Comparative Biochemistry and Physiology, Part A 158 (2011) 201–206

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Regulation of bile acid, cholesterol, and fatty acid synthesis in chicken primary hepatocytes by different concentrations of T0901317, an agonist of liver X receptors

Kan Sato *, Toshihiko Kamada

Animal Science, Department of Biological Production, Tokyo University of Agriculture and Technology, 3-5-8, Saiwai-cho, Fuchu-shi, Tokyo, 183-8509, Japan

ARTICLE INFO

Article history:
Received 21 August 2010
Received in revised form 26 October 2010
Accepted 26 October 2010
Available online 3 November 2010

Keywords:
Liver X receptor
Bile acid
Cholesterol
Fatty acid
Chicken

ABSTRACT

Liver X receptors (LXRs) are members of the nuclear receptor family of transcription factors. They play a crucial role in lipid metabolism processes such as bile acid and fatty acid synthesis, as well as minor or limited roles in the regulation of cholesterol synthesis and uptake in mammals. In avian species, however, little is known about the role of LXRs except for the fact that they are involved in the stimulation of fatty acid synthesis. In this study, we characterize the expression profile of genes related to bile acid, cholesterol, and fatty acid synthesis and VLDL secretion in chicken primary hepatocytes treated with T0901317, a synthetic agonist of LXR. The activity of chicken cholesterol 7α hydroxylase (CYP7A1), a key enzyme in bile acid synthesis, mRNA expression, and bile acid excretion, was stimulated by supplementation of the culture medium with a low concentration (0.01 μM) of T0901317. In contrast, the levels of sterol regulatory element binding protein (SREBP)-1, fatty acid synthase mRNA, and VLDL-triacylglycerol in cells cultured in the presence of a high concentration (10 μM) of T0901317 were higher than those cultured in zero or low concentrations of T0901317. These results suggest that cellular responses to this LXR agonist were similar to those present in mammals. A novel finding of this study concerned changes to the regulation of cholesterol synthesis and uptake in chicken hepatocytes treated with T0901317. Levels of SREBP-2,3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) and low-density lipoprotein receptor (LDLr) mRNA expression increased as a function of increasing T0901317 (up to 1.0 μM), but remained similar to those in cells cultured under control conditions when the concentration of T0901317 was increased to 10 μM. These results suggest that LXRs play an important role in cholesterol synthesis and uptake in chicken hepatocytes and, as such, differ to findings in mammals where the effect of LXR agonists on cholesterol synthesis plays only a minor role in the regulation of cellular sterol homeostasis.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Liver X receptors (LXRs; NR1H3 and NR1H2) belong to the nuclear receptor superfamily of ligand-activated transcription factors (Janowski et al. 1996). These receptors are expressed in metabolically active tissues such as the liver, small intestine, kidney, and adipose tissue (Fan et al. 2008). Endogenous ligands for LXRs include oxysterols such as 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol and 24(S) and 25-epoxycholesterol, but not cholesterol (Janowski et al. 1999). A synthetic ligand for LXRs, T0901317, was first reported by Schultz et al. (2000).

In mammals, LXRs are key regulators of lipid and cholesterol metabolism, such as bile acid and fatty acid synthesis (Wojclcka et al., 2007). The liver is the only site in which substantial amounts of cholesterol are removed from the body by excretion into the bile, either as free cholesterol or after conversion into bile acids, a process that is regulated by the enzyme cholesterol 7alpha hydroxylase (CYP7A1) (Russell and Setchell, 1992). LXR stimulates the expression of CYP7A1 by binding to the LXR response element (LXRE) present in the CYP7A1 promoter (Peet et al. 1998). Fatty acid synthesis in hepatocytes is also regulated by LXR, with LXR agonists markedly stimulating fatty acid synthesis (lipogenesis) via the increased expression of SREBP-1c (Repa et al., 2000; Watanabe et al., 2005). In addition, LXRs directly regulate the expression of several lipogenic enzymes, including fatty acid synthase (Joseph et al., 2002). The liver is also an important site of cholesterol biosynthesis and uptake, which are controlled by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) and low-density lipoprotein receptor (LDLr), respectively (Horton et al., 2003). The administration of T0901317, a synthetic agonist of LXRs, reduces cholesterol biosynthesis in mice (Schultz et al., 2000). In contrast, LXR activation up-regulates SREBP-2 and expression of its regulatory genes, such as HMGR and LDLr, in astrocytes (Abildayeva et al., 2006). T0901317 enhances cholesterol synthesis and increases LDLr expression

Abbreviations: LXR, liver X receptor; VLDL, very low density lipoprotein; Apo B, apolipoprotein B; SREBP, sterol regulatory element binding protein; CYP7A1, cholesterol 7 alpha-hydroxylase; HMGR, 3-hydroxy-3-methylglutaryl CoA reductase; LDLr, low-density lipoprotein receptor; MTP, microsomal triglyceride transfer protein.

* Corresponding author. Tel./fax: +81 42 367 5853.
E-mail address: satokan@cc.tuat.ac.jp (K. Sato).

1095-6433/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2010.10.028

in human hepatoma HepG2 cells (Aravindhan et al., 2006). To this extent, LXRs may be regulators of cholesterol biosynthesis and uptake via the regulation of SREBP-2 activation. Therefore, the interpretation of data concerning the role of LXRs in the regulation of cholesterol synthesis and uptake in mammals is variable. In general, it is assumed that the effect of LXRs on cholesterol synthesis plays only a minor part in the regulation of cellular sterol homeostasis.

In avian species, some reports have shown that LXRs regulate fatty acid synthesis. T0901317 has an effect on acetyl CoA carboxylase hepatic transcription through the LXR-RXR heterodimer (Talukdar and Hillgartner, 2006) and stimulates SREBP-1 and fatty acid synthase (FAS) gene expression as a direct target of LXRs (Demeure et al., 2009). However, there is no information available as yet to provide evidence for a role of LXRs in bile acid and cholesterol synthesis. Lipogenic activity in avian species is much greater in the liver than in adipose tissue. Most of the fats accumulated in adipose tissue are incorporated as triacylglycerols from the LPL-catalyzed hydrolysis of plasma lipoproteins, which are either taken up as triacylglycerols from VLDL secreted and transported from the liver, or obtained from dietary fats (Sato et al., 1999). These results suggest a species-specific difference between chicken and mammalian lipid metabolism and give rise to speculation that the physiological functions of LXRs in chickens are distinct from their mammalian counterparts. In fact, we previously reported that LXRs up-regulate SREBP-2 gene expression in avian follicle cells (Seol et al., 2007) and that the expression profile of SREBP-2 paralleled that of LXRs in the chicken embryo liver (Sato et al., 2008). However, previous studies did not examine the role of LXRs on bile acid synthesis and cholesterol biosynthesis, including gene expression profiles, when the chicken hepatocyte culture medium was supplemented with the LXR agonist T0901317.

The present study was therefore conducted to examine the manner in which exposure of chicken hepatocytes to different concentrations of T0901317 affects bile acid, cholesterol, and fatty acid synthesis, as well as VLDL secretion and expression of related genes.

2. Materials and methods

2.1. Animals

Male broiler chickens (*Gallus gallus*; Ross, obtained from the Matsumoto Hatchery, Ibaragi, Japan) were fed *ad libitum* a commercial grower diet (crude protein 22 g/kg diet, metabolizable energy 13.0 MJ/kg diet) and housed in wire cages under controlled temperature (23 ± 3 °C) conditions. The birds were used for experiments when their body weights were within the range of 1000 to 1200 g at about 4 weeks of age. The Animal Care and Use Committee of the Tokyo University of Agriculture and Technology approved all procedures.

2.2. Chemicals

Bovine glucagon was purchased from Sigma Chemical Co. Ltd (St. Louis, MO, USA). Basal Medium Eagle and antibiotics were obtained from Invitrogen (San Diego, CA, USA). Other reagents were obtained from Wako Pure Chemical Co. Ltd. (Osaka, Japan). Rooster serum was prepared from 10-week-old male broiler chickens.

2.3. Primary culture of chicken hepatocytes

Liver cells were prepared from chickens that had been fasted for 12 h and maintained in a monolayer culture as described previously (Tachibana et al., 2002). Hepatocytes with more than 90% viability (verified by the Trypan blue exclusion test) were used for subsequent plating (5.0×10^5 cells/ϕ 60 mm collagen type I-coated dish) with incubation medium (Basal Medium Eagle supplemented with essential amino acids), containing 75 U/mL penicillin, 75 U/mL streptomycin, 1 μg/mL insulin (bovine), 1 μg/mL glucagon (human recombinant), and 0.5% rooster serum. After 20-h incubation (attachment phase), cultures were preincubated for 1 h in serum- and hormone-free media, followed by a 24-h incubation in serum- and hormone-free media containing 0, 0.01, 0.1, 1.0, or 10.0 μM T0901317. After the 24-h incubation, the culture media and cells were cooled on ice. ApoB levels in the media were then measured and cells were collected for analysis of VLDL-triacylglycerol (TG), total bile acid, mRNA expression, and intracellular protein levels. All analyses were conducted on six independent hepatocyte culture preparations.

2.4. Quantitation of mRNA expression using real-time PCR

Total RNA was extracted from cultured cells using Trizol reagent (Invitrogen, San Diego, CA, USA). To study alterations in the expression of related genes, real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed using the iCycler Real Time Detection System (Bio-Rad Laboratories, Hercules, CA, USA) as described previously (Matsubara et al., 2005; Sato et al., 2008). Briefly, 5 μg of total RNA was reverse transcribed using random primer and M-MLV reverse transcriptase (Invitrogen). Each RT-reaction served as a template in a 50 μL PCR reaction mixture containing 2 mM $MgCl_2$, 0.5 mM of each primer and 0.5× SYBR green master mix (Bio Whittaker Molecular Applications). Temperature cycles were as follows: 94 °C for 3 min followed by 35 cycles of 94 °C for 30 s, 63 °C for 1 min, and 72 °C for 1 min. SYBR green fluorescence was detected at the end of each cycle to monitor the amount of PCR product formed during that cycle. At the end of each run, melting curve profiles were recorded. Oligonucleotide sequences of sense and antisense primers are shown in Table 1. Standard curves of each product followed the calculation of respective gene expressions. Results for each RNA type are presented as a ratio to ribosomal protein S9 (RPS9) corrected for differences in the amounts of template DNA used.

Specificity of the amplification product was verified by electrophoresis on a 0.8% agarose gel and by DNA sequencing.

2.5. Western blot analysis

After incubation of cells with T0901317, the hepatocyte culture media were separated by 6% SDS-PAGE in the absence of reducing agents and then transferred to nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA). Western blotting experiments utilized phosphate-buffered saline solution (PBS) containing 7% non-fat dry milk and strips were incubated with 5 g/mL of chicken apoB-specific monoclonal antibody (CAB4) (Chiba et al., 2003) followed by the corresponding anti-mouse IgG conjugated with horseradish peroxidase. After rinsing 5 times in PBS containing 0.3 % Tween 20, strips were incubated in substrate solution (ECL kit, Amersham Bioscience Corp. Piscataway, NJ, USA) for 5 to 10 min and exposed to Kodak XAR-5 film for 1 min.

2.6. Measurements of VLDL-TG, intracellular protein, and DNA contents

VLDL in culture media were quantitatively collected by ultracentrifugation ($d<1.065$ g/mL) for 16 h using an HITACHI Himac CP56GII ultracentrifuge (Hitachi Koki, Tokyo, Japan) with a RP65T rotor. VLDL-TG was extracted according to the method of Folch and Sloan-Stanly (1957). TG concentrations were quantified by the method of Fletcher (1968).

Total bile acids were measured using a kit from Diagnostic Chemicals Ltd. (Charlottetown, PEI, Canada). Intracellular protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. DNA content was measured using the method of Kissane et al. (1958).

2.7. Statistical analysis

The SAS applications software package (Statistical Analysis System Version 6.03, SAS Institute Inc., Cary, NC, USA) was used for statistical

Download English Version:

https://daneshyari.com/en/article/1972835

Download Persian Version:

https://daneshyari.com/article/1972835

Daneshyari.com