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ORIGINAL ARTICLE

Glucose regulates LXRα subcellular localization and function in rat pancreatic β-cells

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Liver X receptors (LXRs) are members of the nuclear receptor superfamily, which have been implicated in lipid homeostasis and more recently in glucose metabolism. Here, we show that glucose does not change LXRα protein level, but affects its localization in pancreatic β-cells. LXRα is found in the nucleus at 8 mM glucose and in the cytoplasm at 4.2 mM. Addition of glucose translocates LXRα from the cytoplasm into the nucleus. Moreover, after the activation of LXR by its synthetic non-steroidal agonist (T0901317), insulin secretion and glucose uptake are increased at 8 mM and decreased at 4.2 mM glucose in a dose-dependent manner. Furthermore, at low glucose condition, okadaic acid reversed LXRα effect on insulin secretion, suggesting the involvement of glucose signaling through a phosphorylation-dependent mechanism.

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Introduction

Liver X receptors (LXRs) were first identified as orphan members of the nuclear receptor superfamily [1, 2]. LXRα is highly expressed in the liver, intestine, kidney and adipose [3]. The LXRs are activated by physiological concentrations of oxidized hydroxycholesterol, and 22(*R*)-hydroxycholesterol, 27-hydroxycholesterol, 24(*S*), 25-epoxycholesterol [4, 5]. 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 [6]. These ligand-activated transcription factors form obligate heterodimers with the retinoid receptor (RXR) and regulate the expression of target genes containing LXR response elements (LXREs). All LXREs identified are DR-4 hormone response elements (direct repeat of the consensus AGGTCA separated by four nucleotides) [7]. A major advance in the study of LXRs came with the identification of their ligands as a specific class of oxysterol, mostly arising as metabolic derivatives of cholesterol [8]. Recently, a synthetic non-steroidal LXR agonist (T0901317) has also been described [9]. Similar to other members of the nuclear receptor family, these proteins contain a zinc-finger DNA-binding domain and a ligand-binding domain, which promote interaction with coactivator proteins.

To date, more than a dozen LXR target gene have been identified [10]. The discovery of an LXRE within the 7α-cholesterol hydroxylase (*Cyp7a*) gene first suggests that these receptors may serve as the key transcriptional regulator of bile acid synthesis in mice [4]. These findings, together with the generation of LXRα null mice, have provided unequivocal evidence to support that LXRs are key

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regulators of cholesterol homeostasis [11]. LXRs also play a major role in cholesterol efflux from cholesterol loading macrophages by up-regulating two ATP-binding cassette transporter (*abca1* and *abcg1*) [12, 13] and apolipoprotein E as acceptor of cholesterol in the formation of high-density particles [14]. Treatment of highly lipid-loaded macrophages *in vitro* with synthetic LXR ligands leads to dose-dependent increase in LXR target gene expression, suggesting that a similar hyperactivation of the LXR pathway might occur within the vessel wall. Recent work also implicated LXRs in the control of lipogenesis. Although LXRα null mice were noted to be deficient in the expression of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and sterol regulatory element binding protein 1 (SREBP-1) [15]. The demonstration that *srebp-1c*, responsible for the increase in lipogenic gene expression [16], is a direct target for regulation by LXR/RXR heterodimer provided evidence for the ability of LXR ligands to induce hepatic lipogenesis [7, 17]. Moreover, the demonstration of concomitant increase of plasma triglyceride levels in animals treated with LXR agonist (T0901317) revealed that LXRs are key regulators of hepatic lipid metabolism [9]. Inaba *et al*. [18] have shown that hypertriglyceridemia associated with synthetic LXR ligand treatment was due to the overproduction of hepatic ANGPTL3 protein into the plasma, which inhibited hydrolysis of plasma triglyceride in the body.

Recently, some authors suggest that the nuclear receptors LXR play a role in the coordinated regulation of lipid and glucose metabolism. Cao *et al*. [19] have shown 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 [20]. In the same time, Lafitte *et al*. have shown that LXR agonists promote glucose uptake in 3T3-L1 adipocytes and improve glucose tolerance in animal model of diet-induced insulin resistance. The insulin-dependent glucose transporter 4 (GLUT4) has been shown to be a direct target of LXRs in white adipose fat, and its induction would be predicted to result in increased uptake utilization of glucose by this tissue. These results outline a role for LXRs in the coordination of lipid and glucose metabolism. Tobin *et al*. [21] have shown that LXR functions as an essential regulatory component in insulin regulation of both cholesterol homeostasis and triglyceride metabolism. Insulin induces LXRα mRNA level in liver, leading to an increase in the steady-state mRNA level of LXR target genes, such as *srebp-1c* as well as many genes encoding enzymes in fatty acid and cholesterol biosynthesis. In a recent study, a new role for LXRα in the pancreas has emerged. Efanov *et al*. [22] showed that LXRs could control insulin secretion and biosynthesis in the MIN6 pancreatic β-cells. However, it is not known whether LXR activity could be altered by glucose level.

In this paper, we demonstrate, for the first time, that glucose regulates the subcellular localization of LXRα in pancreatic cells and affects its function on insulin secretion as well as the glucose uptake.

Materials and Methods

Cell culture and drug treatments

Cells were maintained in RPMI 1640 medium at 10 mM glucose supplemented with 10 mM HEPES, 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 1 mM sodium pyruvate, 50 μM β-mercaptoethanol, 100 IU/ml penicillin and 100 UG/ml streptomycin in a humidified atmosphere (5% CO_2). Prior to LXR agonist (T0901317) treatment, INS-1 cells were cultured overnight in culture medium containing 5 mM glucose and were washed twice in Krebs Ringer HEPES buffer (KRBH containing 140 mmol/l NaCl, 3.6 mmol/l KCl, 1.5 mmol/l $CaCl_2$, 0.5 mmol/l $MgSO_4$, 0.5 mmol/l NaH_2PO_4, 10 mM HEPES, 2 mM $NaHCO_3$ and 0.1% BSA (pH 7.4)). The culture medium was replaced with KRBH containing 4.2 mM or 8 mM glucose and T0901317. For the inhibition of the protein phosphatase PP1/PP2, the cells were treated for 30 min with okadaic acid (5 nM) (Calbiochem, Switzerland) before the insulin secretion experiments.

Peptide synthesis and immunization

The amino acid sequence of the N-terminal fragment of LXRα used in this work was MSLWLGAPVP DIPPDSAVEL WKPGAQ-DASS QAQGGSSSIL REEARMPHSA GGTAGVGLEA AEP-TALLTRA EPPSEPTEIR PQK. LXRα peptide was synthesized by Boc-benzyl chemistry with an Applied Biosystems 433A-automated solid-phase peptide synthesizer. The purity of the peptide was monitored by reverse-phase high-pressure liquid chromatography and was found be higher than 95%. The peptide identity was confirmed by mass spectrometry.

Rabbits were immunized with LXRα in accordance to the following scheme: (i) 500 μg intradermally on day 0, (ii) the same concentration of the immunogen intradermally on day 15 and (iii) 250 μg of the immunogen intramuscularly on day 30. Then, a new immunization (250 μg of immunogen) was performed every 2 weeks. Rabbits were bled via marginal ear veins on days 37 and 52, and a final bleed was preformed by cardiac puncture at the end of the experiment. Serum was obtained after centrifugation at 1000 × *g* for 15 min.

ELISA and LXRα antibody separation

For antiserum titration, microtiter plates were coated overnight at room temperature with 100 μl of LXRα peptide (5 μg/ml) in 0.1 M sodium carbonate–bicarbonate buffer (pH 9.6). The plates were washed three times with 300 μl of phosphate buffer saline (0.1M, pH 7.4). The plates were blocked for 1 h at 37 °C with 300 μl of 3% BSA/PBS buffer and then were washed six times. Next, 100 μl of

serially diluted serum was added to each well and incubated for 2 h at 37 °C. Unbound antibody was removed by washing four times, and 100 µl of goat anti-rabbit IgG-peroxidase conjugate diluted (1:8000; Chemicon) was added to each well. The plates were incubated for 2 h at 37 °C and washed several times. Prior to developing the enzyme label, 30 mg of *o*-phenylenediamine was dissolved in 20 ml of 0.1 M citrate/phosphate buffer and 20 µl of 30% H_2O_2. Then, 100 µl of enzyme substrate solution was added to each microtiter well. After incubation of 30 min at room temperature in the dark, the reaction was terminated by adding 100 µl HCl 1 M and the absorbance at 492 nm was measured using a microplate photometer (Dynex Technologies). The titer of each serum sample was arbitrarily set as the maximum dilution corresponding to the absorbance equal to 1.

An affinity chromatography step with an activated CH Sepharose™ 4B column was introduced after sodium sulfate precipitation of total immunoglobulin fraction. The collected fraction containing the anti-LXRα antibody was dialyzed and concentrated. The final concentration was estimated by use of BC Assay kit (Interchim).

Preparation of total protein extract

INS-1 cells were washed in cold PBS and harvested by centrifugation (13 000 rpm; 1 h; 4 °C) in lysis buffer (2% (w/v) Triton, 10% deoxycholic acid, 0.1 M sodium pyruvate, 0.2 M sodium vanadate, 1 M sodium fluoride and protease inhibitor cocktail).

Immunoblotting

The protein extract was separated by using 12% SDS-PAGE and transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech, UK) in a transblot electrophoretic cell (Bio-Rad). After transblotting, the membranes were blocked with 10% (wt/vol) non-fat dry milk dissolved in TBST (Tris 0.04 M, NaCl 0.11 M, 0.2% (vol/vol) Tween 20) overnight at 4 °C followed by incubation with polyclonal rabbit anti-LXRα antibody (1:400) raised in our laboratory against the fragment sequence LXRα (N-terminal 83 amino acids) at room temperature for 2 h on a rocking shaker. This antibody does not cross-react with LXRβ. After washing in TTBS (TBS, 0.2% (vol/vol) Tween 20, SDS 3.5 mM, deoxycholic acid 6 mM), the blots were reincubated for 1 h with horseradish peroxidase-conjugated secondary antibodies (Sigma-Aldrich Inc.) diluted 1:10 000 in TBST containing 5% (wt/vol) non-fat dry milk. Blots were developed using an enhanced chemiluminescence system (Amersham Pharmacia Biotech). The optical density was measured by densitometry and the results are shown as a ratio expression for T0901317 treated INS-1 cells versus non-treated cells. All experiments were repeated four times in separated assays.

Electrophoretic mobility shift assay (EMSA)

The nuclear forms of LXRα and RXRα proteins were synthesized *in vitro* using the TNT quick-coupled transcription/translation system (Promega). Double-stranded oligonucleotides were end-labeled with [α-^{32}P]ATP using T4-polynucleotide kinase according to standard procedures. Four microliters of LXRα and RXRα protein was incubated for 15 min at room temperature in a total volume of 20 µl of binding buffer described by Lehmann *et al*. [5] before the radiolabeled probe was added (400 000 c.p.m./well). For supershift assays, 2 µg of polyclonal LXRα antibody was added to the binding reaction. Then, the labeled oligonucleotide was added and the reaction was further incubated for 15 min. The following primer was used: LXRE cyp7a, 5'-TTT GCT TTG GTC ACT CAA GTT CAA GTT ATT-3'. DNA-protein complex was resolved by 6% non-denaturing polyacrylamide gel in 0.25× Tris-borate-EDTA buffer. The gel was then dried and exposed at −80 °C for autoradiography.

Immunofluorescence

After the treatment, INS-1 cells (cultured on glass coverslips) were fixed with 4% (v/v) formaldehyde for 40 min at 4 °C followed by three washes with TN buffer (20 mM Tris, 0.5 M NaCl, (pH 7.4)). The fixed cells were incubated for 1 h in lysine 0.1 M and permeabilized for 5 min in 0.01% Triton X-100 in TN at 37 °C. Then, the cells were washed three times with TN and incubated for 10 min in TNO buffer (TN containing 0.5% ovalbumine) at room temperature. INS-1 cells were incubated with anti-LXRα for 1 h at room temperature. After five washes with TNO, the cells were incubated with Alexa Fluor 488 goat anti-rabbit IgG (Interchim, France) for 1 h at room temperature. For nuclear DNA visualization, cells were stained with the Hoechst dye (bis-benzimide H33342; Sigma-Aldrich) for 30 min at room temperature. Finally, the cells were washed five times with TN, mounted in mowiol and observed with fluorescence microscope.

Insulin secretion analysis

At the end of the treatment, INS-1 cells were washed twice in KRBH. After 10 min, the solution was collected and insulin was measured in the medium by ELISA (Mercodia, Sweden) using rat insulin as standard.

Gene expression analysis

Total RNA was prepared from INS-1 cells using RNeasy™ Total RNA Kit (Qiagen, Germany), treated with DNase I RNase free (Qiagen) and reverse-transcribed with random hexamers using the Omniscript RT kit. The resulting cDNA product was subsequently PCR amplified. Primers for each gene were designed using Vector NTI Suite software (Informax) based on the sequence data available through GenBank: rat *Srebp-1c* (GenBank accession no. AF286470), forward 5'-GGA GCC ATG GAT TGC ACA TT-3' and reverse 5'-AGG AAG GCT TCC AGA GAG GA-3'; rat FAS (GenBank accession no. NM_017332), forward 5'-GCA CAC AGG TGG CAA AGG GGT GG-3' and reverse 5'-TCT GGG CAG AAG GTC TTG GAG ATG G-3'.

Glucose uptake analysis

After incubating the INS-1 cells for 24 h at 37 °C in 4.2 mM or 8 mM glucose KRBH with or without T0901317, uptake of glucose was determined as described previously [23]. Cells were then incubated for 30 min in glucose-free KRBH. After two additional washes with KRBH, the cells were incubated with 2-deoxy-D-[^{3}H]glucose (1 µCi/ml; Amersham Life Science) at room temperature for 15 s, 1 min, 5 min and 10 min in KRBH containing 4.2 mM or 8 mM glucose. Other cells were incubated with 2-deoxy-D-[^{3}H] in the presence of unlabeled 2-deoxy-glucose (Sigma Chemical Co.), which was added in 10 000-fold excess to block specific uptake of the labeled 2-deoxyglucose. The uptake of radiolabeled glucose was terminated by washing the cells rapidly with ice-cold PBS containing 0.2 mM phloretin (Sigma Chemical Co.). The cells were solubilized in 0.1% SDS, and aliquots were counted and assayed for protein content by using Bio-Rad protein assay (Bio-Rad).

Statistical analyses

One-way ANOVA (SPSS Release 7.5 for Windows; SPSS Institute) was used to evaluate the effects. A p-value <0.05 was considered to be statistically significant.

Results

Characterization of the polyclonal LXRα antibody

It was evident that the similarity (78%) of the human LXRα and -β proteins causes a specificity problem for generating antibodies. In order to overcome this problem, we have selected the N-terminal 83 amino acids. Within this region, the similarity of LXRα and LXRβ amino acid sequences is relatively low (35% identity). Recently, Watanabe *et al*. [24] published the establishment of a monoclonal antibody for human LXRα using the same region: N-terminal 94 amino acids. Moreover, the results of immunoblotting indicated that our antibody is able to specifically recognize *in vitro* translated human (TNT) LXRα but not LXRβ as shown in Figure 1A. Additional experiments using supershift assay indicate that our LXRα antibody causes a supershift of the bounded complex LXR/RXR to the consensus DR4 oligonucleotides (Figure 1B).

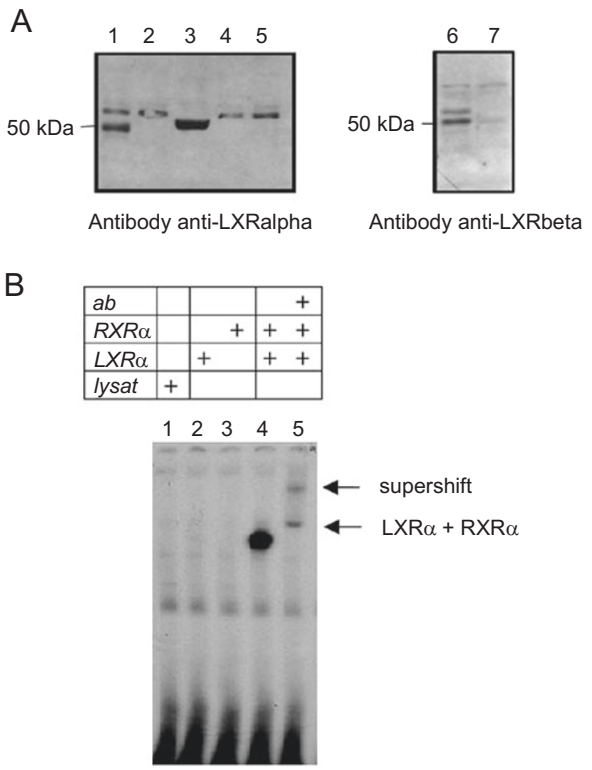

Figure 1 (A) Immunoblot detection of LXRα protein using the polyclonal anti-LXRα antibody. Protein extracts were separated on a SDS-polyacrylamide gel (12%), subjected to electrophoresis and transferred to nitrocellulose membrane. Lanes: 1, TNT LXRα (pcMX vector); 2, lysat (pcMX vector); 3, rat liver; 4, TNT LXRβ ($pcDNA_3$ vector); 5, lysat ($pcDNA_3$ vector); 6, TNT LXRβ ($pcDNA_3$ vector); and 7, lysat (pc DNA_3 vector). **(B)** EMSAs were performed with *in vitro* transcribed/translated human LXRα, RXRα or unprogrammed reticulocytes lysate (indicated by *lysat*) and the labeled oligonucleotide cyp7a LXRE (lanes 1–5). For supershift assays, 2 μg of polyclonal LXRα antibody was added to the binding reaction (lane 5). ab, antibody.

Glucose does not regulate LXRα protein expression in INS-1 cells

First, we showed that LXRα protein is expressed in rat pancreas at lower level than in rat liver by Western blot (Figure 2A). The quantitation of LXRα was evaluated as the ratio of LXRα to β-actin protein. Moreover, we demonstrated that LXRα is expressed in rat pancreatic β-cells, INS-1 (Figure 2A). The INS-1 cellular extract analysis by Western blot showed that LXRα protein expression is not altered by glucose in both conditions (Figure 2B).

Glucose affects LXRα localization in INS-1 cells

To test the effect of the glucose on the LXRα subcellular localization, fluorescence microscopy was used for LXRα protein analysis in both glucose conditions (4.2 mM and 8 mM). At low glucose concentration (Figure 3Aa), LXRα is found in the cytoplasm, apparently excluded from the nucleus, even though LXRα is localized in the nucleus at a higher glucose concentration (Figure 3Ab assessed by the Hoechst staining assay in (Figure 3Ac). To test whether high glucose concentration could induce a translocation of LXRα from the cytoplasm to the nucleus, glucose was added to cells incubated for 24 h in 4.2 mM glucose where LXRα is localized in the cytoplasm (Figure 3Ba). Fluorescence is slightly detectable in the nucleus within 1 min (Figure 3Bb) and becomes entirely nuclear within 10 min (Figure 3Bc). These results demonstrated clearly that the subcellular localization of LXRα is regulated by glucose.

LXRα is functional in pancreatic β-cells at 4.2 mM and 8 mM glucose

We showed that LXR is functional in these cells by analyzing its described hepatic target genes. Pancreatic expression of LXRα gene did not change significantly upon treatment with the agonist (data not shown), but expression of LXRα target genes involved in *de novo* lipogenesis was increased. The expression of rat pancreatic β-cell *srebp-1c* mRNA was induced by LXRα agonist in both conditions of 4.2 mM (4.3 fold-induction) and 8 mM glucose (6.3 fold-induction) (Figure 4). *Fas* mRNA was also induced by 1.9- and three-fold in the two glucose conditions (Figure 4). In these experiments, the basal expression of *Srebp-1c* and *Fas* mRNA in INS-1 cells is not significantly affected by glucose. In conclusion, our data show clearly that LXRα is functional in INS-1 cells in both glucose concentrations.

We showed that in 4.2 mM glucose and in the presence of T0901317, a small part of LXRα is translocated into the

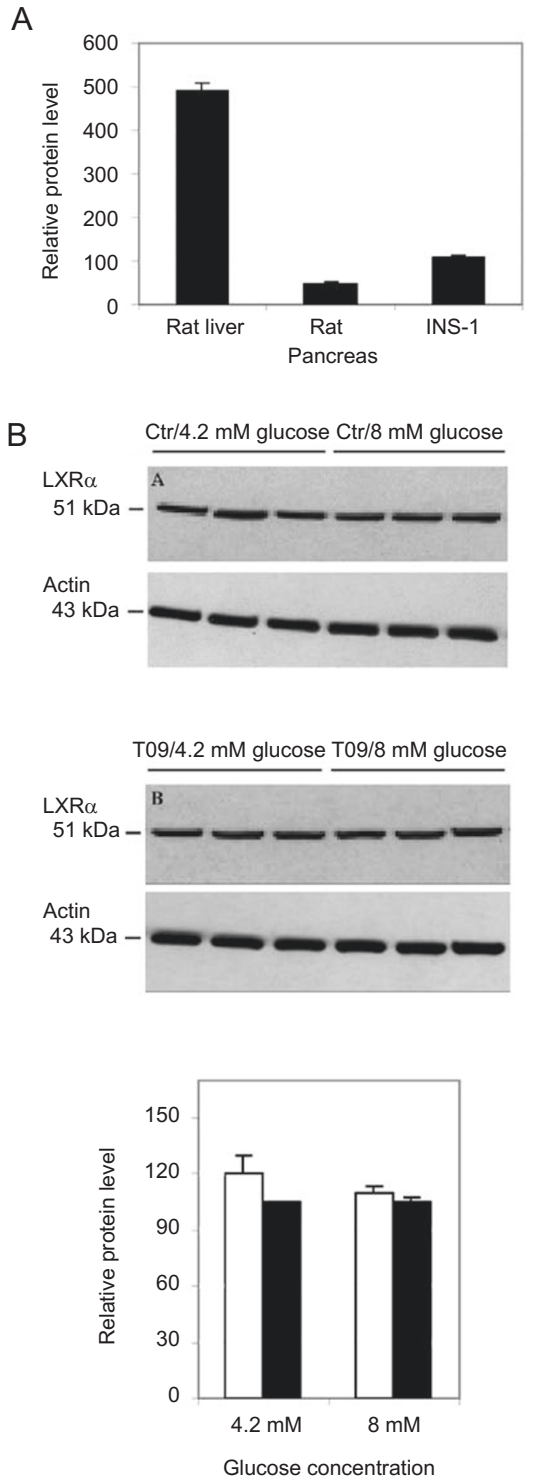

Figure 2 (A) Relative LXRα protein accumulation in rat liver and pancreas analyzed by Western blot (n=3). **(B)** Glucose does not regulate LXRα protein accumulation in INS-1 cells. Western blot analysis of LXRα and actin expression in INS-1 cells treated for 24 h with DMSO (open bars) or T0901317 (filled bars) in the presence of 4.2 mM or 8 mM glucose. Quantitation of LXRα expressed as the ratio of LXRα to actin protein.

nucleus (Figure 5A and 5 B). In 8 mM glucose, after LXR ligand treatment, the localization of LXRα remains nuclear (Figure 5C), as judged by the Hoechst staining assay (Figure 5D). These results could explain why the transcriptional activity on LXRα target genes is lower at 4.2 mM compared to that obtained with 8 mM glucose. These results suggest that the cell could regulate LXRα function by controlling its transport into and out of the nucleus.

Glucose alters the effect of LXRα on insulin secretion and glucose uptake in rat pancreatic β-cells

To evaluate whether glucose could affect the physiological function of LXRα, we analyzed the effect of the treatment with T0901317 ranging from 0 to 1.5 μM at high and low glucose concentrations. The results showed that insulin release was modulated in a dose-dependent response causing an inhibition at 4.2 mM glucose and a stimulation of the insulin secretion at 8 mM glucose (Figure 6A and 6B). Furthermore, to evaluate whether LXR regulates carbohydrate metabolism, we measured glucose uptake in INS-1 cells. We treated INS-1 cells with 1.5 μM T0901317 for 24 h in the presence of 8 mM glucose, after which the rate of glucose uptake was determined. The rate of glucose uptake was significantly increased (approx. 1.7-fold) compared to untreated cells (Figure 6D). The significant increase in glucose transport was attenuated for long time exposure to radiolabeled glucose. The same experiment was performed with INS-1 cells treated for 24 h with 1.5 μM T0901317 in 4.2 mM glucose. In this case, the rate of glucose uptake was significantly decreased (approx. 0.7-fold) (Figure 6C).

Effect of okadaic acid on insulin secretion in the presence of T0901317 in INS-1 cells

To determine how glucose affects LXRα function, we evaluate whether a phosphorylation signaling could be involved in the modulation of insulin secretion by LXR agonist. We used okadaic acid, a highly specific inhibitor of protein phosphatase PP1/PP2 known to be implicated in the glucose effect. The cells were incubated for 24 h in the absence or presence of 1.5 μM LXR agonist (T0901317) and treated for 30 min in a medium containing 5 nM okadaic acid. In the absence of T0901317, in both glucose concentrations (4.2 mM and 8 mM), okadaic acid inhibited insulin release, respectively, by 22% and 10% (Figure 7A and 7B). However, at low glucose concentration (4.2 mM), the inhibition of insulin release observed with LXRα ligand T0901317 is reversed in the presence of okadaic acid resulting in an increase of insulin secretion by 66% (Figure 7A). At 8 mM glucose concentration, okadaic acid enhanced the LXR-stimulated insulin secretion by 54% (Figure 7B). These data indicate that a phosphorylation/dephosphoryla-

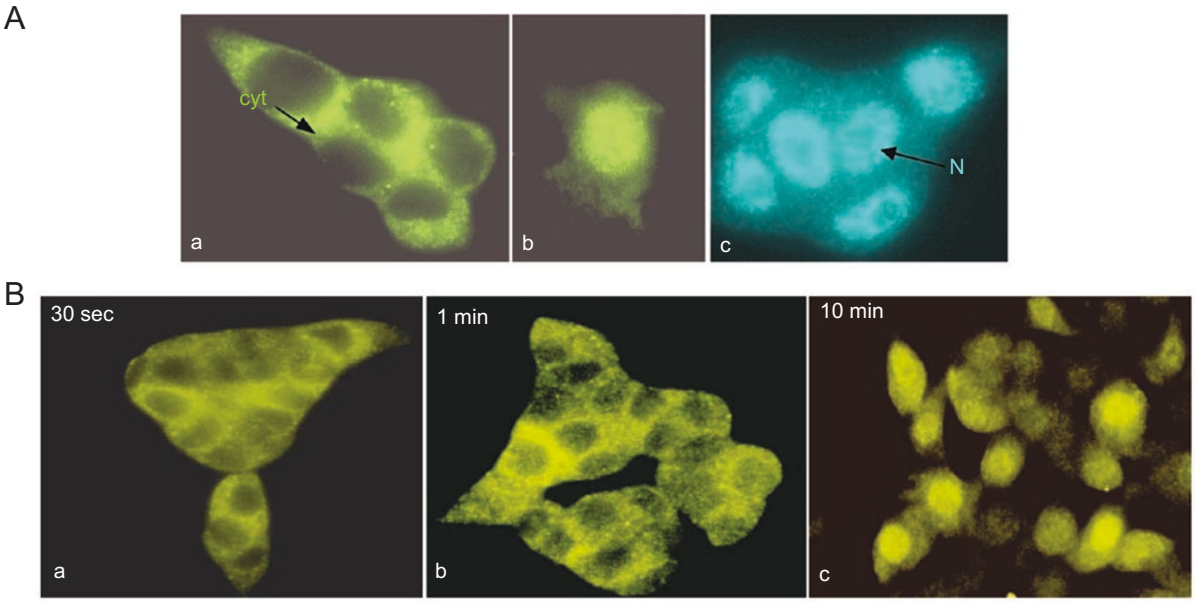

Figure 3 (A) Subcellular localization of LXRα at 4.2 mM glucose (a) or 8 mM glucose (b) conditions. Nuclear chromatin was stained with Hoechst 33258 (c). **(B)** Time course of LXRα translocation in the nucleus. INS-1 cells were grown in standard culture medium and placed for 24 h in 4.2 mM glucose before the incubation in 8 mM glucose (a: 30 s, b: 1 min and c: 10 min). Arrows indicate examples of cytoplasmic localization (cyt) and nuclear localization (N).

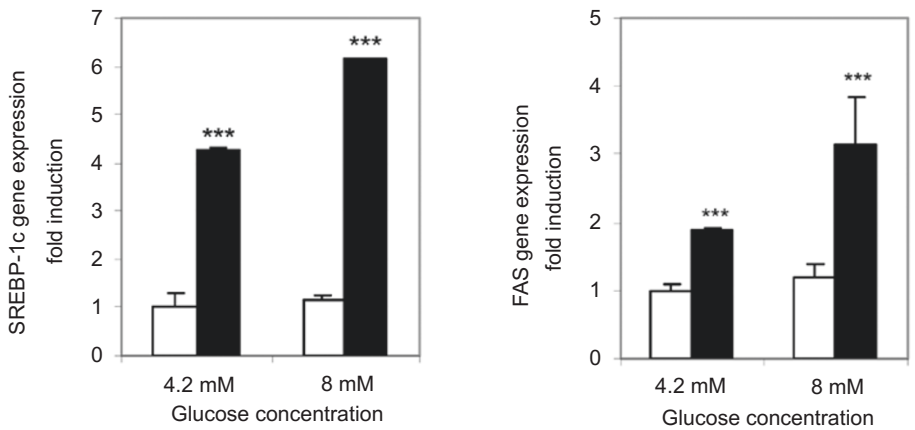

Figure 4 *Srebp-1c* and *Fas* gene expression in INS-1 cells. *Srebp-1c* mRNA expression in cells treated in the absence (open bars, n=3) or presence of 1.5 μM T0901317 (filled bars, n=3) was measured by RT-PCR. Results were normalized to cyclophiline mRNA levels. Statistically significant differences between groups versus control (without T0901317) were obtained with a one-way ANOVA test and indicated by asterisks (***p<0.001).

tion mechanism through glucose signaling might control LXRα function.

Discussion

Studies in recent years have suggested that nuclear receptors are intimately linked to pathophysiology of diabetes. The antidiabetic thiazolinediones have been identified as ligands of peroxisome proliferator-activated receptor γ (PPARγ) [25, 26]. RXR ligands have also been shown to lower plasma glucose levels in rodent diabetic models [26, 27].

Since LXRα has been reported to be expressed in the human pancreas [28], Efanov *et al*. [22] have confirmed its

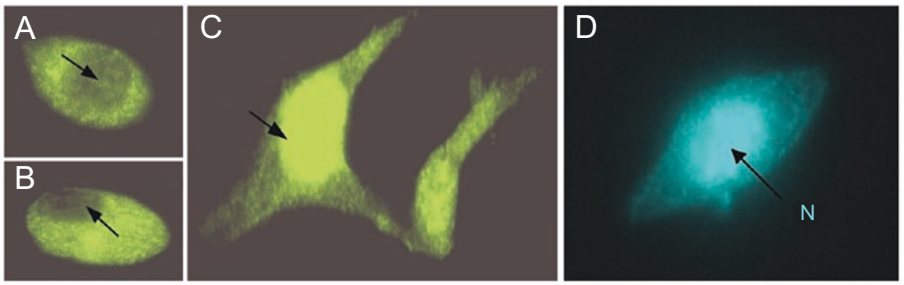

Figure 5 Subcellular localization of LXRα at 4.2 mM glucose **(A, B)** or 8 mM glucose **(C)** in the presence of T0901317. Nuclear chromatin was stained with Hoechst 33258 **(D)**. Arrows indicate examples of nuclear localization (N).

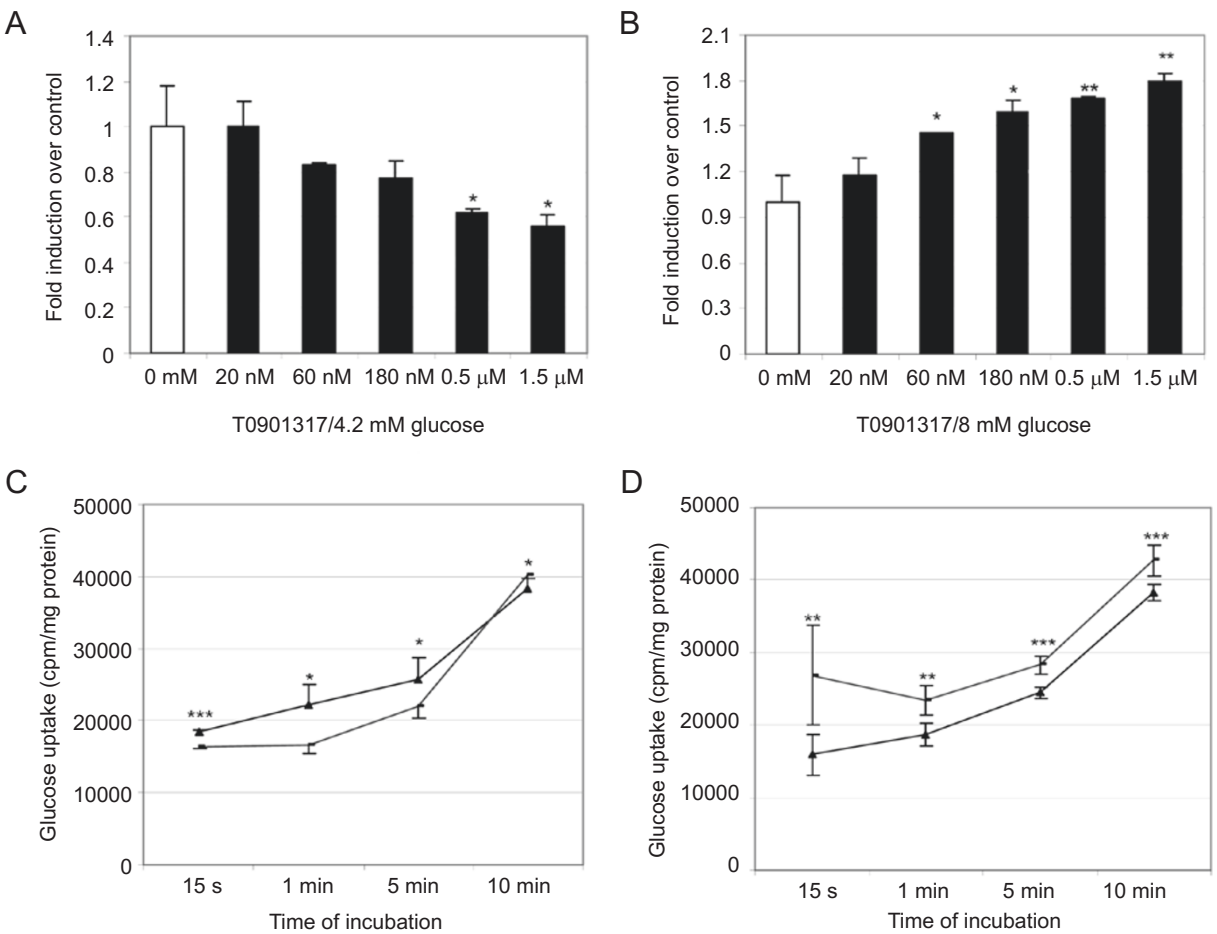

Figure 6 Dose response of insulin secretion in INS-1 cells upon exposure to T0901317 (filled bars, *n*=3) or vehicle (open bar, *n*=3) in medium containing, respectively, 4.2 mM **(A)** and 8 mM **(B)** glucose. Statistically significant differences between groups versus control (0 nM) were obtained with a one-way ANOVA test and indicated by asterisks (**p*<0.05; ***p*<0.01). Glucose uptake in INS-1 cells treated with T0901317 (bar, *n*=4) or vehicle (triangle, *n*=4) in 4.2 mM **(C)** and 8 mM **(D)** glucose. Statistically significant differences between groups versus control (without T0901317) were obtained with a one-way ANOVA test and indicated by asterisks (**p*<0.05; ***p*<0.01; ****p*<0.001).

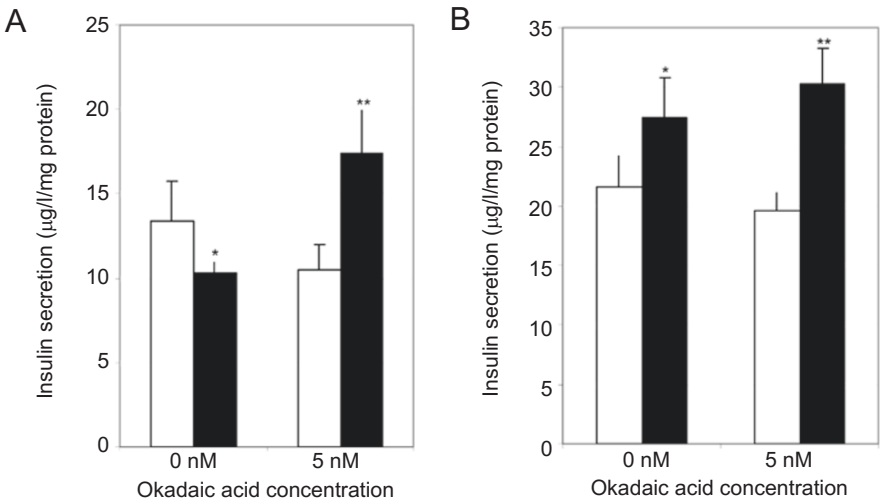

Figure 7 Effect of okadaic acid on insulin secretion in INS-1 cells **(A, B)**. Cells were maintained in standard culture medium containing 4.2 mM **(A)** or 8 mM **(B)** glucose in the presence (filled bars, *n*=3) or absence (open bars, *n*=3) of T0901317 for 24 h and treated for 30 min with 5 nM of okadaic acid. Cells were assayed for insulin secretion after glucose stimulation. Statistically significant differences between groups versus control (without T0901317) were obtained with a one-way ANOVA test and indicated by asterisks (*p<0.05; **p<0.01).

expression in mouse and rat islets as well as in glucagon-secreting cell lines. In our study, we compared LXRα protein accumulation in cells cultured in either low (4.2 mM) or high (8 mM) glucose-containing media. Thus, we showed that LXRα level is not regulated by glucose in INS-1 cells. In agreement with this, it has been recently reported that LXR expression level in the liver was not different between fasted and fed animals, even though *Srebp-1c* expression was strongly regulated in these same animals [20]. However, we showed that the nuclear localization of LXRα is regulated by glucose. At high glucose concentration, LXRα is found in the nucleus, while at low concentration, it is found in the cytoplasm. The addition of glucose causes its rapid translocation from the cytoplasm to the nucleus. The estrogen receptor and the nuclear receptor family are generally found in the nucleus, both in hormone-stimulated and hormone-free cells. However, there are important exceptions, and new findings, particularly with regard to sub-nuclear localization. Hager *et al*. [29] proposed that the intracellular distribution of both receptor classes is dependent not only on subcellular localization signals directly encoded in the receptors but also on the nature and composition of the large macromolecular complexes formed by each receptor. Another nuclear receptor, PPARα, was detected primarily in the cytoplasmic compartment, indicating that PPARα protein resides predominantly in the cytoplasm of macrophages [30]. These authors suggested that the low activation of PPARα by Wy14,643 might be due, in part, to the small amount of PPARα in the nucleus compared to PPARγ. Moreover, some of the receptors including the glucocorticoid receptors are expressed in the cytoplasm and ligand causes receptor translocation to the nucleus. Relatively little is known about the machinery that regulates steroid receptor movement through the cytoplasm and into the nucleus. Several studies suggest that movement is influenced by reversible phosphorylation. Dean *et al*. [31] showed that the serine/threonine protein phosphatase participates in the regulation of GR nucleocytoplasmic shuttling and that the GR-induced transcriptional activity results from the nuclear accumulation of GR, yet still requires agonist to elicit maximal transcriptional activation.

Then, we analyzed the efficacy of LXR activation by T0901317 in pancreatic β-cells by examining lipogenic gene expression in both glucose conditions. Indeed, increases in activity of LXRα target genes such as FAS and ACC accelerates the production of malonyl-CoA, fatty acid CoA and diacylglycerol. Elevated levels of these metabolic factors results in increased insulin secretion and insulin biosynthesis [32]. The expression of rat pancreatic β-cell *Srebp-1c* and *Fas* mRNA was induced by LXRα agonist in both conditions. *Srebp-1c* and *Fas* gene expression were increased less at 4.2 mM glucose than at 8 mM glucose. Then, we showed that at 4.2 mM glucose under T0901317 treatment, only a small amount of LXRα is translocated from the cytoplasm into the nucleus and this correlates with the lower transcriptional activity observed. In this low

glucose concentration, *Srebp-1c* and *Fas* gene expression are also increased, whereas insulin secretion is decreased by 20%. Then, we compared LXRα protein accumulation in cells cultured in either low (4.2 mM) or high (8 mM) glucose-containing media. Thus, we showed that LXRα protein level is not regulated by glucose in INS-1 cells. Our data suggest that the effect of LXRα activation on insulin secretion could not be explained by the overexpression of its lipogenic target genes. The results presented here raise the possibility to separate the beneficial effects of LXR agonists on insulin secretion from the effects on SREBP1-c expression and lipogenesis.

We evaluated whether the glucose concentration could alter the physiological role of LXRα. It has been reported that in insulin-secreting MIN6 cells, LXRα increased insulin secretion [22]. We showed that there is a bimodal effect of LXR agonist, (T0901317) depending on the glucose concentration. Indeed, T0901317 decreased and increased the insulin secretion, respectively, at 4.2 mM and at 8 mM glucose in a dose-dependent manner. These glucose concentrations are commonly used to estimate the effect of glucose in cell culture studies and also reflect physiological and diabetic glucose levels in human plasma. Accumulated data for T0901317 administrated to animals indicate that the compound increased hepatic triacylglycerol accumulation, depending on the dose used and the length of the study, resulted in variable elevation of plasma triacylglycerols. The mechanisms implicated seem to be more complex than simple changes in gene expression and additional parameter like defective energy metabolism as observed in diabetes or obesity could be favored. Cao *et al*. [19] showed the LXR agonist (T0901317) exerts antidiabetic effects through suppression of the hepatic gluconeogenic process through the inhibition of PEPCK and glucose-6 phosphate dehydrogenase. They observed dose-dependent plasma and liver triglycerides increases in both db/db mice and ZDF rats, which is consistent with previous reports in C57BL6 mice. Despite the increase in triglyceride levels, hyperglycemia was reduced dose dependently.

Both glucose uptake and glucose metabolism are required for insulin secretion. Consistent with this idea, we have shown that the LXR agonist (T0901317) promotes glucose uptake in INS-1 cells at a high glucose concentration (8 mM glucose) and decreases it at a basal glucose concentration (4.2 mM glucose). These effects are positively correlated with insulin release after 24 h of T0901317 treatment. Recently published data showed that LXR agonist increased glucose uptake in 3T3-L1 cells and improved glucose tolerance in a model of diet-induced insulin resistance [20]. Cao *et al*. [19] showed a significant difference in plasma glucose level in peripheral blood samples, even though no differences were detected in cardiac blood samples [33]. Thus, Chisholm *et al*. proposed that the major plasma glucose-lowering effect of LXR ligands results from increased peripheral tissue glucose uptake.

Since the glucose signaling involves the activation of protein phosphatases, we addressed the question whether the phosphorylation/dephosphorylation pathway could be implicated in the modulation of insulin secretion induced by T0901317. For this, we used okadaic acid, a selective inhibitor of protein phosphatases PP1/PP2A, which are involved in the glucose effect. Recent studies have described the inhibitory effect of okadaic acid on insulin secretion in rat islets [34] by decreasing the magnitude of the Ca^{2+} signal in β-cells. Another group reported that okadaic acid alone has no secretory response but micromolar okadaic acid enhances the basal insulin secretion in permeabilized islets [35, 36]. In our current study, with low dose of okadaic acid (5 nM), there was a slight inhibitory effect on basal insulin release. The addition of okadaic acid to the treatment with LXRs ligand stimulates insulin secretion in both glucose concentrations and thus reversed T0901713 inhibitory effect at 4.2 mM glucose. In summary, we could hypothesize that the glucose signaling through a phosphorylation/dephosphorylation mechanism regulates LXRα protein subcellular localization and therefore its function on insulin secretion.

This new additional metabolic pathway regulated by LXRα provides evidence that LXRα could be an interesting target in diabetic treatment. However, the role of LXR signaling in this context is not clear and is a subject of ongoing investigation. Pharmacologic activation of LXRs *in vivo* by efficacious synthetic ligands leads to a number of favorable changes in lipid and glucose metabolism [37], but increasing in lipogenesis and plasma triglycerides is an obstacle that needs to be cleared. An alternative approach, for both cardiovascular and diabetic diseases, is to develop a selective modulator that does not lead to the accumulation of liver triglycerides.

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