Liver X receptor (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 p-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. 

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 liver function, increase the risk of vascular diseases. The major lipid abnormalities in diabetic patients are increased triglycerides and decreased HDL cholesterol plasma concentrations (1). 

Liver X receptors (LXRs) and LXRB, bind and get activated by hydroxysterolsteroids, 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.

**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 Ca2+-free medium. Islets and cells were cultured for 1–4 days as reported (7). Insulinaoma 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 a extraction buffer (75% ethanol, 1.5% HCl, 23.5% water) overnight at 4°C, and insulin in the extract was analyzed.

**Cytosolic free Ca2+ measurements.** Cytosolic free Ca2+ ([Ca2+]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 CTGCCAGGCAACAGTGAAC and CTGCTTGGCAGATTCCTCCC (expected
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. 1A and B). Expression of both isoforms was also detected in glucagon-secreting cell line (Fig. 1C and D). Regulation of LXR expression was studied in MIN6 cells by immunoblotting with polyclonal antibody raised against the COOH-terminal of LXRα (Fig. 1E). 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. 2A). 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. 2B).

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 secretion because of an increase in survival of islets during 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 measured 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. 2C). After 48 h a slight increase in insulin secretion in islets incubated with the LXR agonist was observed. The elevation of insulin secr-
tion in the presence of T0901317 reached statistical significance after 72 h pretreatment and still persisted after 96 h incubation (Fig. 2C).

Insulin secretion is triggered by an increase in \([\text{Ca}^{2+}]_i\) in pancreatic \(\beta\)-cells. Changes in \([\text{Ca}^{2+}]_i\) were therefore studied in clusters of islet cells incubated with and without 1 \(\mu\text{mol}/l\) T0901317 for 3 days, followed by a 16.7 mmol/l glucose challenge. The glucose-induced \([\text{Ca}^{2+}]_i\) 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 \(\mu\text{mol}/l\) 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 \(\mu\text{mol}/l\) 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 \(\beta\)-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
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 free fatty acids and triglycerides and eventually stimulation of lipogenic gene expression can result in accumulation of fatty acid-CoA and diacylglycerol. Elevated levels of these metabolic factors result in increased insulin secretion and insulin biosynthesis (11,17–19).

Stimulation of lipogenesis with LXR activation can be beneficial for β-cells 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 introduction 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.

REFERENCES