Effect of Linoleic Acid on Gluconeogenesis In Primary Cultured Chicken Hepatocyte: Involvement of $cPLA_2$, COX_2 and PPARδ pathways

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INTRODUCTION

Liver plays a major role in regulating blood glucose level by maintaining the balance between the storage and release of glucose (Kim et al., 2007). FFA levels are often increased in obese individuals in both the fed and fasted states and have been implicated as critical players in the progression of obesity to type II diabetes (Baldeweg et al., 2000; Bolinder et al., 2000; Boden et al., 1997; Henry et al., 1995; Kelley et al., 2000; Lewis et al., 2002). FFA has been proposed to directly regulate hepatic gluconeogenesis independent of hormones in several ways. First, FFA promotes gluconeogenesis by serving as a source of substrates and energy, including acetyl-CoA, NADH, and ATP. Second, FFA may directly regulate gluconeogenesis through transcription of key gluconeogenic genes (Lam et al., 2003). Gluconeogenesis is involved several signal pathways. The intermediary protein kinase Akt2/protein kinase B (PKB)-β, elicits the phosphorylation of PGC-1, promoting gluconeogenesis in the liver of type 2 diabetes mellitus patients (Li et al., 2007). p38 MAPK is necessary for FFA-induced activation of the gluconeogenesis on primary rat hepatocyte (Collins et al., 2006). Also, AMP-activated protein kinase (AMPK) is considered as a “fuel-gauge” on gluconeogenesis in metabolic diseases such as obesity and type II diabetes mellitus (Daval et al., 2006). Activities of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) are known to contribute to gluconeogenesis transcriptional regulation in most models of diabetes (Hall et al., 2000; Schmoll et al., 2000; Argaud et al., 1996). Although PPARs are ligand-activated transcription factors involved in physiological issues, such as energy balance, lipid metabolism, and glucose control (Lee et al., 2003), the functions of PPARβ/δ in hepatocytes gluconeogenesis exposed to linoleic acid are largely unknown. Therefore, we hypothesize that PPARδ may plays an important role in the gluconeogenesis of primary cultured chicken hepatocytes.

A primary culture of hepatocytes has been used by many biophysiological studies on the liver function because it retains many of the liver-specific functions and responds to various hormones through the expression of the liver-specific functions (Lee et al., 2006; Suh et al., 2007). The primary chicken hepatocytes culture system used in this study also retains the *in vitro* differentiated phenotype that is typical of the liver, including albumin expression (Hou et al., 2001), P450 1A induction (Hou et al., 2001), tyrosine aminotransferase expression (Sasaki et al., 2000), and ascorbate recycling (Sasaki et al., 2001). Therefore, this study examined the effect of linoleic acid on gluconeogenesis and its related pathways in primary cultured chicken hepatocytes.

MATERIALS AND METHODS

Materials

Two week-old male White Leghorn chickens were obtained from Dae Han Experimental Animal Co, Ltd. (Chungju, Korea). All the procedures for animal management were performed according to the standard protocols at Seoul National University. The appropriate management

of the experimental samples and quality control of the laboratory facility and equipment were maintained. The Class IV collagenase and soybean trypsin inhibitor were purchased from Life Technologies (Grand Island, NY, USA). The fetal bovine serum was supplied by Gibco (Rockville, MD, USA). The linoleic acid, mepacrine, $AACOCF_3$, indomethacin, and monoclonal anti β-actin were obtained from Sigma Chemical Company (St. Louis, MO, USA). The cPLA2, COX2, and PPARδ antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The goat anti-rabbit IgG was purchased from Jackson Immunoresearch (West Grove, PA, USA). All the other reagents were of the highest purity commercially available.

Methods

Primary culture of chicken hepatocytes

The chicken liver cells were prepared and maintained as a monolayer culture, as described elsewhere (Hou et al., 2001). Briefly, the chicken hepatocytes were isolated by perfusion with 0.05% collagenase from a liver that had been starved for 3 hr. Hepatocytes with > 90% viability, as verified by a trypan blue exclusion test, were used for subsequent plating. The hepatocytes were plated (5.0×10^5 cells/ 60-mm collagen-coated dish) with an incubation medium (Basal Medium Eagle supplemented with essential amino acids), containing 75 U/ml penicillin and 75 U/ml streptomycin, 1 μg/ml insulin, 10^{-12} M dexamethasone, 5 μg/ml transferrin, 10^{-8} M T_3, and 5% calf serum, and incubated for 4 hr at 37°C in 5% CO_2. The medium was then replaced with fresh incubation medium, and the hepatocytes were incubated for a further 20 hr in order to achieve the monolayer culture.

Measurement of glucose production

Glucose production from primary cultured chicken hepatocytes was measured as previously described (Collins et al., 2006). Briefly, cells were washed three times with warm phosphate-

buffered saline to remove glucose, followed by the treatment with 0.1 mM linolein acid for 4 hr in the glucose-free medium (Gibco 11966-025) with containing gluconeogenic substrates (20 mM sodium lactate and 2 mM sodium pyruvate). Glucose concentration was determined with a glucose assay kit from Roche Applied Science (cat. No. 0716251) and normalized to the cellular protein concentrations. Total glucose production was derived from both glycogenolysis and gluconeogenesis. Glucose production from glycogenolysis was measured in the absence of gluconeogenic substrates. The amount of glucose production by gluconeogenesis is defined as the difference between total glucose production and glucogenolysis.

Assay of AA release

AA release from the cultures was determined by a modification of the method of Xing et al. To summarize, confluent monolayers of chicken hepatocytes were incubated for 24 hours in basal medium containing 0.5 mCi [^{3}H]AA/ml, as well as the three growth supplements. The monolayers were washed three times with William's medium (pH 7.4), and incubated (at 37°C) for 1 hour in incubation medium containing specified agents. At the end of the 1 hour incubation period, the incubation medium was removed, and transferred to ice-cold tubes containing 100 ml of 55 mM EGTA and EDTA (final concentration, 5 mM each). The incubation medium was then centrifuged at 12,000g to eliminate cell debris. To determine radioactive levels, aliquots of the samples were placed in scintillation vials containing scintillation fluid, and the radioactivity was counted using a liquid scintillation counter. The cells which remained attached to the plates were scraped into 1 ml of 0.1% SDS, and 900 ml of the resulting cell lysate was utilized for scintillation counting. The remaining 100 ml of the cell lysate was utilized for protein determinations. The quantity of [^{3}H]AA release in each condition (determined as described above) was then standardized with respect to protein. Subsequently, this standardized level of released [^{3}H]AA was compared percentage-wise to the level of total [^{3}H]AA which had been incorporated into the cells at the start of the 1 hour incubation period

with the specified agents (equivalent to the quantity of total released radioactivity plus the total cell-associated radioactivity at the end of the 1 hour incubation period).

PGE_2 Assay

Chicken hepatocytes plated on 60-mm culture plates were grown in a FBS-free medium for 24 hours and divided into groups according to the experimental protocol. The PGE_2 concentration in the culture medium was measured using an enzyme-linked immunosorbent assay (ELISA) with a PGE_2 High Sensitivity Immunoassay kit (R&D Systems, Minneapolis, MN, USA).

PPARδ small interfering ribonucleic acid transfection

The cells were grown in each dish until they reached 75% confluence. They were then transfected for 24 hours with either a SMART pool of the small interfering RNAs specific to PPAR δ (200 pmol/L) or a non-targeting small interfering RNA (as negative control; 200 pmol/L; Dharmacon, Inc., Lafayette, CO) using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

RNA isolation and RT-PCR

Total RNA was extracted from primary cultured chicken hepatocytes using STAT-60, monophasic solution of phenol and guanidine isothiocyanate from Tel-Test, Inc. (Friendwood, Tex., USA). Reverse transcription was conducted with 3 μg RNA using reverse transcription system kit (AccuPower® RT PreMix, Korea) with oligo-dT_{18} pri-mers. After that, 5 μl of RT products was amplificated with PCR kit (AccuPower® PCR PreMix, Korea) followed by: denaturation at 94 °C for 5 min and 35 cycles at 94 °C for 15 sec, 55 °C for 1 min and 72 °C for 30 sec followed by 5 min extension at 72 °C. Amplifications of G6Pase, PEPCK, and PPAR (α, δ, γ) cDNAs were performed in cells using primers described in Table 1. PCR of β-actin

was also performed as control for quantity of RNA.

Real-time RT-PCR

The total RNA was extracted from the cells treated with each designated agent using STAT-60, which is a monophasic solution of phenol, and guanidine isothiocyanate, which was purchased from Tel-Test, Inc. (Friendwood, Tex., USA). The real-time quantification of RNA targets was then performed in the Rotor-Gene 2000 real-time thermal cycling system (Corbett Research, NSW, Australia) using the QuantiTect SYBR Green RT-PCR kit (QIAGEN, CA, USA). The reaction mixture (20 μl) contained 200 ng of total RNA, 0.5 μM of each primer, appropriate amounts of enzymes, and fluorescent dyes as recommended by the supplier. The Rotor-Gene 2000 cycler was programmed as follows: 30 min at 50°C for reverese transcription; 15 min at 95°C for DNA polymerase activation; 15 sec at 95°C for denature; 45 cycles of 15 sec at 94°C, 30 sec at 55°C, 30 sec at 72°C. The data collection was carried out during the extension step (30 sec at 72°C). The PCR reaction was followed by a melting cure analysis to verify specificity and identity of the RT-PCR products, which can distinguish the specific PCR products from the non-specific PCR product resulting from primer-dimer formation. The primers used were described in Table 1. The temperature of PCR products was elevated from 65 to 99 °C at a rate of 1 °C/5 sec, and the resulting data were analyzed by using the soft-ware provided by the manufacturer.

Preparation of cytosolic and total membrane fraction

The medium was removed, and the cells were washed twice with ice-cold PBS, scraped, harvested by microcentrifugation and resuspended in buffer A (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, 2.5 mM EDTA, 1 mM dithiothreitol, 0.1 mM PMSF, 10 μg/ml leupeptin, pH 7.5). The resuspended cells were lysed mechanically on ice by trituration using a 21.1-gauge needle. The lysates were first centrifuged at 1,000 x g for 10 min

at 4°C. The supernatants were further centrifuged at 100,000 x g for 1 hr at 4°C to prepare the cytosolic and total particulate fractions. The particulate fraction containing the membrane fraction was washed twice and resuspended in buffer A containing 1% Triton X-100. The protein level in each fraction was quantified using the Bradford procedure (Bradford. 1976).

Western blot analysis

The cell homogenates (40 μg of protein) were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose paper. The blots were then washed with TBST (10mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween-20). The membrane was blocked with 5% skim milk for 1hr and incubated with the appropriate primary antibody at the dilutions recommended by the supplier. The membrane was then washed, and the primary antibodies were detected with goat anti-rabbit-IgG conjugated to horseradish peroxidase. The antibodies were incubated at 4°C. The bands were visualized using enhanced chemiluminescence (Amersham Pharmacia Biotech, England).

Statistical analysis

The results are expressed as the mean ± the standard error (S.E.). All the experiments were analyzed by ANOVA. In some experiments, a comparison of the treatment means was made with the control using a Bonnferroni-Dunn test. A p-value < 0.05 was considered significant.

Table 1. Primers used for PCR

Gene		Primer sequence 5'- 3'	Product size bp
G6Pase	Sense antisense	GTGAATTACCAAGACTCCCAG GCCCATGGCATGGCCAGAGGG	305 bp
PEPCK	Sense antisense	CTGGTTCCGGAAAGACAAAA GCTCGGAGCTCCCTCTCTAT	350 bp
PPARα	Sense antisense	GACGAATGCCAAGATCTGAGAAG CG CTCTTTGTA GTGCTGTCAGC	374 bp
PPARδ	Sense antisense	ACCTGCAGATGGGCTGTCAC GTCTCGATGTCGTGGATCAC	483 bp
PPARγ	Sense antisense	AAGAGCTGACCCAATGGTTG TCCATAGTGGAAGCCTGATGC	314 bp
β-actin	Sense antisense	AGCCATGTACGTAGCCATCC CTCTCAGCTGTGGTGGTGAA	230 bp

RESULTS

Effect of FFA on gluconeogenesis

The effective treatment time and concentration of linoleic acid on glucose production were determined by observing the chicken hepatocytes at various times (0-24 hr) and concentrations (0-1M). As shown in Fig. 1A and B, 10^{-4} M linoleic acid for 4 hr was chosen in the present study. To compare the effect of linoleic acid on gluconeogenesis with other fatty acids, the cells were treated with 10^{-4} M linoeic acid (double-unsaturated, long chain), oleic acid (monounsaturated, long chain), and palmitic acid (long chain), or caproic acid (short chain) for 4 hr and then glucose production were determined. Linoleic acid, oleic acid, palmitic acid promoted glucose production whereas caproic acid failed to promoted glucose production (Fig.1C).

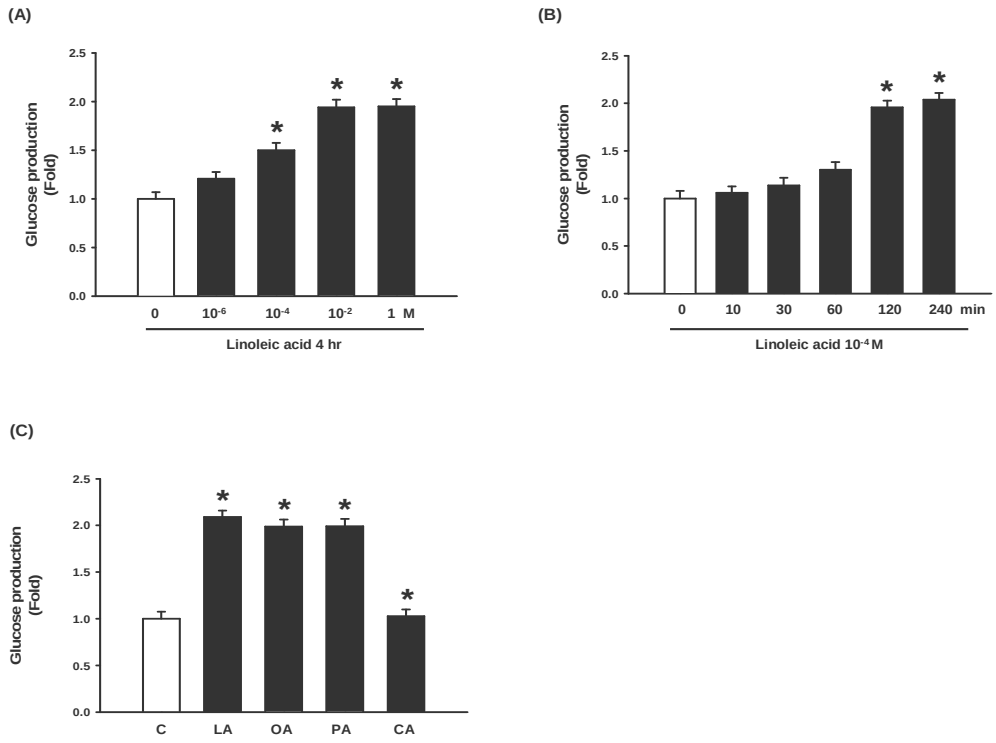

Figure 1. FFA induction of glucose production in primary cultured chicken hepatocytes. **(A, B)** Primary cultured chicken hepatocytes were incubated with different concentrations (0-1 M) and times (0-240 min) of linoleic acid and then glucose production was measured. The values represent the mean ± S.E. of 4 independent experiments with triplicate dishes. *P< 0.05 vs. control. **(C)** Cells were incubated with 10^{-4}M linoleic acid, oleic acid, palmitic acid, or caproic acid for 4 hr. Then glucose production was measured. The values represent the mean ± S.E. of 4 independent experiments with triplicate dishes. *P< 0.05 vs. control.

Involvement of $cPLA_2$ and COX_2 in linoleic acid-induced gluconeogenesis

The level of $cPLA_2$ phosphorylation was also increased from the 10 minutes treatment with linoleic acid (Fig. 2A). Indeed, as shown in Figure 2C, $AACOCF_3$ (10^{-6} M) and mepacrine (10^{-6} M) significantly blocked the linoleic acid-induced increase of [^{3}H]AA release (Fig. 2C). The cells were treated with $AACOCF_3$ and mepacrine before adding linoleic acid to examine the role of $cPLA_2$ pathway in linoleic acid-induced gluconeogenesis. As shown in Fig. 2B, $AACOCF_3$ and mepacrine significantly blocked the linoleic acid-induced increase glucose production. The COX (1, 2) expression was examined as a function of the time in order to determine if linoleic acid can induce the expression of the COX (1, 2) proteins. The results showed that linoleic acid increased the level of COX-2 expression in a time-dependent manner ($\geq$ 10 min) but had no effect on COX-1 (Fig. 3A). Moreover, these increases were inhibited by either $AACOCF_3$ or mepacrine (Fig. 3C) which suggests that $cPLA_2$ is upstream signal molecules of COX_2. Indeed, as shown in Fig. 3D, linoleic acid increased PGE_2 production, which were blocked by $AACOCF_3$ and indomethacin (10^{-6} M). The cells were treated with indomethacin before adding linoleic acid to examine the role of COX2 pathway in linoleic acid-induced gluconeogenesis. As shown in Fig. 3B, indomethacin significantly blocked the linoleic acid-induced increase glucose production.

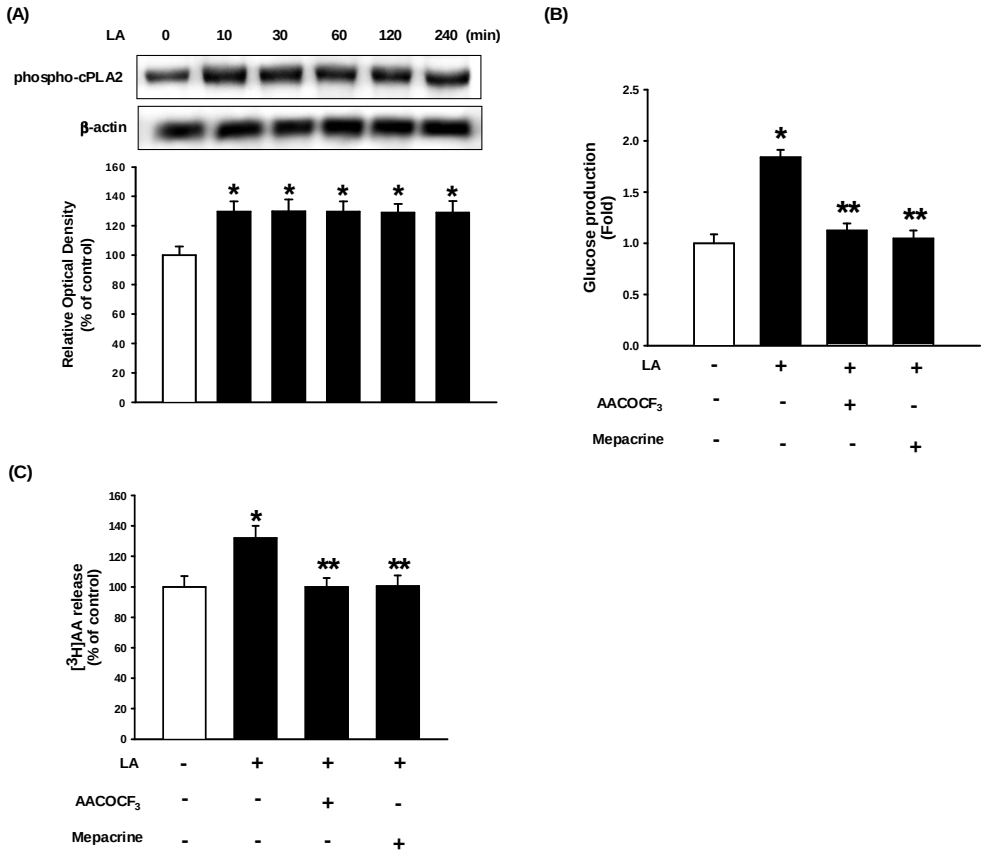

Figure 2. Involvement of $cPLA_2$ in linoleic acid-induced gluconeogenesis. Primary cultured chicken hepatocytes were isolated and cultured as detailed under "Materials and Methods". **(A)** Cells were treated with linoleic acid for 0-240 min and then harvested. The total protein was extracted and blotted with the phospho $cPLA_2$ or β-actin antibodies. The lower panels denote the means ± S.E. of three experiments for each condition determined from densitometry relative to β-actin. *P < 0.05 vs. control. **(B, C)** Cells were treated with $AACOCF_3$ (10^{-6} M) or mepacrine (10^{-6} M) for 30 min prior to treatment of linoleic acid for 4hr and then glucose production and [^{3}H]AA release were measured. The values represent the mean ± S.E. of 3 independent experiments with triplicate dishes. *P < 0.05 vs. control, **P < 0.05 vs. linoleic acid.

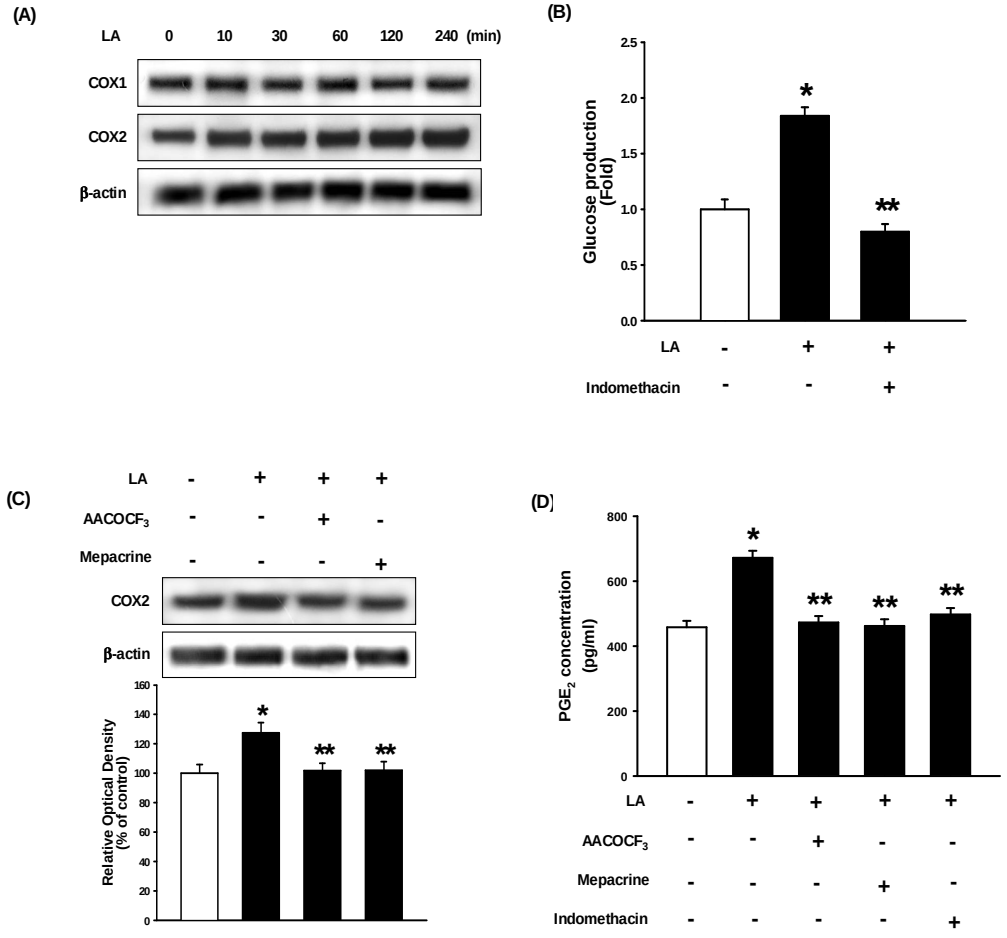

Figure 3. Involvement of COX_2 on linoleic acid-induced gluconeogenesis. Primary cultured chicken hepatocytes were isolated and cultured as detailed under "Materials and Methods". **(A)** Cells were treated with linoleic acid for 0-240 min and harvested. The total protein was

extracted and blotted with the COX_1, COX_2, or β-actin antibodies. The lower panels denote the means ± S.E. of four experiments for each condition determined from densitometry relative to β-actin. *P < 0.05 vs. control. **(B)** Cells were treated with indomethacin (10^{-6} M) for 30 min prior to treatment of linoleic acid for 4hr and then glucose production was measured. The values represent the mean ± S.E. of 4 independent experiments with triplicate dishes. *P < 0.05 vs. control, **P < 0.05 vs. linoleic acid. **(C)** To examine the upstream signal molecule, cells were treated with $AACOCF_3$ and mepacrine for 30 min prior to treatment of linoleic acid for 1 hr. The total protein was extracted and blotted with the COX_2 or β-actin antibodies. The lower panels denote the means ± S.E. of three experiments for each condition determined from densitometry relative to β-actin. *P < 0.05 vs. control, **P<0.05 vs. linoleic acid. **(D)** Cells were treated with $AACOCF_3$, mepacrine, and indomethacin for 30 min prior to treatment of linoleic acid for 2hr and then PGE_2 production was detected. The values represent the mean ± S.E. of 4 independent experiments with triplicate dishes. *P < 0.05 vs. control, **P < 0.05 vs. linoleic acid.

Involvement of PPARδ in linoleic acid-induced gluconeogenesis

The expression of PPAR isotypes were detected by RT-PCR to determine if the PPARs are involved in the linoleic acid-induced gluconeogenesis. As shown in Fig. 4A, PPARα, δ, and γ were expressed in chicken hepatocytes. Indeed, linoleic acid increased the level of the PPARδ proteins in a time-dependent manner (≥ 10 min) (Fig. 4B), which was blocked by $AACOCF_3$, mepacrine, and indomethacin (Fig. 4D). To determine if the PPARδ are involved in the linoleic acid-induced gluconeogenesis, the cells were transfected with the PPARδ specific siRNAs (200 pmole) then glucose production was measured. PPARδ specific siRNA blocked linoleic acid-induced glucose production whereas non specific siRNA had no effect (Fig. 4C). Linoleic acid

increased glucose-6-phosphatase and PEPCK (gluconeogenic enzymes) mRNA level (Fig. 5A). These results indicate that linoleic acid can directly elevate levels of gluconeogenesis gene transcripts in hepatocytes. PPARδ specific siRNA blocked linoleic acid-induced gluconeogenic genes mRNA expression which suggest relevancy (Fig. 5B).

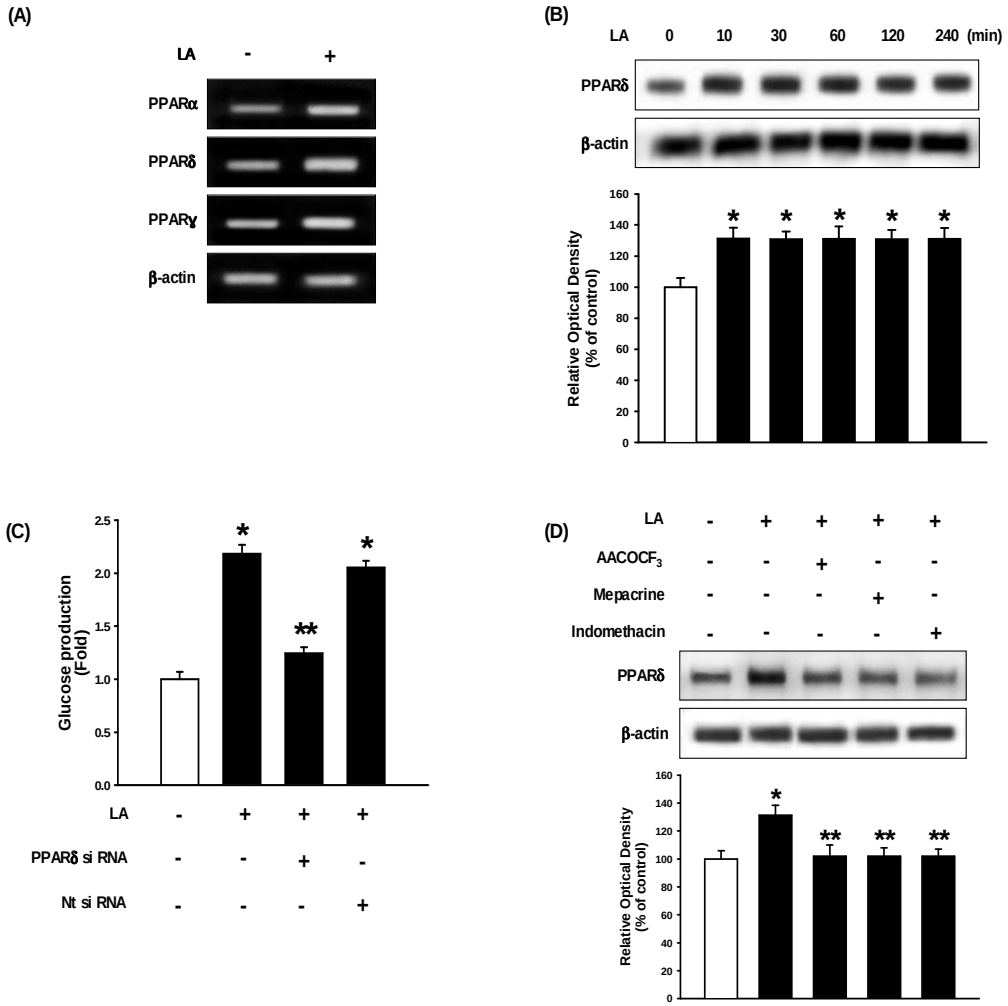

Figure 4. Involvement of PPARδ in linoleic acid-induced gluconeogenesis. Primary cultured chicken hepatocytes were isolated and cultured as detailed under "Materials and Methods". **(A)** Cells were treated with linoleic acid for 2 hr, and the PPAR α, δ, γ gene expression levels were

then analyzed by RT-PCR. **(B)** Cells were incubated with linoleic acid for 0-240 min and then harvested. The nuclear fraction was extracted and blotted with the PPARδ or β-actin antibodies. The lower panels denote the means ± S.E. of five experiments for each condition determined from densitometry relative to β-actin. *P < 0.05 vs. control. **(C)** Cells were transfected for 24 hours with either a SMARTpool of PPAR (siRNAs (200 pmol/L) or a non-targeting control siRNA (200 pmol/L) using LipofectAMINE 2000 prior to linoleic acid treatment for 2 hr. Then glucose production was measured. The values represent the mean ± SE of 4 independent experiments with triplicate dishes. *P<0.05 vs. control, **P<0.05 vs. linoleic acid. **(D)** To examine the upstream signal molecule, cells were treated with $AACOCF_3$, mepacrine, and indomethacin for 30 min prior to treatment of linoleic acid for 1 hr. The nuclear fraction was extracted and blotted with the PPARδ or β-actin antibodies. The lower panels denote the means ± S.E. of four experiments for each condition determined from densitometry relative to β-actin. *P<0.05 vs. control, **P<0.05 vs. linoleic acid.

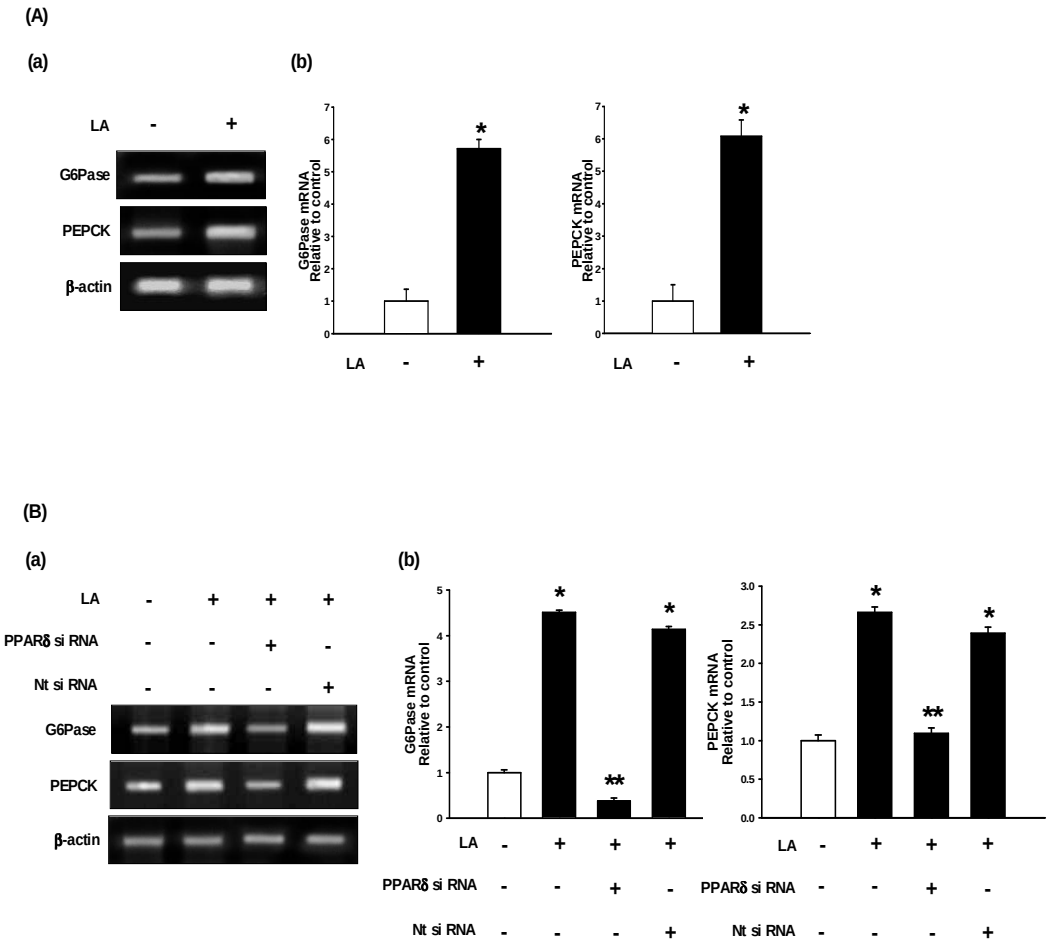

Figure 5. Involvement of PPARδ ioin gluconeogenic enzymes. (A) Primary cultured chicken hepatocytes treated with linoleic acid for 4 hr and the G6Pase and PEPCK mRNA levels were analyzed by RT-PCR or real-time RT-PCR. The values represent the mean ± SE of 3 independent experiments. *P<0.05 vs. control. **(B)** Cells were transfected for 24 hours with either a SMART pool of PPAR (siRNAs (200 pmol/L) or a non-targeting control siRNA (200 pmol/L) using LipofectAMINE 2000 prior to linoleic acid treatment for 4 hr. The G6Pase and PEPCK mRNA levels were analyzed by RT-PCR or real-ime RT-PCR. The values represent the mean ± SE of 4 independent experiments. *P<0.05 vs. control, **P<0.05 vs. linoleic acid.

DISCUSSION

In the present study, we showed linoleic acid (double-unsaturated, long chain), oleic acid (monounsaturated, long chain), and palmitic acid (long chain) increased glucose production, whereas caproic acid (short chain) did not increase. These results suggest long chain FFA (saturated or unsaturated) can stimulate glucose production. Linoleic acid plays its roles through in membrane lipids, as ligands for receptors and transcription factors that regulate gene expression, precursor for eicosanoids, in cellular communication, and through direct interactions with proteins. Adverse fatty acid alters the fatty acid composition of membrane phospholipids and storage triglycerides with the potential to disrupt cellular environments, and program structure and function (Innis, 2007). Therefore, we selected linoleic acid as a ligand.

In the present study, linoleic acid leads the activation of $cPLA_2$ and AA release. High dietary linoleic acid led to a high heart membrane AA, and Ca2+ dependent cPLA2 with a marked increase in pigs (Ghosh et al., 2007). PLA_2 enzyme acts as the catalyst the hydrolysis of cellular phospholipids at the sn-2 position to liberate arachidonic acid (Nanda et al., 2007). In the present study, treatment of linoleic acid significantly increased the expression of COX-2 protein levels. These results consistent with a previous study, linoleic acid induced expression of COX-2 as well as PGE_2 in retinal pigment epithelial cell (Nanda et al., 2007). COX, including COX-1 and COX-2, is the rate-limited enzyme catalyzing the conversion of AA into endoperoxide intermediates, which are ultimately converted by specific synthases to prostanoids, including PGE_2 (Gupta and Dubois, 2001; Smith and Langenbach et al., 2001). Prostaglandins are oxygenated metabolites of the 20-carbon polyunsaturated fatty acid molecule AA, which is released from the membrane phospholipids through the action of phospholipases, mainly phospholipase A_2 (Higa et al., 2007). In the present study, the concentration of PGE_2 generated through COX increased glucose production of chicken

hepatocytes obtained under linoleic acid treatment. We also provide direct evidence for an involvement of $cPLA_2$ and COX_2 in the stimulation of hepatocytes glucose production by linoleic acid.

In the present study, we demonstrate that linoleic acid up-regulates PPARδ expression. Thus, we hypothesize that overexpression of PPARδ in chicken hepatocytes is a sufficient condition to gluconeogenesis. We reproduced this observation using a PPARδ specific siRNA system to validate PPARδ as the mediator of gluconeogenesis. The present result is consistent with a previous report that PPARs, after binding peroxisome proliferation compounds or fatty acids, are activated and regulate the expression of genes related to lipid metabolism, such as gluconeogenesis (Panadero et al., 2005). The physiological and pharmacological roles of PPARδ are just beginning to emerge. It has recently become clear that PPARδ has a function in epithelial tissues, but inconsistent reports leave the situation controversial. These data raise the possibility of a model in which impaired activation of PPARδ may alter the lipid signaling required for gluconeogenesis. It is well known that both G6Pase and PEPCK are rate limiting enzymes of gluconeogenesis. In the present study, the expression of both G6Pase and PEPCK are stimulated by FFA, which is dependent of PPARδ subsequently with previous studies (Yamagata et al., 2007; Davies et al., 2001; Tontonoz et al., 2007). They share many common features in their gene transcription (Collins et al., 2006). For example, their transcription are stimulated glucagons but suppressed by insulin (Barthel et al., 2003; Hanson et al., 1997). However, there are some distinctions in the regulation of these two genes. The signals from the central nervous system regulate the expression of the G6Pase gene but do not influence the transcription of the PECK gene in the liver (Lam et al., 2005). Because PEPCK is the earliest rate-limiting enzyme in the process of gluconeogenesis, it may play a more important role in the regulation of gluconeogenesis.

CONCLUSION

In conclusion, linoleic acid induced gluconeogenesis via $cPLA_2$, COX_2, and PPARδ pathways in cultured chicken hepatocytes.

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논문제목	한글 : 초대 배양된 닭 간세포에서 linoleic acid 에 의한 포도당신생과 연관된 cPLA2, COX2, 그리고 PPARδ 신호 전달 경로 영문: Effect of Linoleic acid on gluconeogenesis in primary cultured chicken				

	hepatocyte: Involvement of $cPLA_2$ COX_2 and PPARδpathways

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건 아래 -조선대학교가 저작물을 이용할 수
있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집・형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포・전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송・출력을 허락함.

2008 년 2 월 일

저작자: Hoang Thi Huong (서명 또는 인)

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