two or three different doses of CGP 52608, BRL 49653 (racemate), or the reference compound prednisolone. Fig. 4 shows the development of the inflammatory edema in the adjuvant-injected hind paw (primary lesion) and in the noninjected hind paw (secondary lesion) during drug treatment.

CGP 52608 inhibited edema development in both hind paws significantly and dose-dependently with very low doses (0.01 and 0.1 mg/kg). The higher dose of 0.1 mg/kg was at least as effective as prednisolone at 10 mg/kg and caused a nearly total suppression of the primary and secondary inflammatory edema after 3–4 weeks of treatment.

The same low doses of BRL 49653 (0.01 and 0.1 mg/kg) did not inhibit arthritis development and progression. A 10-fold higher dose (1.0 mg/kg) reduced the edema in the injected hind paw only slightly and transiently by 15–25% from day 18 to 28. The secondary lesion was not significantly inhibited.

Prednisolone (1–10 mg/kg), as expected, dose-dependently inhibited the inflammatory edema in both hind paws.

Our results show potent anti-inflammatory activity in rat
adjugent arthritis for the PPARγ-activating thiazolidinedione derivative CGP 52608 and the GR ligand prednisolone, whereas the PPARγ ligand BRL 49653 did not exhibit significant effects in this chronic model for autoimmune diseases.

Discussion

Several lines of evidence indicate that the antidiabetic thiazolidinedione BRL 49653 (Berger et al., 1996; De Vos et al., 1996; Kallen and Lazar, 1996) and the antiarthritic thiazolidinedione derivative CGP 52608 (Wiesenber et al., 1995; Missbach et al., 1996) exert their pharmacological effects at least in part via specific activation of the nuclear hormone receptors PPARγ and RORA. Our results obtained in transiently transfected Drosophila SL-3 cells confirm PPARγ-specific gene activation for BRL 49653 and the putative natural ligand 15d-PGJ2 and RORA-specific gene activation for CGP 52608 and the putative natural ligand melatonin (Fig. 2).

Nuclear hormone receptors are involved in the regulation of many pathophysiological processes, and it is well known that there exists a complex cell- and tissue-specific interplay between different members of the receptor superfamily. To further investigate the functional role of PPARγ and RORA, we compared the pharmacological properties of BRL 49653 and CGP 52608 in vitro and in vivo models that had been used in previous studies to demonstrate typical effects of these compounds.

The leptin (ob) gene is exclusively expressed in adipocytes. The ob/ob mice, which carry a nonsense mutation in the ob gene, do not produce leptin and develop a profound obesity, often accompanied by diabetes (Zhang et al., 1994). Although a functional PPARγ response element (PPRE) has not been identified in the promoter of the ob gene (De Vos et al., 1996), BRL 49653 and structurally related antiobdiabetic thiazolidinediones inhibit leptin mRNA synthesis in vitro and in vivo and increase food intake and adipose tissue weight in rats (De Vos et al., 1996; Kallen and Lazar, 1996). Our data show that low nanomolar concentrations of BRL 49653 cause a profound suppression of immunoreactive leptin in the supernatants of differentiated adipocytes, whereas CGP 52608 and structurally related thiazolidinedione derivatives were completely inactive at 10 μM (Table 1).

Pharmacological doses of glucocorticosteroids induce ob gene expression in rat adipose tissue within 24 hr (De Vos et al., 1995). This early event is followed by complex metabolic changes resulting in a decrease in food consumption, a reduction in body weight gain, and the development of insulin resistance with enhanced blood glucose and triglyceride levels. BRL 49653 counterregulated the dexamethasone-induced insulin resistance, whereas CGP 52608 exhibited steroid-like effects in this model (Fig. 3).

Finally, BRL 49653 and CGP 52608 were tested in a chronic autoimmune model, in rat adjuvant arthritis, and the GR ligand prednisolone was taken as reference compound. Glucocorticosteroids are potent immunosuppressive and anti-inflammatory drugs, and down-regulation of nuclear factor-κB-induced genes such as interleukin (IL)-1, IL-2, IL-3, IL-6, IL-8, tumor necrosis factor-α, granulocyte-macrophage colony-stimulating factor, interferon-γ, class I and II MHC, and cell adhesion molecules by transcriptional activation of the IκB gene has been recently discovered to be a key mechanism in mediating their therapeutic effects (Auphan et al., 1995). Fig. 4 shows that prednisolone (1–10 mg/kg) dose-dependently suppressed adjuvant arthritis and that CGP 52608 (0.01–0.1 mg/kg) exhibited a 10–100 times higher antiarthritic potency, indicating a highly specific and efficacious mechanism of action. On the other hand, BRL 49653 was nearly inactive in the dose range tested (0.01–1 mg/kg), demonstrating again the different pharmacological properties of CGP 52608 and BRL 49653.

Nuclear signaling via RORA is suggested to be a key mechanism in mediating the anti-inflammatory and antiarthritic effects of CGP 52608 and structurally related derivatives (Missbach et al., 1996) and may be a promising therapeutic supplement and/or alternative to glucocorticosteroids in the treatment of rheumatoid arthritis and related autoimmune diseases. The molecular mechanisms that occur after RORA activation and finally cause the therapeutic effects of CGP 52608 and analogues are currently unknown. First, RORA-regulated genes have been identified (Steinhilber et al., 1995; Schraeder et al., 1996); potentially interesting candidates are human 5-lipoxygenase, an important enzyme in the control of allergic and inflammatory reactions, and human and mouse p21WRAP/CIP1, a cell cycle inhibitor that could play a role in suppressing autoimmune processes. However, it is likely that other target genes central to the immunoinflammatory process also are involved.

Cross-talk between nuclear receptors resulting in either synergistic or antagonistic effects is a common regulatory principle in controlling gene transcription. PPAR interactions with thyroid hormone receptors, C/EBP receptors, and COUP-TF have been shown to regulate genes involved in lipid metabolism (Hunter et al., 1996). PPARγ-activating thiazolidinediones counteract glucocorticoid-induced metabolic changes and reverse glucocorticoid-induced insulin resistance (De Vos et al., 1995; Turner, 1996; current study), indicating that GR and PPARγ are major players with antagonistic properties in the regulation of carbohydrate and lipid homeostasis and energy balance. Whether the observed steroid-like effects of CGP 52608 on triglyceride levels and body weight are ROR-mediated effects of physiological relevance is unknown.

Recent progress has been made in the identification of PPAR ligands. The availability of radiolabeled thiazolidinediones (Lehmann et al., 1995; Berger et al., 1996) and the discovery of GW 2331, a fibrate derivative with high affinity for PPARα and PPARγ (Kliwer et al., 1997), facilitates the screening for PPAR subtype-selective ligands. First results are available showing that certain naturally occurring fatty acids and arachidonic acid metabolites (leukotriene B4, 8-hydroxy-eicosatetraenoic acid, 15d-PGJ2, prostacyclin) are either nonselective or subtype-specific ligands of PPARα and PPARγ (Devcand et al., 1996; Hertz et al., 1996; Forman et al., 1997; Kliwer et al., 1997). The fact that arachidonic acid metabolites that are produced in inflamed tissues via the cyclooxygenase pathway (15d-PGJ2, prostacyclin) or the lipoxygenase pathway (leukotriene B4, 8-hydroxy-eicosatetraenoic acid) are not only potent inflammatory mediators but also PPAR ligands has generated the interesting hypothesis that activation of PPARs might be involved in the control and limitation of inflammatory reactions (Devcand et al., 1996; Kliwer et al., 1997). Our results with the PPARγ ligand BRL 49653 show that this compound did not inhibit
inflammation and joint destruction in a chronic arthritis model. However, PPARα ligands might be more promising candidates, but we are not aware of systematic studies investigating their effects in inflammatory models. In contrast, GR ligands and RORA activating thiazolidinedione derivatives (Missbach et al., 1996; current study) are potent inhibitors in experimental arthritis and autoimmune models and may have synergistic therapeutic effects in rheumatoid arthritis and related autoimmune diseases.

In summary, our results with the thiazolidinedione BRL 49653 and the thiazolidinedione derivative CGP 52608 support the concept that specific activation of PPARγ or RORA results in distinctly different pharmacological effects. Both compounds are prototypes of novel therapeutic agents for the treatment of NIDDM or rheumatoid arthritis and related autoimmune diseases. They also are valuable tools for further investigation of the pathophysiological role of PPARγ and RORA and their potential interplay with other members of the superfamily of nuclear receptors.

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