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Evidence for an indirect transcriptional regulation of glucose-6-phosphatase gene expression by liver X receptors ☆

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Abstract

Liver X receptor (LXR) paralogues α and β (LXRα and LXRβ) are members of the nuclear hormone receptor family and have oxysterols as endogenous ligands. LXR activation reduces hepatic glucose production in vivo through the inhibition of transcription of the key gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (G6Pase). In the present study, we investigated the molecular mechanisms involved in the regulation of G6Pase gene expression by LXR. Both T0901317, a synthetic LXR agonist, and the adenoviral overexpression of either LXRα or LXRβ suppressed G6Pase gene expression in H4IIE hepatoma cells. However, compared to the suppression of G6Pase expression seen by insulin, the decrease of G6Pase mRNA by LXR activation was delayed and was blocked by cycloheximide, an inhibitor of protein synthesis. These observations, together with the absence of a conserved LXR-binding element within the G6Pase promoter, suggest an indirect inhibition of G6Pase gene expression by liver X receptors.

Keywords: Glucose-6-phosphatase; Insulin; Diabetes; Gluconeogenesis; LXR; Liver; T0901317

The key gluconeogenic enzyme glucose-6-phosphatase (G6Pase) catalyses the hydrolysis of glucose-6-phosphate to free glucose, the final step of hepatic glucose production. The activity of this enzyme is regulated mainly at the level of gene transcription. The expression of G6Pase is increased in diabetes, which probably contributes to the hyperglycemia associated with this disease [1,2]. Thus, characterization of the molecular mechanisms controlling G6Pase gene expression could offer new pharmacological approaches for the inhibition of hepatic glucose production in diabetes. Insulin, the most important hormone decreasing G6Pase enzymatic activity, inhibits G6Pase gene expression through the activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) pathway and the subsequent phosphorylation of Foxo1 [3]. The insulin effect is mediated in part via the insulin response unit (IRU) within the promoter of G6Pase.

The phorbol ester PMA exerts its inhibitory effects by activation of extracellular signal-regulated kinase 1/2 (ERK 1/2) [4]. TNFα decreases the G6Pase gene expression by activation of nuclear factor κB (NFκB) [5]. Recently, the activation of liver X receptor (LXR) by synthetic agonists, such as T0901317, has also been shown to decrease G6Pase gene expression and to lower blood glucose levels in vivo [6,7]. This regulation was not observed in LXRα$^{-/-}$β$^{-/-}$ mice [8].

☆ *Abbreviations:* bt$_2$cAMP, N^6,2′-*O*-dibutyryl cAMP; Dex, dexamethasone; EMSA, electromobility shift assay; ERK, extracellular signal-regulated kinase; G6Pase, glucose-6-phosphatase catalytic subunit; GSK3, glycogen synthase kinase 3; IRU, insulin response unit; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor κB; PGC-1α, peroxisomal proliferator-activated receptor γ coactivator 1α; PEPCK, phosphoenolpyruvate carboxykinase; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PMA, phorbol-12-myristate-13-acetate; TNFα, tumor necrosis factor α.

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The LXRα and LXRβ paralogues belong to the family of nuclear receptors. The ligands for these transcription factors are oxidized derivatives of cholesterol, so-called "oxysterols." LXRα is expressed predominantly in liver, kidney, intestine, adipose tissue, and macrophages, whereas LXRβ is expressed ubiquitously. LXRs form heterodimers with the nuclear transcription factor retinoid X receptor (RXR) and regulate a number of genes involved mainly in cholesterol and fatty acid metabolism [9]. Many of the target genes that are activated by LXRs harbour a common LXR response element (LXRE) within their promoters. This element contains two halfsites, each with the consensus sequence AG(G/T)TCA, which are separated by four nucleotides (also called DR4 element) [10].

Here, we studied the molecular mechanism of LXR signalling that mediates the regulation of G6Pase gene expression. Our data suggest that LXR regulates G6Pase gene expression via an indirect mechanism.

Materials and methods

Reagents and cell culture. Anti-GAPDH antibody was obtained from Abcam. Anti-LXR antibody (H-144) was purchased from Santa Cruz Biotechnology. T0901317 was obtained from Calbiochem and was diluted in DMSO. An appropriate amount of DMSO was used in parallel incubations as a control. H4IIE rat hepatoma cells and HEK293 cells were cultured in DMEM containing 10% FCS and antibiotics (penicillin/streptomycin).

Plasmids and transient transfections. H4IIE cells were transfected with 8.5 μg plasmid DNA/dish of the reporter gene vector pGL3-G6Pase

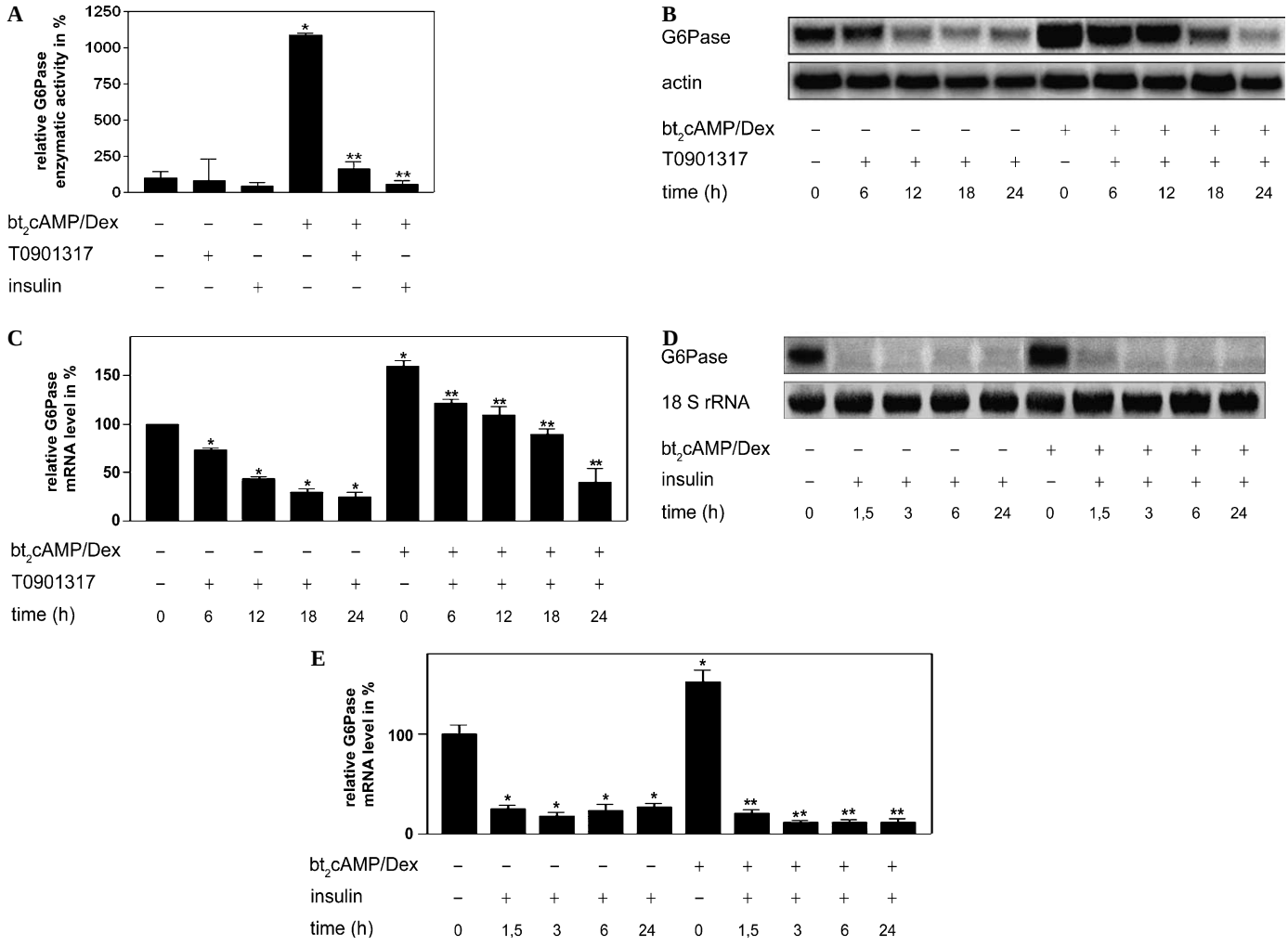

Fig. 1. T0901317 represses basal and induced gene expression and activity of glucose-6-phosphatase in H4IIE cells. (A) G6Pase enzymatic activity. H4IIE cells were incubated with DMSO, T0901317 (3 μM) or insulin (10 nM) in the presence or absence of bt_2cAMP (500 μM)/dexamethasone (Dex; 1 μM). After 24 h, microsomes were prepared as described and G6Pase activity was measured. All values represent means ± SEM ($n = 3$). *$P < 0.05$ compared with basal activity, **$P < 0.05$ compared with induced activity. (B) Northern blot. H4IIE cells were incubated with DMSO or T0901317 for the indicated times in the absence or presence of bt_2cAMP/dexamethasone, prior to isolation of RNA. Fifteen micrograms of RNA was electrophoresed, transferred onto nylon membranes, and hybridized using a random-primed ^{32}P-labelled probe specific for G6Pase. Actin was used as a normalization control. (C) Densitometric analysis of the blots. All values represent means ± SEM ($n = 3$). *$P < 0.05$ compared with basal expression, **$P < 0.05$ compared with induced expression. (D) Northern blot. H4IIE cells were incubated with DMSO or insulin for the indicated times in the absence or presence of bt_2cAMP/dexamethasone, prior to isolation of RNA. G6Pase mRNA was detected as described before. As a normalization control 18S RNA was used. (E) Densitometric analysis of the blots. All values represent means ± SEM ($n = 3$). *$P < 0.05$ compared with basal expression, **$P < 0.05$ compared with induced expression.