Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 329 (2005) 391–396
BBRC
www.elsevier.com/locate/ybbrc

Regulation of pyruvate dehydrogenase kinase expression by the farnesoid X receptor

Rajesh S. Savkur [a], Kelli S. Bramlett [a,b], Laura F. Michael [a], Thomas P. Burris [a,b,*]

[a] *Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, IN 46285, USA*
[b] *Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, USA*

Received 25 January 2005

Abstract

The pyruvate dehydrogenase complex (PDC) functions as an important junction in intermediary metabolism by influencing the utilization of fat versus carbohydrate as a source of fuel. Activation of PDC is achieved by phosphatases, whereas, inactivation is catalyzed by pyruvate dehydrogenase kinases (PDKs). The expression of PDK4 is highly regulated by the glucocorticoid and peroxisome proliferator-activated receptors. We demonstrate that the farnesoid X receptor (FXR; NR1H4), which regulates a variety of genes involved in lipoprotein metabolism, also regulates the expression of PDK4. Treatment of rat hepatoma cells as well as human primary hepatocytes with FXR agonists stimulates the expression of PDK4 to levels comparable to those obtained with glucocorticoids. In addition, treatment of mice with an FXR agonist significantly increased hepatic PDK4 expression, while concomitantly decreasing plasma triglyceride levels. Thus, activation of FXR may suppress glycolysis and enhance oxidation of fatty acids via inactivation of the PDC by increasing PDK4 expression.
© 2005 Elsevier Inc. All rights reserved.

Keywords: PDK4; Glucocorticoids; PPAR; TRB-3; FOXO; Bile acids; Diabetes; Lipid; Glucose

The pyruvate dehydrogenase complex (PDC) plays a pivotal role in intermediary metabolism and is regulated by dietary substrates and hormones. Under conditions of abundant food supply, the PDC is functionally active and promotes the utilization of glucose for the formation of acetyl- and malonyl-CoA and the synthesis of fatty acids. Conversely, in the starved state, the PDC is inactivated thereby limiting glucose oxidation and promoting fatty acid oxidation as the source of fuel. Thus, the activity of PDC serves as an important metabolic switch for fuel selection [1,2]. Activity of the PDC is primarily regulated by reversible phosphorylation of the complex that is mediated by specific kinases and phosphatases. Inactivation of the complex is catalyzed by four pyruvate dehydrogenase kinase (PDK) isozymes (PDK1–PDK4) that are expressed in varying amounts in a tissue-specific manner [3].

Previous studies have demonstrated that an increase in hepatic PDK activity upon acute starvation occurs in conjunction with an increase in expression of both PDK2 and PDK4 [4–6]. However, the mechanisms involved in the regulation of hepatic PDK4 expression differ from those of PDK2. Peroxisome proliferator-activated receptor-α (PPARα) has been implicated to play a critical role in regulating the expression of PDK4 [7–10], but not that of PDK2 [11]. Furthermore, PPARα-null mice exhibit dysregulated hepatic lipid and carbohydrate metabolism [6], consistent with the observation that PPARα agonists increase the expression of PDK4 [11,12]. In addition to the role of PPARα in PDK4 regulation, glucocorticoid receptor (GR) agonists have also been shown to induce the expression of PDK4, but not PDK2 [11]. Since glucocorticoids also induce the expression of PPARα [13], it is conceivable that

* Corresponding author. Fax: +1 317 276 1414.
E-mail address: burris_thomas_p@lilly.com (T.P. Burris).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.01.141

the induction of PDK4 expression by glucocorticoids could involve a pathway mediated by PPARα.

The farnesoid X receptor (FXR; NR1H4) is a member of the superfamily of nuclear hormone receptors [14] that was recently identified as the physiological receptor of bile acids [15,16]. FXR is predominantly expressed in the liver, kidney, and intestine, and plays a critical role in the regulation of bile acid synthesis [17–19]. Furthermore, FXR-null mice display additional deficiencies in lipid metabolism implicating a broader role for the bile acid receptor in the regulation of lipoprotein metabolism [20,21]. We have recently demonstrated that FXR also plays an important role in carbohydrate metabolism [22]. Since the expression of PDK4 is regulated by both PPARα and the FOXO proteins [23], and FXR plays a role in carbohydrate metabolism via PPARα and the FOXO proteins [22], we investigated the ability of FXR agonists to regulate the expression of PDK4.

In this study, we demonstrate that FXR agonists increase the expression of PDK4 in vitro as well as in vivo. Induction of PDK4 via activation of FXR is conserved between human primary hepatocytes, rat hepatoma cultured cells, and mice.

Materials and methods

Cell culture and reagents. H4IIE rat hepatoma cells and cryopreserved human primary hepatocytes (Cambrex, Walkersville, MD) were maintained as previously described [22]. Dexamethasone (DEX), chenodeoxycholic acid (CDCA), and WY14,643 were purchased from Sigma Chemical (St. Louis, MO). FXR agonists, GW4064 and fexaramine (FEX), were synthesized using standard organic chemistry synthetic methods.

mRNA measurements. H4IIE cells were seeded in 6-well plates at 1×10^6 cells per well in DMEM 10% FBS and allowed to attach overnight. The following day cells were washed once in serum free DMEM and serum starved for 24 h prior to treatment with FXR agonists, DEX, or WY14,643. In experiments examining mRNA expression from primary human hepatocytes, cells were treated with FXR and GR ligands, as indicated, for 8 h. Quantitative PCR was performed as previously described using TaqMan reagents and instrumentation [22].

Immunoblot analysis. After treatment of H4IIE cells for 24 h with DEX (1 μM), GW4064 (2.5 μM), CDCA (40 μM) or FEX (20 μM), total cellular protein was isolated and a Western blot was performed as previously described [22]. Relative expression was determined by densitometry. Polyclonal antibodies to the C-terminus of PDK4 (AP7041b; Abgent, San Diego, CA) and GAPDH (ab9485; Abcam, Cambridge, MA) were used for immunoblot analysis.

Analysis of the effects of GW4064 in mice. C57BL/6 mice (7–9 weeks of age) were treated with GW4064 (50 mg/kg BID; IP) or 5% acacia vehicle (4 animals/group). After 7 days of treatment, the mice were sacrificed and total RNA from liver was isolated. The expression of PDK4 was assessed by TaqMan Q-PCR, and plasma triglycerides were measured as previously described [24].

Statistical analysis. Statistical analysis was assessed using the Mann–Whitney test or ANOVA followed by a Tukey test. Values of $p < 0.05$ were considered significant. All values are reported as means ± SEM.

Results and discussion

Previous studies using a rat hepatoma cell line demonstrated that the GR agonist, dexamethasone (DEX), induces the expression of PDK4 [11]. To determine whether FXR ligands affect the expression of PDK4, we treated H4IIE rat hepatoma cultured cells with the potent non-steroidal FXR agonist, GW4064. As illustrated in Fig. 1A, Q-PCR analysis of RNA isolated from H4IIE cells treated with GW4064 significantly increased the expression of PDK4. This increase in PDK4 mRNA expression was comparable to that observed when the cells were treated with DEX (2.0-fold). As illustrated in Fig. 1B, GW4064 dose-dependently increased the expression of PDK4. To ascertain whether the increase in PDK4 mRNA also resulted in an increase in the expression of PDK4 protein, immunoblot analysis was performed using total protein isolated from the H4IIE cells that were treated with three FXR agonists, CDCA, FEX, and GW4064, and the GR agonist, DEX. As shown in Fig. 1C, all the three FXR agonists increased the expression of PDK4 protein in the range of 5- to 9-fold. This increase in PDK4 protein expression was comparable to that observed with cells that were treated with DEX (5-fold).

To investigate whether the increase in PDK4 expression observed with FXR ligands could be detected in cells from human origin, cultured human primary hepatocytes were treated with either GW4064 or DEX for 24 h followed by isolation of total RNA. As illustrated in Fig. 2, both GW4064 and DEX were effective in inducing the expression of PDK4. The induction of PDK4 mRNA was 7.5-fold and 20-fold for 100 nM and 1 μM GW4064, respectively, and 7-fold for 1 μM DEX. The difference in the magnitude of PDK4 induction between human primary hepatocytes and rat hepatoma cells may be due to the differences in sensitivity between the species and the transformed nature of the hepatoma cells, or alternatively, may be a function of the sensitivity of the particular donor of human primary hepatocytes. Nevertheless, these results indicate that the induction of PDK4 expression by FXR agonists is conserved between rat and human.

To determine whether activation of FXR leads to an increase in PDK4 expression in vivo, we treated C57BL/6 mice with the FXR agonist, GW4064. Similar to previous observations [25], activation of FXR by a 7-day treatment with GW4064 resulted in a 52.5 ± 8% reduction in the levels of plasma triglyceride levels (Fig. 3). Consistent with the induction of PDK4 expression in human and rat cultured cells upon treatment with FXR agonists, expression of PDK4 mRNA was increased 340 ± 140% in comparison with the vehicle treated animals (Fig. 3). Thus, activation of FXR in vivo resulted in an increase in PDK4 expression that was associated with a concomitant decrease in plasma

Download English Version:

https://daneshyari.com/en/article/10771837

Download Persian Version:

https://daneshyari.com/article/10771837

Daneshyari.com