Fibroblast growth factor 21 (FGF21) is an effective metabolic regulator of glucose and lipid homeostasis in the context of insulin resistance, glucose intolerance and dyslipidemia in diabetic rodents and monkeys.3) The expression of FGF21 is most abundant in the liver and it is induced by fasting and starvation in a peroxisome proliferator-activated receptor α (PPARα)-dependent manner in rodents.3) Tentative PPAR responsive elements (PPREs) are located in the promoter regions of both mouse and human FGF21 genes, and hepatic FGF21 mRNA expression is robustly induced by PPARα agonists as well as by fasting and starvation in mice. However, recent findings suggest that the effects and regulation of FGF21 qualitatively differ between rodents and humans. Here, we examined the effects of PPARα and PPARγ agonists on FGF21 mRNA expression in the mouse liver and in cultured hepatocytes. Intraperitoneal injection of both bezafibrate and pioglitazone induced FGF21 mRNA expression in the mouse liver. Rosiglitazone and pioglitazone as well as bezafibrate significantly induced FGF21 mRNA expression in cultured mouse hepatocytes. On the other hand, both rosiglitazone and pioglitazone significantly induced pyruvate dehydrogenase kinase 4 mRNA expression, suggesting that HepG2 cells are sensitive to bezafibrate like the mouse liver. Our findings suggest that PPARγ-activating antidiabetic drugs such as rosiglitazone and pioglitazone induce FGF21 expression in mouse and human hepatocytes, and that PPARγ rather than PPARα might play an important role in human FGF21 production.

Key words fibroblast growth factor 21; rosiglitazone; pioglitazone; bezafibrate; HepG2; mouse liver

MATERIALS AND METHODS

Six-week-old male Slc:1CR mice (Japan SLC Inc., Hama-matsu, Japan) were housed under a 12 h light–12 h dark cycle and given food and water ad libitum. Bezafibrate (Sigma, U.S.A.), rosiglitazone (Cayman Chemical Co., MI, U.S.A.) and pioglitazone (Toronto Research Chemicals Inc., Ontario, Canada) were dissolved in warm (ca. 40°C) sterile corn oil (Sigma) at a concentration of 3 mg/ml and administered as a single intraperitoneal (i.p.) dose of 30 mg/kg body weight. Sterile corn oil served as the control injection. After 4 h, the mice were sacrificed and tissues were dissected, quickly frozen and stored at −80°C. All animal experiments and handling proceeded under the authorization of the Animal Care and Use Committees of AIST (Permission #2010-020).

Mouse primary hepatocytes were obtained from the Primary Cell Co., Ltd. (Ishikari, Japan) as attached cells in 24-well plates in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 U/ml penicillin, 10 μg/ml streptomycin, 0.5 μg/ml insulin, 1 μM dexamethasone, 10 ng/ml epidermal growth factor, 200 μM ascorbic acid, and 10 μM nicotinamide. HepG2 cells were cultured in DMEM (Sigma) supplemented with 10% FBS (Invitrogen) and antibiotics in a 5% CO₂ atmosphere. The cells were incubated with 100 μM of PPAR ligands or vehicle (0.01% dimethyl sulfoxide (DMSO)) for 4 h, washed with ice cold phosphate buffered saline (PBS), then harvested in RNAiso (Takara Bio Inc., Otsu, Japan) and stored at −80°C.

Total RNA was extracted using RNAiso. Single-strand cDNA was synthesized using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara Bio Inc., Otsu, Japan). Real-time reverse transcription-polymerase chain reaction (RT-PCR) proceeded using the SYBR® Premix Ex Taq™ II (Takara Bio Inc., Otsu, Japan) using a LightCycler™ (Roche Diagnostics, Mannheim, Germany). The reaction conditions were 95°C for 10 s followed by 45 cycles of 95°C for 5 s, 57°C for 10 s and 72°C for 10 s. The sequence of primer pairs were as follows: mouse FGF21, 5’-ATGGATGGATTGAGATCTAGAGTTGG-3’ and 5’-TCTTGTCTGCTCATCTGGTGAGAGG-3’; mouse pyruvate dehydrogenase kinase 4 (PDK4), 5’-CACATGCTTTGCAACTCTTGAAG-3’ and
Expression in Mouse Liver

Fig. 1. Acute Effects of PPARα and PPARγ Ligands on FGF21 mRNA Expression in Mouse Liver

Bezafibrate (BEZ), rosiglitazone (RSO) and pioglitazone (PIO) were administered as a single intraperitoneal (i.p.) dose of 30 mg/kg body weight. After 4 h, the mice were decapitated and the liver tissues were dissected. Value for control (CTRL) mice is expressed as 100% and other values are presented as means ± S.E.M. (n=6–8). Data were statistically evaluated using the one-way analysis of variance (ANOVA). Values of *p<0.05 and **p<0.01 were considered statistically significant. Significant differences compared with value from control mice are indicated as *p<0.05 and **p<0.01.

5′-TGATTGTAAGGCTCTCTTTCCCCAG-3′; mouse 18S ribosomal RNA (rRNA), 5′-GTAACCCGTTGAACCCCAATT-3′ and 5′-CCATCCAATGAGTACGAG-3′; human FGF21, 5′-GAGTCAGACATCAGTTCTCTC-3′ and 5′-GACTTTTCCCCGCCAGGTGAG-3′; human PDK4, 5′-CTGTCATGAAAGCTCTAC-3′ and 5′-TCCACCAAATCCATCGGGCTCTG-3′; human 18S rRNA, 5′-CCGACCACCATCCAG-3′ and 5′-GAATACGAACCTCTGATTCCCGT-3′. The amount of target mRNA was normalized relative to that of 18S rRNA.

RESULTS AND DISCUSSION

Intraperitoneal injection of both bezafibrate and pioglitazone significantly induced, whereas that of rosiglitazone did not affect FGF21 mRNA expression in the mouse liver (Fig. 1). Rosiglitazone and pioglitazone as well as bezafibrate significantly induced FGF21 mRNA expression in cultured mouse hepatocytes (Fig. 2A). On the other hand, both rosiglitazone and pioglitazone significantly induced, whereas bezafibrate did not affect FGF21 mRNA expression in the human liver carcinoma cell line HepG2 (Fig. 2B). However, bezafibrate significantly induced pyruvate dehydrogenase kinase 4 (PDK4) mRNA expression, suggesting that HepG2 cells are sensitive to bezafibrate like the mouse liver. Our findings suggest that PPARγ-activating anti-diabetic drugs such as rosiglitazone and pioglitazone induce FGF21 expression in both mouse and human hepatocytes, and that PPARγ plays a critical role in the regulation of FGF21 expression especially in the human liver.

High levels of PPARγ are expressed in the fatty liver induced by a high-fat or ketogenic diet, the latter of which induces FGF21 expression in a PPARα-independent manner.7,8 FGF21 seems to be a novel biomarker for non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH).9 Therefore, PPARγ activation might be responsible for hepatic FGF21 expression under conditions of NAFLD or NASH. On the other hand, Gälman et al. reported that serum levels of FGF21 varied 250-fold among 76 healthy individuals and did not relate to age, gender, body mass index, serum lipids, or plasma glucose.3) Further elucidation of the molecular mechanism regulating FGF21 expression by thiazolidinediones especially in human tissues will be of great interest.

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