Antidiabetic Action of a Liver X Receptor Agonist Mediated By Inhibition of Hepatic Gluconeogenesis

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The oxysterol receptors LXR (liver X receptor)-α and LXRβ are nuclear receptors that play a key role in regulation of cholesterol and fatty acid metabolism. We found that LXRs also play a significant role in gluconeogenesis. Treatment of diabetic rodents with the LXR agonist, T0901317, resulted in dramatic reduction of plasma glucose. In insulin-resistant Zucker (fa/fa) rats, T0901317 significantly improved insulin sensitivity. Activation of LXR did not induce robust adipogenesis but rather inhibited the expression of several genes involved in hepatic gluconeogenesis, including phosphoenolpyruvate carboxykinase (PEPCK). Hepatic glucose output was dramatically reduced as a result of this regulation. Nuclear run-on studies indicated that transcriptional repression was primarily responsible for the inhibition of PEPCK by the LXR agonist. In addition, we show that the regulation of the liver gluconeogenic pathway by LXR agonists was a direct effect on hepatocytes. These data not only suggest that LXRs are novel targets for diabetes but also reveal an unanticipated role for these receptors, further linking lipid and glucose metabolism.

Type II diabetes mellitus is a prevalent metabolic disease in developed countries, with insufficient therapies for treatment and prevention (1, 2). Studies in recent years have suggested that nuclear receptors are intimately linked to the pathophysiology of diabetes. The anti-diabetic thiazolidinediones have been identified as ligands of peroxisome proliferator-activated receptor γ (PPARγ) (3, 4). Retinoid X receptor (RXR) ligands have also been shown to lower plasma glucose levels in rodent diabetic models (3–5).

Originally identified as orphan members of the nuclear receptor superfamily, liver X receptors exist as two isoforms, LXRα and LXRβ. The two isoforms display distinct patterns of expression with LXRα being primarily expressed in liver, intestine, and kidney, whereas LXRβ is expressed ubiquitously (6). Oxysterols were identified as the putative physiological ligands for the LXRs (7), and additional studies have demonstrated that these receptors act as sensors for these cholesterol metabolites and are essential components of a physiological feedback loop regulating cholesterol metabolism and transport (8). Consistent with their role in regulation of these metabolic pathways, several LXR-regulated genes involved in lipid metabolism and cholesterol transport have been identified including ABCA1, ABCG1, ABCG5, ABCG8, ApoE, CETP, Cyp7a1, LPL, SREBP1c, and FAS (8–14).

As a result of the close relationship between lipid and carbohydrate metabolism, we examined the potential role LXRs may play in glucose homeostasis by using a specific LXR agonist, T0901317, in rodent models of diabetes. Our findings indicated that T0901317 dose-dependently lowered plasma glucose level in both db/db and Zucker diabetic fatty (ZDF) rat models. In the fa/fa insulin-resistant rat model, T0901317 significantly improved insulin sensitivity. Examination of the liver gluconeogenesis pathway revealed dramatic repression of key genes involved in this pathway. As a result, hepatic glucose output was dramatically suppressed. PEPCK mRNA suppression appeared to originate primarily from transcriptional repression as indicated by the nuclear run-on experiments. Further studies in cultured hepatocytes indicated that hepatic activation of LXRs was sufficient to mediate the suppression of the hepatic gluconeogenesis pathway. Moreover, in an in vitro adipocyte differentiation assay, we showed that LXR agonists only minimally induced adipocyte differentiation compared with the robust effect by classic PPARγ agonists.

MATERIALS AND METHODS

In Vivo Glucose-lowering Studies—Five-week-old male db/db mice were purchased from Harlan (Madison, WI) and acclimated for 2 weeks prior to the start of the study. Mice were provided Purina 5008 food ad libitum, and the compounds were dosed once daily via oral gavage for 7 days. Blood samples were taken 1 h after dosing via the tail vein, and plasma glucose and triglyceride levels were measured on a Hitachi 912 clinical chemistry analyzer. Animals were sacrificed in the morning, 1 h after the eighth dose, and tissues were collected and frozen in liquid nitrogen for processing. A similar protocol was used for ZDF rats that were purchased from Charles River Genetic Models, Inc. (Zionville, IN). The rats were 8 weeks old at the start of the study.

Oral Glucose Tolerance Study in fa/fa Rats—Obese insulin-resistant female Zucker (fa/fa) rats (Charles River Genetic Models, Inc.), 10 weeks of age, were orally gavaged for 9 days with either vehicle or T0901317 (3 mg/kg/d). A pair-fed group was also included, to ascertain the effects of a mild reduction in food consumption noted in the T0901317 group. Eight hours after the last dose, animals were fasted overnight and on the following morning subjected to an oral glucose tolerance test. Briefly, blood was obtained from the animals in the conscious state, via the tail vein, at time 0 and times 15, 30, 60, and 120 min. Plasma glucose was measured as described above.

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min after an oral glucose challenge (2.5 g of glucose/kg body weight). Plasma glucose and insulin levels were analyzed on all samples, and the results are expressed as the product of glucose AUC and insulin AUC.

**Glucose Output with ZDF Rat Liver Slices**—Precision-cut liver slices were generated from control, T0901317-treated (10 and 30 mg/kg/d for 7 days), and pair-fed to male ZDF rats following 7 days of treatment and an overnight fast. After preincubation and wash phases, the slices were incubated for 2 h at 37°C in Krebs-Henseleit bicarbonate buffer containing 40 mM mannitol in either the presence or absence of 10 mM lactate. Incubation media glucose levels were assessed at the 2-h time point. Lactate-stimulated glucose output for each condition was derived by subtracting the basal rate of glucose output per gram of liver tissue from the substrate-stimulated rate of glucose output per gram of liver tissue. This rate of glucose output largely reflects the gluconeogenic rate because no group displayed a net increase in glycogen formation during the incubation period (data not shown).

**Nuclear Run-on Experiment**—A nuclear run-on experiment was performed essentially as described (15). Briefly, db/db mice were treated by T0901317 as described above. Liver samples were collected after animals were sacrificed. Nuclei were isolated, and in vitro elongation reaction was performed. The radiolabeled RNA was then subjected to slot blot to probes.

**mRNA Measurement**—Total RNAs were prepared from frozen tissue samples or cells with TRizol reagent (Invitrogen) or Qiagen RNA prep kit. Mouse PEPCK and G6P mRNA were measured by RNase protection assay and quantified with a Molecular Dynamics Phosphorimager Model 51. Rat mRNA was subjected to reverse transcription reactions using the Omniscript reverse transcriptase kit (Qiagen) according to the manufacturer’s directions. The resulting cDNA was amplified using TaqMan 2× PCR master mix (Applied Biosystems). The PCR products were detected in real time using an ABI-7900HT sequence detection system (Applied Biosystems). The rat PEPCK bDNA was performed as described (16).

**RESULTS**

In 7-week-old male diabetic db/db mice, the specific LXR agonist T0901317, dose-dependently lowered plasma glucose (Fig. 1a). The maximum efficacy in plasma glucose-lowering achieved with T0901317 was comparable with rosiglitazone (Invitrogen; Avandia®) treatment. Food consumption and body weight gain were similar to control in all dose groups with the exception of the 100 mg/kg T0901317 treatment, which trended downward (Table I). Subsequently we tested T0901317 in the
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Male ZDF model. Eight-week-old ZDF rats were treated orally with various doses of T0901317 for 7 days. Consistent with the data from the db/db model, plasma glucose levels were significantly reduced at 3 and 10 mg/kg doses. At a greater dose of T0901317 (30 mg/kg) a more striking reduction in plasma glucose was noted but was associated with a significant decrease in food consumption and weight loss, possibly as a result of the high dose of compound used (Table II). Both plasma and liver triglycerides in db/db and ZDF rat studies were significantly reduced at 3 and 10 mg/kg doses. At a greater dose of T0901317 the effect was reduced but was associated with a significant decrease in food consumption and weight loss, possibly as a result of the high dose of compound used (Table II). Both plasma and liver triglycerides in db/db and ZDF rat studies increased dramatically (Tables I and II), which is consistent with earlier reports in C57B6 mice (11). There was no significant change in plasma insulin levels in these studies (Tables I and II). Treatment of normal C57BL6 mice resulted in no glucose-lowering effects (Fig. 1d). Very similar trends were observed for lactate utilization as the vehicle control; pair-fed groups displayed the greatest rates, followed distantly by T0901317 at 10 mg/kg and T0901317 at 30 mg/kg, which utilized essentially no lactate. These results indicate that the LXR agonist, T0901317, improves glucose homeostasis in diabetic rodents, at least in part, through down-regulation of key enzymes in the hepatic gluconeogenesis pathway.

To investigate the mechanism of PEPCK mRNA reduction upon LXR action, we performed nuclear run-on experiments with liver samples from db/db mice treated with either T0901317 or vehicle (Fig. 2e). The results suggested that reduction of PEPCK mRNA upon T0901317 treatment in db/db mice was largely from transcriptional repression. To determine whether the aforementioned alterations were the result of T0901317 acting directly on hepatocytes, we treated rat hepatoma Fao cells with either 0.2 nM insulin or 100 nM T0901317 or a combination of both for 24 h. The mRNA levels of PEPCK, G6P, pyruvate carboxylase, and fructose 1,6-bisphosphatase were reduced dose-dependently and correlated well with their respective described LXR potencies (11, 18). These results suggest that the LXR agonist, T0901317, improves glucose homeostasis in diabetic rodents, at least in part, through down-regulation of key enzymes in the hepatic gluconeogenesis pathway.

As a result of recent studies indicating that LXR agonists are a target of PPARγ (19, 20), we further explored the mechanisms of LXR action by comparing the effects of LXR agonists and a PPARγ agonist, rosiglitazone, on adipocyte differentiation in vitro. Although rosiglitazone induced dramatic adipocyte differentiation, both the natural LXR ligand, 22(R)-hydroxycholesterol, and T0901317 failed to induce robust adipocyte differentiation as assessed by Oil Red O staining (Fig. 4a). Similar results were obtained in C3H10T1/2 cells where adipocyte dif-

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differentiation was quantitated (Fig. 4b). Examination of aP2 mRNA in 3T3L1 cells did not reveal any regulation by the LXR agonist, T0901317 (data not shown). These results show that although LXRα is a direct target gene of PPARγ, LXR agonists do not merely mimic PPARγ action in adipocytes, suggesting a unique mechanism for LXR-mediated antidiabetic action. Our results however, do not rule out the potential involvement of LXR-mediated contributions in peripheral tissues.

DISCUSSION

Our studies reveal for the first time that an LXR agonist, T0901317, exerts antidiabetic effects through suppression of hepatic gluconeogenesis. In vivo administration of T0901317 down-regulated the hepatic gluconeogenesis pathway, as evidenced by decreased expression of PEPCK and G6P mRNAs (Fig. 2). Lactate-stimulated glucose production from liver slices derived from T0901317-treated ZDF rats was significantly reduced compared to control groups (Fig. 2d).

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the hepatic gluconeogenic process. Because inhibition of hepatic glucose production has been identified as an effective approach for lowering hyperglycemia (2), LXR potentially represent novel targets for treating diabetes.

LXR and RXR function as permissive heterodimers (21), and our results suggest that the glucose-lowering effect of rexinoids may be mediated, at least partially, through decreased hepatic gluconeogenesis via activation of the LXR/RXR heterodimer. Recent studies indicated that LXR/RXR heterodimers regulate a spectrum of important gene products involved in lipid metabolism. One of the target genes, SREBP1c, has been identified as the master transcription factor controlling the entire fatty acid biosynthetic pathway (22, 23). In our studies, we observed dose-dependent plasma and liver triglyceride increases in both db/db mice and ZDF rats (Tables I and II), which is consistent with previous reports in C57BL6 mice. It is interesting to note that despite the increase in triglyceride levels, hyperglycemia was reduced dose-dependently. This observation is strikingly similar to the previous report on the effects of RXR agonists (24).

Although LXRs have been regarded as potential targets for mediating cardiovascular benefits, the induction of hypertri-
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glyceridemia and liver steatosis has severely hampered its development. A selective modulator that does not lead to accumulation of liver triglycerides will be essential if therapeutic potentials of LXRs for both cardiovascular and diabetic diseases can be realized.

In contrast to previous reports that have identified positively regulated target genes for LXR, we have identified several gene products that are down-regulated by LXR activation. Traditional LXR target genes possess an LXR-responsive element (LXRE) in their promoter or intron; however, it is unclear whether LXR represses genes through a negative LXRE or through indirect regulation similar to farnesoid X receptor repression of cholesterol 7α-hydroxylase expression (25, 26).

Our novel findings suggest that LXR activation alters liver metabolism in a manner reminiscent of insulin, increased lipogenesis and decreased gluconeogenesis. Despite the similarities, T0901317 does not appear to work through the classic insulin signaling cascade because we found that LXR activation does not alter AKT phosphorylation or PGC-1 expression (data not shown). In contrast, insulin has been shown to regulate LXRs in hepatocytes, and thus it is plausible that the effects of insulin on lipogenesis and gluconeogenesis may be regulated, at least in part, through changes in LXR expression.

In summary, we have discovered an additional metabolic pathway regulated by LXRs. Activation of this pathway by an LXR agonist leads to a significant reduction in hyperglycemia and an improvement in insulin sensitivity in preclinical models. These studies strongly implicate LXRs as alternative targets for intervention in diabetes mellitus.

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