Liver X Receptor Activation Stimulates Insulin Secretion via Modulation of Glucose and Lipid Metabolism in Pancreatic β-Cells

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Liver X receptors (LXRs) α and β, transcription factors of a nuclear hormone receptor family, are expressed in pancreatic islets as well as glucagon-secreting and insulin-secreting cell lines. Culture of pancreatic islets or insulin-secreting MIN6 cells with a LXR specific agonist T0901317 caused an increase in glucose-dependent insulin secretion and islet insulin content. The stimulatory effect of T0901317 on insulin secretion was observed only after >72 h of islet culture with the compound. In MIN6 cells, T0901317 increased protein expression of lipogenic enzymes, fatty acid synthase, and acetyl-CoA carboxylase. LXR activation also produced an increase in glucokinase protein and pyruvate carboxylase (PC) activity levels. The PC inhibitor phenylacetic acid abolished the increase in insulin secretion in cells treated with T0901317. The results suggest that LXRs can control insulin secretion and biosynthesis via regulation of glucose and lipid metabolism in pancreatic β-cells. *Diabetes* 53 (Suppl. 3):S75–S78, 2004

Development of type 2 diabetes is associated with increased levels of lipids, which places diabetic patients at an increased risk for cardiovascular diseases. The major lipid abnormalities in diabetic patients are increased triglyceride and decreased HDL cholesterol plasma concentrations (1). Cholesterol accumulation in the body can negatively affect cellular viability and is tightly regulated by a complex signaling system. Liver X receptors (LXRs), members of a nuclear hormone receptor family of transcription factors, control cholesterol homeostasis in the body through regulation of reverse cholesterol efflux out of the cell to the acceptor plasma apolipoproteins (2). Both isoforms of the receptor, LXRα and LXRβ, bind and get activated by hydroxycholesterols, naturally occurring cholesterol metabolites (3). Serving as a cholesterol sensor in the cells, LXRs activate cholesterol transport via induction of expression of a number of genes, including cholesterol transporters, cholesterol metabolizing enzymes, and apolipoproteins (2). In addition, LXRs stimulate synthesis of fatty acids and triglycerides, which are secreted and used for formation of lipoproteins (2).

Recently, it has been shown that LXRs play a role in the control of glucose homeostasis in the body. Activation of LXRs with the selective synthetic agonist T0901317 significantly decreases blood glucose levels in a rodent model of type 2 diabetes (4). T0901317 improves insulin sensitivity by downregulation of expression of genes important for liver gluconeogenesis (phosphoenolpyruvate carboxykinase and glucose 6-phosphate dehydrogenase) and upregulation of the glucose transporter GLUT4 in adipose tissue (4,5).

In this study we have examined effects of the LXR agonist T0901317 on insulin secretion in pancreatic β-cells and demonstrated that activation of LXR receptors stimulates insulin biosynthesis and secretion.

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9cRA, 9-*cis*-retinoic acid; ABCA1, ATP-binding cassette protein transporter A1; ACC, acetyl CoA-carboxylase; $[Ca^{2+}]_i$, cytosolic free Ca^{2+}; FAS, fatty acid synthase; GK, glucokinase; GLP-1, glucagon-like peptide 1; LXR, liver X receptor; SREBP-1, sterol response binding element-1; PAA, phenylacetic acid; PC, pyruvate carboxylase; RXR, retinoid X receptor.

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RESEARCH DESIGN AND METHODS

Cell preparation and culture. Pancreatic islets were isolated from male Wistar rats by ductal collagenase injection, digestion, and density gradient separation (6). Islet cells were prepared from freshly isolated islets by shaking in Ca^{2+}-free medium. Islets and cells were cultured for 1–4 days as reported (7). Insulinoma MIN6 cells were cultured as described elsewhere (8). T0901317 (Cayman Chemical, Ann Arbor, MI) and 9-*cis*-retinoic acid (9cRA) (Sigma-Aldrich, St. Louis, MO) were added to the culture medium as dimethyl sulfoxide (DMSO) stock solutions.

Insulin secretion and insulin content measurements. Cultured pancreatic islets were starved in Earle's balanced salt solution (EBSS) containing 2.8 mmol/l glucose for 30 min. Groups of three islets were selected and transferred into tubes with 0.3 ml of EBSS medium with test compounds. Islets were incubated for 1 h at 37°C, supernatant was collected, and insulin was measured. MIN6 cells were seeded in 96-well plate (30,000 cells per well) and cultured for 3 days. Cells were starved in EBSS with 2.8 mmol/l glucose for 30 min and then stimulated with substances of interest. Insulin in supernatant was measured. For measurements of islet insulin content, islets were incubated in an extraction buffer (75% ethanol, 1.5% HCl, 23.5% water) overnight at 4°C, and insulin in the extract was analyzed.

Cytosolic free Ca^{2+} measurements. Cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) was monitored with a fluorescent probe fura 2 (Molecular Probes, Eugene, OR) as reported previously (9).

PCR analysis. Total RNA from pancreatic islets and cell lines was isolated using RNeasy Mini Kit (Qiagen, Valencia, CA). One microgram of total RNA was used for reverse transcription using random hexamer primers and Superscript II (Invitrogen, Carlsbad, CA). PCR was performed under the following conditions: 3 min at 94°C followed by 35 cycles of 45 s at 94°C, 45 s at 60°C, and 60 s at 72°C. Primers for human and rodent LXRα were CTGCCCAGCAACAGTGTAAC and CTGCTTTGGCAAAGTCTTCCC (expected

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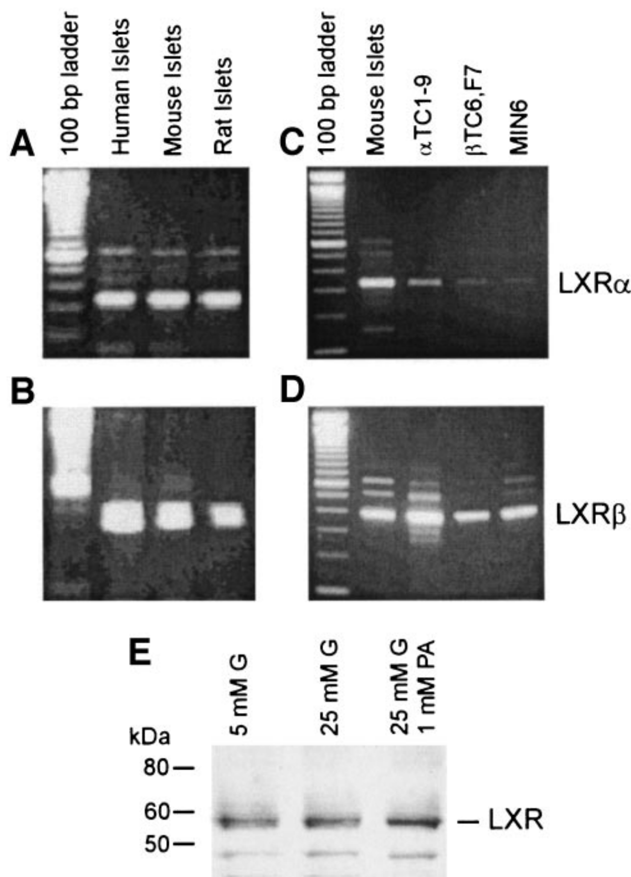

FIG. 1. Liver X receptor expression in pancreatic islets and pancreatic cell lines. *A*: mRNA expression of LXRα in pancreatic islets. *B*: mRNA expression of LXRβ in pancreatic islets. *C*: mRNA expression of LXRα in pancreatic cell lines. *D*: mRNA expression of LXRβ in pancreatic cell lines. *E*: LXR protein expression levels in MIN6 cells incubated for 48 h at 5 mmol/l, 25 mmol/l glucose (G), and 25 mmol/l glucose with 1 mmol/l palmitic acid (PA).

fragment 333 bp) and for human and rodent LXRβ were CGCTACAACCAC GAGACAG and GCAGCTTCTTGTCCTGGAG (expected fragment 393 bp). Both primer sets span an intron. Products were analyzed on a 1% agarose gel.

Immunoblotting. MIN6 cells were lysed in a M-Per buffer (Pierce Biotechnology, Inc., Rockford, IL) containing a protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Postnuclear protein homogenates were collected and protein content was measured. For detection of LXR protein expression, nuclear protein fractions were prepared using ProteoExtract subcellular proteome extraction kit (Calbiochem; EMD Biosciences, La Jolla, CA). Protein samples were separated by SDS-PAGE, transferred to polyvinylidine fluoride (PVDF) membrane and probed with antibodies against following proteins: LXRα, sterol response binding element-1 (SREBP1), and glucokinase (GK) (Santa Cruz Biotechnology, Santa Cruz, CA), ABCA1 and acetyl-CoA carboxylase (Upstate, Charlottesville, VA), fatty acid synthase (BD Biosciences Pharmingen, San Diego, CA), and eIF4E (Cell Signaling Technology, Beverly, MA). Western Lightning ECL kit (PerkinElmer, Boston, MA) was used to visualize protein expression.

Pyruvate carboxylase activity measurements. Pyruvate carboxylase (PC) activity in MIN6 cell homogenates was analyzed as reported previously (10).

RESULTS

The expression of LXR receptor isoforms was studied in pancreatic islets and pancreatic cell lines with RT-PCR. Human and rodent pancreatic islets express both LXRα and LXRβ isoforms of the receptor (Fig. 1*A* and *B*). Expression of both isoforms was also detected in glucagon-secreting αTC1–9 as well as insulin-secreting βTC6, F7 and MIN6 cells. Levels of LXRβ expression were comparable in glucagon- and insulin-secreting cell lines, whereas expression of LXRα was higher in the glucagon-secreting cell line (Fig. 1*C* and *D*). Regulation of LXR protein expression was studied in MIN6 cells by immunoblotting with polyclonal antibody raised against the COOH-terminal of LXRα (Fig. 1*E*). MIN6 cells were incubated in the presence of 5 mmol/l glucose, 25 mmol/l glucose, or 25 mmol/l glucose plus 1 mmol/l palmitic acid for 2 days. Similar incubation conditions have been reported to alter expression of genes involved in lipogenesis, with some of those genes being LXR targets (11). Chronic exposure of MIN6 cells to high glucose or combination of high glucose and free fatty acid did not produce significant changes in LXRα expression.

T0901317, the synthetic LXR agonist, has been employed to study the role of LXRs in regulation of pancreatic β-cell function (12). Isolated rat pancreatic islets have been incubated in the absence and the presence of 1 μmol/l T0901317 or in combination of 1 μmol/l T0901317 with 10 μmol/l 9cRA. 9cRA is an agonist of the retinoid X receptor (RXR), which forms functional heterodimers with LXRs. It has been reported previously that long-term incubation of pancreatic islets with 9cRA produces an increase in insulin secretion (13). Combination of the two ligands produces hyperactivation of LXR/RXR dimers (14). Islets preincubated with T0901317 demonstrated an increased glucose and glucagon-like peptide-1 (GLP-1)-induced insulin secretion, as compared with islets cultured in the absence of the compound (Fig. 2*A*). Interestingly, pretreatment of islets with T0901317 and 9cRA did not elevate GLP-1–induced insulin secretion and even inhibited the response to high glucose concentration. T0901317 and 9cRA did not change insulin secretion when added acutely to pancreatic islets (data not shown).

An increase in insulin secretion with T0901317 was also observed in insulin-secreting MIN6 cells. Incubation of MIN6 cells with 1 μmol/l T0901317 for 3 days produced higher levels of insulin secretion in the presence of 2.8 mmol/l glucose, 16.7 mmol/l glucose, and 16.7 mmol/l glucose with 25 mmol/l KCl (Fig. 2*B*).

We next investigated the effects of T0901317 and 9cRA on insulin synthesis (or gene expression) in rat pancreatic islets. Incubation of islets with 1 μmol/l T0901317 for 3 days resulted in an increase in insulin content, from 42 ± 1 ng/islet in control islets to 56 ± 5 ng/islet in T0901317-incubated islets ($P < 0.05$, $n = 4$). Insulin secretion from the same islet preparations was measured and normalized to the islet insulin content. After normalization, islets incubated in the presence of T0901317 still demonstrated a 40% increase in glucose-induced insulin secretion (data not shown). Incubation of islets with combination of 1 μmol/l T0901317 and 10 μmol/l 9cRA did not cause an increase in insulin content as compared with control conditions: insulin content was 44 ± 4 ng/islet in the presence of T0901317 and 9cRA.

We next investigated the time course of the stimulatory effect of T0901317 on glucose-induced insulin secretion. Glucose-induced insulin secretion was accessed in islets incubated with the agonist for 1, 2, 3, and 4 days. Incubation of islets with T0901317 for 24 h did not produce any effect on insulin secretion (Fig. 2*C*). After 48 h a slight increase in insulin secretion in islets incubated with the LXR agonist was observed. The elevation of insulin secre-

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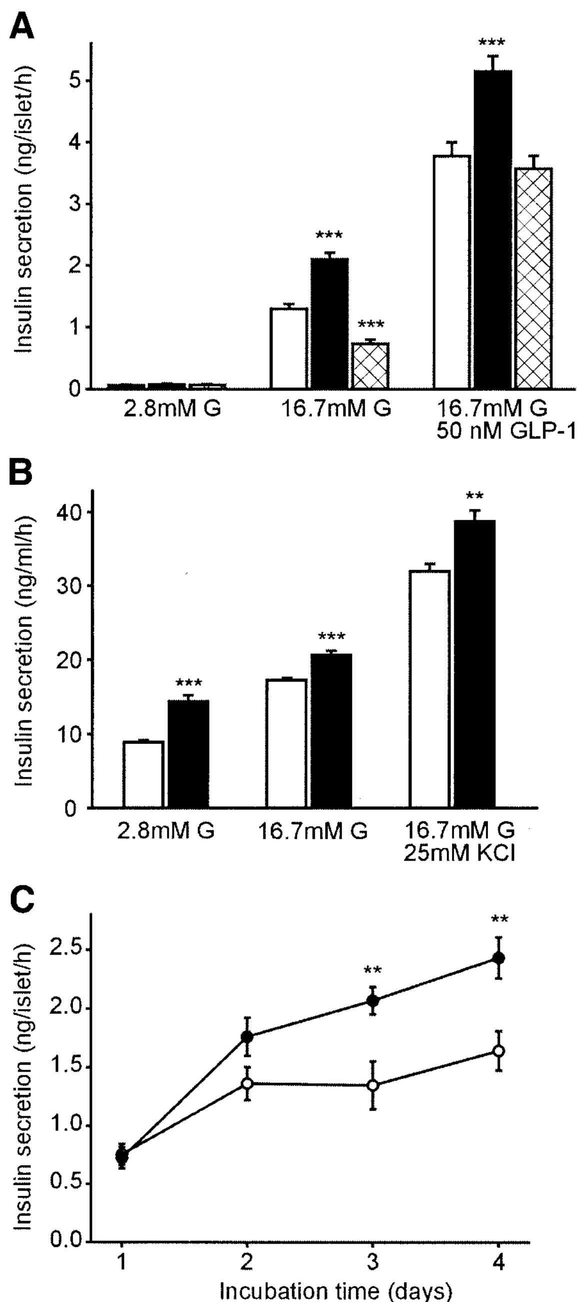

FIG. 2. Activation of LXRs modulates insulin secretion in pancreatic β-cells. *A*: Effects of T0901317 and 9cRA on insulin secretion in isolated rat pancreatic islets. Islets were incubated for 72 h alone (□), with 1 μmol/l T0901317 (■), or 1 μmol/l T0901317 with 10 μmol/l 9cRA (☒) and were then challenged with glucose (G) and GLP-1. *B*: Effects of T0901317 on insulin secretion in MIN6 cells. Insulin-secreting cells were incubated for 72 h alone (□) or with 1 μmol/l T0901317 (■). *C*: Time course of the stimulatory effect of T0901317 on insulin secretion induced by 16.7 mmol/l glucose in isolated rat pancreatic islets. Islets were incubated for the indicated number of days alone (○) or with 1 μmol/l T0901317 (●) and then challenged with 16.7 mmol/l glucose. Data are means ± SEM for eight experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. insulin secretion in cells incubated under control conditions.

tion in the presence of T0901317 reached statistical significance after 72 h pretreatment and still persisted after 96 h incubation (Fig. 2*C*).

Insulin secretion is triggered by an increase in $[Ca^{2+}]_i$ in pancreatic β-cells. Changes in $[Ca^{2+}]_i$ were therefore studied in clusters of islet cells incubated with and without

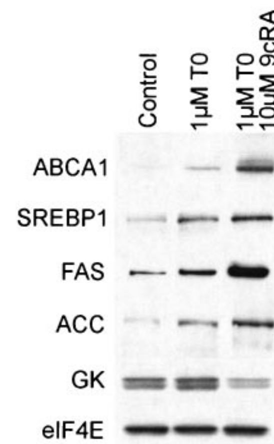

FIG. 3. Modulation of protein expression by LXR/RXR agonists in MIN6 cells. MIN6 cells were incubated with 1 μmol/l T0901317 (T0) or combination of 1 μmol/l T0901317 with 10 μmol/l 9cRA for 72 h. Protein expression was analyzed by immunoblotting. eIF4E was used as a control to demonstrate similar protein loading in different lanes.

1 μmol/l T0901317 for 3 days, followed by a 16.7 mmol/l glucose challenge. The glucose-induced $[Ca^{2+}]_i$ increase was not significantly modified in cells pretreated with the LXR agonist (data not shown).

Expression levels of proteins known to be targets for LXRs or involved in the regulation of insulin secretion were investigated with immunoblotting in insulin-secreting MIN6 cells incubated with T0901317 and 9cRA. Expression of ATP-binding cassette protein transporter A1 (ABCA1) responsible for the cholesterol efflux out of the cell is known to be governed by LXR activation (2). Activation of LXR/RXR in MIN6 induced a strong elevation in ABCA1 protein expression (Fig. 3). A lipogenic transcription factor, SREBP-1, is a direct target gene of LXR/RXR (15). T0901317 and its combination with 9cRA produced an increase in SREBP-1 protein expression (Fig. 3). Expression of other proteins that are induced by LXR and SREBP-1 activation, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), was strongly upregulated in MIN6 cells treated with T0901317 and 9cRA (Fig. 3). Interestingly, incubation of MIN6 cells with 1 μmol/l T0901317 induced a moderate increase in GK protein levels. However, combination of T0901317 and 9cRA did not further elevate GK levels but produced a decrease in GK expression (Fig. 3).

PC is expressed in insulin-secreting cells and mediates transport of glucose carbons into citric acid cycle in mitochondria. PC-mediated anaplerotic transport of carbon atoms is crucial for glucose-induced insulin secretion (16). In MIN6 cells incubated for 3 days with 1 μmol/l T0901317, PC activity was elevated by 61 ± 13% ($P < 0.05$, $n = 4$). Inhibiting the PC activity with an enzyme inhibitor, phenylacetic acid (PAA), reduced glucose-induced insulin secretion to the same levels regardless of the previous incubation conditions (Fig. 4).

DISCUSSION

The present study shows that activation of LXRs in pancreatic β-cells leads to stimulation of insulin secretion and insulin biosynthesis. These effects are achieved via stimulation of glucose and lipid metabolism. Induced by T0901317, increases in GK protein and PC activity enhance

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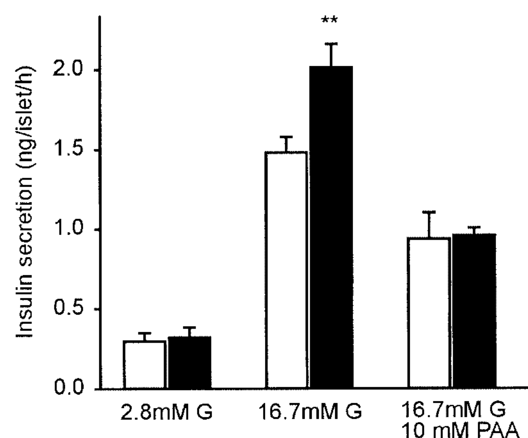

FIG. 4. Effects of PAA on glucose-induced insulin secretion in isolated rat pancreatic islets. Islets were incubated in the absence (□) or the presence (■) of 1 μmol/l T0901317 for 72 h and then challenged with glucose (G) or combination of glucose with 10 mmol/l PAA. Data are means ± SEM for 16 experiments. **P < 0.01 vs. insulin secretion in islets incubated under control conditions.

glucose flux through glycolysis and TCA cycle as well as stimulate anaplerosis with resulting elevation of ATP, NAD(P)H, and anaplerosis-derived metabolic coupling factors. In addition, increases in FAS and ACC activity accelerate production of malonyl-CoA, fatty acid-CoA, and diacylglycerol. Elevated levels of these metabolic factors result in increased insulin secretion and insulin biosynthesis (11,17–19).

Interestingly, exposure of β-cells to elevated glucose concentrations produces changes in gene expression identical to those induced by LXR activation (19). Pancreatic β-cells exposed to elevated glucose display improved glucose responsiveness and secretory phenotype, which are achieved through enhancement of anaplerosis and cataplerosis. LXRs may therefore play a role in activation of gene transcription under conditions of long-term glucose exposure. Although we observed no changes in LXR expression in cells cultured at various glucose concentrations, it still remains to be determined whether LXR activity alters at different glucose levels.

Stimulation of lipogenesis with LXR activation can be beneficial for β-cell function. However, prolonged activation of lipogenic gene expression can result in accumulation of free fatty acids and triglycerides and eventually β-cell dysfunction (20,21). It has been demonstrated that induced expression of SREBP-1c, a target gene for LXR, results in blunted glucose-stimulated insulin secretion and increased rates of apoptosis (21,22). In this study, hyperactivation of lipogenesis with combination of the LXR/RXR agonists did not produce any beneficial effects on insulin secretion and insulin content but rather inhibited glucose-induced insulin secretion in pancreatic islets.

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