p38 Mitogen-activated Protein Kinase Mediates Free Fatty Acid-induced Gluconeogenesis in Hepatocytes*‡¶1

Received for publication, March 7, 2006, and in revised form, June 22, 2006 Published, JBC Papers in Press, June 27, 2006, DOI 10.1074/jbc.M602177200

Qu Fan Collins‡, Yan Xiong§, Edgar G. Lupo, Jr.,†, Hui-Yu Liu§, and Wenhong Cao‡¶1

From the ‡Division of Biological Sciences, Endocrine Biology Program, CIIT Centers for Health Research, Research Triangle Park, North Carolina 27709, the ‡Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha 410078, Hunan, China, and the †Division of Endocrinology, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27708

Free fatty acids (FFA) are considered as a causative link between obesity and diabetes. In various animal models and in humans FFA can stimulate hepatic gluconeogenesis. Although the in vivo role of FFA in hepatic gluconeogenesis has been clearly established, the intracellular role of FFA and related signaling pathway remain unclear in the regulation of hepatic gluconeogenic gene transcription. In this study, we have identified p38 mitogen-activated protein kinase (p38) as a critical signaling component in FFA-induced transcription of key gluconeogenic genes. We show in primary hepatocytes that both mid- and long-chain fatty acids (saturated or unsaturated) could activate p38 and increase levels of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase, and peroxisome proliferator-activated receptor γ coactivator α (PGC-1α) gene transcripts. The FFA-induced expression of PEPCK and PGC-1α genes and gluconeogenesis in isolated hepatocytes could be blocked by the inhibition of p38. Furthermore, PGC-1α phosphorylation by p38 was necessary for FFA-induced activation of the PEPCK promoter. Additionally, FFA stimulated phosphorylation of cAMP-response element-binding protein (CREB) through p38. The overexpression of the dominant-negative CREB prevented FFA-induced activation of the PEPCK promoter. Finally, we show that FFA activation of p38 requires protein kinase Cδ. Together, our results indicate that p38 plays a critical role in FFA-induced transcription of gluconeogenic genes, and the known gluconeogenic regulators, PGC-1α and CREB, are also integral parts of FFA-stimulated transcription of gluconeogenic genes.

Hepatic gluconeogenesis, which is the de novo synthesis of glucose from non-hexose carbohydrate precursors, is essential for maintaining blood glucose levels during fasting. However, excessive gluconeogenesis is a major source of hyperglycemia in both type I and type II diabetes (1, 2). Gluconeogenesis is directly controlled by rate-limiting gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK),2 glucose-6-phosphatase (G6Pase), and fructose-1,6-bisphosphatase (see Ref. 3 for review). The function of these enzymes is mainly regulated at their transcriptional levels by the pancreatic hormones insulin and glucagon (3). Insulin suppresses, whereas glucagon stimulates, transcription of these gluconeogenic genes in the liver (3). In addition to hormonal control, hepatic gluconeogenesis has been shown to be directly regulated by some nutrients such as free fatty acids (FFA) (see Refs. 4 and 5 for review).

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 (6–12). The regulatory role of FFA in gluconeogenesis is complicated, because it can be direct and indirect. The indirect role of FFA is through insulin secretion and insulin action. FFA-stimulated insulin secretion from pancreatic islets suppresses gluconeogenesis (see Refs. 13 and 14 for review). But on the other hand, exposure of the liver to FFA can desensitize insulin signaling and dampen its suppression of gluconeogenesis, with the net effect of elevating hepatic gluconeogenesis (15–25). In addition, 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 (see Ref. 26 for review). Although this direct role of FFA in hepatic gluconeogenesis has been suggested in previous studies (27, 28), the components of the signaling cascade through which FFA operates have not been systematically investigated and remain largely unknown.

p38 mitogen-activated protein kinase (p38) has been recently linked to energy metabolism in adipocytes, skeletal muscle, cardiomyocytes, and hepatocytes (29–36). In particular, p38 plays a regulatory role in the function of peroxisome proliferator-
activated receptor γ coactivator-1α (PGC-1α). This coactivator is known to be an important stimulator of hepatic gluconeogenesis (37, 38). In this study, we examined the role of p38 in FFA induction of gluconeogenic gene expression in hepatocytes. Our results show that FFA stimulated phosphorylation of p38 and transcription of PGC-1α, PEPCK, and G6Pase genes. The inhibition of p38 blocked FFA-induced transcription of PGC-1α and PEPCK genes and gluconeogenesis in isolated hepatocytes. Together, we have identified p38 as a mediator of FFA-induced transcription of hepatic gluconeogenic genes, and have provided new insight into understanding of fatty acid regulation of hepatic gluconeogenesis.

**MATERIALS AND METHODS**

**Chemicals and Antibodies**—SB203580 (SB) and rottlerin were from Calbiochem. Glucagon was from Sigma. Antibodies against p38, phosphorylated p38 (cat. no. 9211s), phosphorylated ATF-2 (cat. no. 9221s), and phosphorylated CREB (serine 133, cat. no. 9191s) were from Cell Signaling Technology. Antibodies against p38, phosphorylated p38 (cat. no. 9221s), and phosphorylated ATF-2 (cat. no. 9221s) were from Cell Signaling Technology. The β-actin antisera was from Sigma (cat. no. A-5441). The siRNA duplexes against PKCδ were purchased from Super Array Bioscience Corp. (cat. no. RM-0951). The PGC-1α promoter (2 kb) construct was purchased from Addgene (www.addgene.com, plasmid no. 8887). The PEPCK promoter construct was a gift from Dr. Jianhua Shao. The A-CREB construct was a kind gift from Dr. Charles R. Vinson. The expression vectors of wild-type PGC-1α and phosphorylation-deficient mutant of PGC-1α (PGC-1α-A3) were kind gifts from Dr. Bruce Spiegelman.

**Isolation of Primary Hepatocytes**—Primary hepatocytes were isolated from C57BL/6 mice as previously described (39). (All the mice used for isolation of hepatocytes were fed under normal chow diet and regular schedule unless otherwise noted.) Briefly, under anesthesia with pentobarbital (intraperitoneal, 30 mg/kg body weight), livers were perfused with Ca²⁺-free Hanks’ balanced solution (Invitrogen) at 5 ml/min for 8 min, followed by continuous perfusion with serum-free Williams’ medium containing collagenase (Worthington, type II, 50 units/ml, Invitrogen), HEPES (10 mM), and NaOH (0.004 n) at 5 ml/min for 12 min. Hepatocytes were harvested and purified with Percoll. The viability of hepatocytes was examined with trypan blue exclusion. Only cell isolates with viability >5% were used. Hepatocytes were inoculated into collagen-coated 6-well plates (5 × 10⁵/well) in Williams’ medium with 10% fetal bovine serum and were incubated for 24 h before any experimentation.

**Preparation of FFA and Treatment of Hepatocytes with FFA**—FFA solutions were prepared as previously described (40). Briefly, palmitate and capric acids were dissolved with methanol and then diluted with DMEM medium (containing 5.5 mM glucose) to 10 mM. Oleate, linoleate, and caproic acid were all purchased as liquid solutions, and were freshly diluted with DMEM medium (containing 5.5 mM glucose) to 100 mM. The serum-free DMEM media used for the treatment of cells with FFA were supplemented with 5.5 mM glucose and 2% FFA-free bovine albumin (BSA).

**Transfection of Hepatocytes**—Primary hepatocytes were transfected as previously described (36). Briefly, cells were settled down in Williams’ medium for 24 h before the transfection. Hepa1c1c7 cells were maintained in minimal essential medium (Invitrogen) with 10% fetal bovine serum. Plasmid DNAs were introduced into primary hepatocytes and Hepa1c1c7 cells by using Lipofectamine 2000 (Invitrogen). The transfection efficiency was monitored by introducing an identical amount of green fluorescent protein plasmid DNA into the control cells simultaneously, followed by observation under a fluorescence microscope. Approximately 50% of cells were transfected.

**Measurement of Glucose Production in Primary Hepatocytes**—Primary hepatocytes were isolated from mice, which had been fasted for 24 h to deplete glycogen in the liver. Glucose production from the primary hepatocytes was measured as previously described (41). Briefly, cells were washed three times with warm phosphate-buffered saline to remove glucose and pre-treated with 5 μM SB (30 min) as noted, followed by the treatment with 0.5 mM oleate (4 h) in the glucose-free medium containing gluconeogenic substrates (20 mM sodium lactate and 2 mM sodium pyruvate). Glucose concentrations were 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 glycolysis and gluconeogenesis. Glucose production from glycolysis 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 glycolysis.

**Immunoblotting**—Immunoblotting analyses were performed as previously described (29, 30, 36, 42). Briefly, cell lysates were prepared by homogenization and sonication, followed by addition of 2× Laemmli sample buffer. Aliquots (5 μg/well) were resolved with mini Tris-glycine gradient gels (4–20%, Invitrogen) and transferred to nitrocellulose membranes. Levels of phosphorylated and total p38 or phosphorylated CREB were detected with a 1:1,000 dilution of each specific primary antiserum, followed by incubation with a 1:10,000 dilution of goat anti-rabbit immunoglobulin G conjugated with alkaline phosphatase (RPN5783, Amersham Biosciences). Fluorescence bands were visualized with a Typhoon PhosphorImager (Molecular Dynamics).

**RNA Isolation, Semi-quantitative RT-PCR, and TaqMan Real-time PCR**—Total RNAs from hepatocytes were prepared by using RNA purification kits from Qiagen. Semi-quantitative RT-PCR reactions were performed according to the manuals from the manufacturer. Real-time RT-PCR TaqMan probes and reaction agents were purchased from Applied Biosystems. Reactions were performed according to manuals from the manufacturer. Catalogue numbers for the probes were as follows: PEVK (Mm00440636-m1), G6Pase (Mm00839363-m1), and PGC-1α (Ahs0017304-m1).

**Introduction of siRNA Duplexes into Primary Hepatocytes**—The siRNA duplexes were introduced into primary hepatocytes as previously described (31). Briefly, siRNA duplexes as indicated in each experiment were mixed with 4 μl of Lipofectamine 2000 (Invitrogen) in OPTI medium (Invitrogen) and added to the primary hepatocytes, which had been seeded in 6-well plates 24 h earlier.

**Measurement of Promoter Activities**—The PEVK or PGC-1α promoter was introduced via transient transfection.
into Hepa1c1c7 hepatoma cells together with an expression vector for β-galactosidase as an internal control (29, 30, 36), and cells were treated as noted in each experiment. Promoter activities were measured by luciferase assays and normalized to the β-galactosidase internal control.

Measurements of p38 Activity—Activity of p38 was measured as previously described (31). In brief, cells were washed twice with phosphate-buffered saline and lysed with a buffer containing 5 mM β-glycerophosphate, 1 mM sodium orthovanadate, 25 mM Tris-HEPES, 150 mM NaCl, 5 mM β-glycerophosphate, 1 mM sodium orthovanadate, 5 mM sodium pyrophosphate, 5 mM EDTA, 5 mM EGTA, 0.9% Triton X-100, 0.1% IGEPA, 10% glycerol, and proteinase inhibitors (Roche Applied Science, cat. no. 11836153001, 1 tablet per 10 ml). Cell lysates (20 µl) were incubated at 37 °C for 30 min with 4 µg of GST-ATF-2 (Cell Signaling Technology, cat. no. 9224) and 200 µM ATP in 100 µl of kinase reaction buffer (20 mM HEPES, 20 mM MgCl₂, 25 mM β-glycerophosphate, 2 mM dithiothreitol, and 0.1 mM sodium orthovanadate). Glutathione-Sepharose 4B (50 µl/reaction) was used to precipitate GST-ATF-2, which was immunoblotted with antisera against phosphorylated ATF-2 (1:1,000 dilution, Cell Signaling Technology, cat. no. 9221). Phosphorylated ATF-2 was finally detected with goat anti-rabbit immunoglobulin G conjugated with alkaline phosphatase (1:10,000 dilution, Amersham Biosciences, cat. no. RPN5783) and visualized with a Typhoon PhosphorImager.

RESULTS

*FFA Stimulates p38 Phosphorylation in Primary Hepatocytes—*FFA can stimulate hepatic gluconeogenesis in a variety of animal models and in humans (15, 16, 19, 20, 22, 23). After an overnight fast plasma levels of FFA fluctuate between 350 and 740 µM and can reach 1–2.5 mM during prolonged fasting and in diabetes, in which hepatic gluconeogenesis is elevated (43–45). In cardiac myocytes and endothelial cells FFAs have been previously shown to activate p38 (46, 47), and we have recently reported that p38 plays a key role in glucagon-induced hepatic gluconeogenesis (36). Therefore, we postulated that FFA might activate p38 to induce hepatic gluconeogenesis. To test this hypothesis, we first examined the effect of unsaturated FFA on p38 activation in primary hepatocytes. As shown in Fig. 1, both oleate (monounsaturated, long chain) and linoleate (double-unsaturated, long chain) stimulated p38 phosphorylation in a dose-dependent manner. Next, the role of saturated FFA in p38 activation was examined. As shown in Fig. 2, palmitate (long chain) and capric acid (mid chain) could both stimulate p38 phosphorylation, whereas caproic acid (short chain) failed to do so. Together, these results suggest that both mid- and long-chain FFA (saturated or unsaturated) can stimulate phosphorylation of p38 in primary hepatocytes.

*FFA Induces Gluconeogenesis in a p38-dependent Manner—*Although the role of FFA in hepatic gluconeogenesis has been well established (15, 16, 19, 20, 22, 23), the direct role of FFA on gluconeogenesis has not been determined in primary hepatocytes. Therefore, we treated isolated primary hepatocytes with oleate in the presence or absence of p38 inhibition with SB. (Oleate is one of the most abundant FFA in blood (48).) As shown in Fig. 3, oleate promoted glucose production via gluconeogenesis in primary hepatocytes but was blocked by the inhibition of p38. These results indicate that FFA can directly stimulate gluconeogenesis in isolated hepatocytes through activation of p38.

To determine the role of FFA in transcription of gluconeogenic genes, isolated hepatocytes were treated with increasing amounts of FFA, followed by measuring transcripts of key gluconeogenic genes. As shown in Fig. 4, the effect of oleate on expression of PEPCK and G6Pase genes was biphasic. Specifically, oleate elevated levels of both PEPCK and G6Pase transcripts at 50 µM, but this elevation was reduced to a level, which

![FIGURE 1. p38 activation by unsaturated FFA in primary hepatocytes.](image-url)
levels of PEPCK and G6Pase transcripts at 50 \mu M, and this increase continued at 100 \mu M for PEPCK and 500 \mu M for G6Pase. Elevation of PEPCK and G6Pase transcripts by capric acid started to decrease when concentrations of capric acid reached 500 \mu M (for PEPCK) or 1000 \mu M (for G6Pase). The effect of palmitate (saturated FFA) on PEPCK and G6Pase genes were significantly milder in comparison to oleate and capric acid. Palmitate started moderately to increase levels of PEPCK and G6Pase transcripts only at high concentrations (\geq 500 \mu M). Together, these results indicate that various FFAs can directly elevate levels of gluconeogenic gene transcripts at different concentrations in primary hepatocytes.

To determine the role of p38 in FFA-induced elevation of gluconeogenic gene transcripts, primary hepatocytes were treated with oleate in the presence or absence of p38 inhibitors. As shown in Fig. 5, oleate increased levels of both PEPCK and G6Pase transcripts. The increase in PEPCK transcripts was blocked by the inhibition of p38 with either SB or dominant-negative p38 (dn-p38), whereas, interestingly, levels of G6Pase transcripts were not affected by the blockade of p38. Similar results were observed when palmitate instead of oleate was used (data not shown). Together, these results indicate that FFA induction of the rate-limiting enzyme, PEPCK, is p38-dependent, whereas FFA induction of the G6Pase gene is independent of p38.

To further investigate the role of p38 in FFA-induced elevation of gluconeogenic gene transcripts, the PEPCK promoter

FIGURE 2. p38 activation by saturated FFA in primary hepatocytes. Primary hepatocytes were isolated and cultured as detailed under “Materials and Methods” and were treated for 20 min with increasing amounts of palmitate (A and B), capric acid (C and D), or caproic acid (E and F) in serum-free DMEM media containing 5.5 mM glucose and 2% FFA-free BSA. Levels of p38 in cell lysates were detected by immunoblotting with antisera against p38 (p38-T) or phospho-p38 (p38-P). Results of p38-P were normalized to p38-T, and represent three independent experiments. *, p < 0.05 compared with 0 mM palmitate; #, p < 0.01 compared with 0 mM capric acid.

FIGURE 3. FFA induces gluconeogenesis in primary hepatocytes in a p38-dependent manner. Isolated hepatocytes were incubated with oleate (0.5 mM) in the presence or absence of SB (5 \mu M) for 4 h. The treatment with oleate was performed in serum-free DMEM media containing 2% FFA-free BSA. Glucose production from gluconeogenesis was quantified as detailed under “Materials and Methods.” Results represent mean \pm S.D. of two independent experiments, each in triplicate. *, p < 0.05 compared with all other treatments. #, p > 0.05 compared with treatment with SB alone.

is still significantly higher than the basal one (p < 0.05), at 500 \mu M, followed by another increase at 1000 \mu M. We repeatedly observed this biphasic effect of oleate, but we have not been able to explain this phenomenon yet. Capric acid started to increase levels of PEPCK and G6Pase transcripts at 50 \mu M, followed by another increase at 500 \mu M. We repeatedly observed this biphasic effect of oleate, but we have not been able to explain this phenomenon yet. Capric acid started to increase
was introduced into Hepa1c1c7 hepatoma cells, which were subsequently treated with oleate in the presence of p38 inhibitor SB or constitutive activator (M KK6E). As shown in Fig. 6, activation of the PEPCK promoter by oleate was suppressed by SB but further enhanced by the overexpression of MKK6E. These results further support a role for p38 in FFA stimulation of PEPCK gene transcription.

p38 Mediates FFA-induced Transcription of the PGC-1α Gene—PGC-1α and FFA both play critical roles in hepatic gluconeogenesis (36, 37, 49). Levels of PGC-1α gene transcription and plasma FFA are coincidently increased during fasting and in diabetes (6–12, 36, 37). We have recently shown that p38 mediates glucagon-induced transcription of the PGC-1α gene (36). Our results in Figs. 1 and 2 show that FFA can activate p38 in hepatocytes. Therefore, we postulated that FFA activation of p38 might influence expression of the PGC-1α gene. To test this hypothesis, isolated hepatocytes were treated with oleate in the presence or absence of p38 inhibitors, followed by measurements of PGC-1α transcripts. As shown in Fig. 7A, levels of PGC-1α transcripts were increased by oleate but blocked by the inhibition of p38 with either SB or dominant-negative p38α.

To further study the effect of p38 on the PGC-1α gene, we introduced the PGC-1α promoter into Hepa1c1c7 cells, which were subsequently stimulated by oleate in the presence of inhibitor or activator of p38. As shown in Fig. 7B, oleate activated the PGC-1α promoter, but this activation was blocked by the inhibition of p38. In contrast, the overexpression of p38 activator MKK6E further enhanced the promoter activity induced by oleate. Together, these results demonstrate that p38 is a mediator of FFA-induced transcription of the PGC-1α gene.

PGC-1α Phosphorylation by p38 Is a Component of FFA-induced Transcription of the PEPCK Gene—We and others have previously shown that the co-activating strength of PGC-1α activity is dependent upon p38-mediated phosphorylation (30, 32). Therefore, we introduced the PEPCK promoter together with either the wild-type or phosphorylation-deficient PGC-1α into Hepa1c1c7 hepatoma cells, followed by the treatment with oleate. As shown in Fig. 8, the PEPCK promoter was activated by oleate and further enhanced by the overexpression of the wild-type PGC-1α. Interestingly, the co-expression of the phosphorylation-deficient PGC-1α-A3 not only failed to enhance the activation of the PEPCK promoter but also suppressed the oleate-induced promoter activity. Together, these results suggest that the phosphorylation of PGC-1α by p38 is another element in the regulation of the PEPCK gene by FFA.
CREB Is Activated by FFA in Primary Hepatocytes through p38, and Activity of CREB Is Required for FFA-induced Transcription of the PEPCK Gene—CREB is another critical player in the control of hepatic gluconeogenesis (50). We have previously shown that activation of CREB in the liver by fasting and in isolated hepatocytes by glucagon is p38-dependent (36). It is established that CREB is an indirect substrate of p38 (51–53). To determine whether CREB is required for FFA regulation of gluconeogenesis, primary hepatocytes were treated with oleate in the presence of increasing amounts of SB, followed by measurements of CREB phosphorylation. Phosphorylation of CREB was stimulated by oleate (Fig. 9A) and other fatty acids, including palmitate and capric acid (data not shown), but blocked by the inhibition of p38. These results indicate that FFA can activate CREB in hepatocytes through p38.

To determine whether CREB activity is required for FFA-induced expression of gluconeogenic genes, the PEPCK promoter was introduced into Hepa1c1c7 cells and stimulated by oleate in the presence or absence of dominant-negative CREB (A-CREB). As expected, the PEPCK promoter was activated by oleate but inhibited by the overexpression of A-CREB (Fig. 9B). These results suggest that CREB activation is necessary for FFA-induced transcription of the PEPCK gene.

PKCδ Is Required for Fatty Acid Activation of p38 in Primary Hepatocytes—FFA has been previously shown to activate certain isoforms of PKC in adipocytes (54). Among these isoforms, PKCδ is an established activator of p38 (55, 56). Therefore, we chose to examine the possible involvement of PKCδ in the activation of p38 by FFA. As shown in Fig 10A, the activation of p38 by oleate was completely blocked by rottlerin, which is a relatively specific inhibitor of PKCδ. Further evidence is that the specific siRNA was used to silence the PKCδ gene. The PKCδ gene was knocked down by ~70% with the siRNA (Fig. 10B), and this reduction of the PKCδ transcripts significantly decreased the level of p38 activity induced by oleate in comparison to the control siRNA. Together, these results suggest that PKCδ is an upstream activator FFA activation of p38 in hepatocytes.

DISCUSSION
Although FFA have been firmly established in many in vivo animal models and in humans as a promoter of hepatic gluconeogenesis (15, 16, 19, 20, 22, 23), the mechanism by which FFAs promote transcription of gluconeogenic genes at cellular and molecular levels remains unclear. In this study, we have identified a critical role for p38 in transcription of hepatic gluconeogenic genes induced by FFA. Our results also indicate that PKCδ is an upstream activator, whereas PGC-1α and CREB are downstream effectors of p38 in FFA regulation of hepatic gluconeogenesis.
FFAs, p38 MAPK, and Hepatic Gluconeogenesis

Graphical representation of the PGC-1α phosphorylation by p38 is required for FFA stimulation of the PEPCK promoter. The PEPCK promoter was introduced to Hepa1c1c7 cells and stimulated by 0.5 mM oleate for 6 h in the presence or absence of overexpression of wild-type PGC-1α or phosphorylation-deficient PGC-1α (PGC-1α-A3) via transient transfection. Treatment with oleate was performed in serum-free minimal essential medium containing 5.5 mM glucose and 2% FFA-free BSA. Promoter activities were measured by luciferase assays and normalized to the β-actin, or oleate plus PGC-1α-A3. #, p < 0.01 compared with basal, PGC-1α-A3 or oleate plus PGC-1α-A3.

Previous studies have implicated a link between FFA and p38 in the control of hepatic gluconeogenesis under physiological and diabetic conditions. First, levels of both plasma FFA and p38 phosphorylation in the liver are increased during the post absorption stage or fasting when hepatic gluconeogenesis is elevated (4, 17, 36, 43, 49, 57, 58). Second, levels of both FFA and p38 phosphorylation are elevated in obese animals with or without diabetes (43, 54, 57), which is characterized by exaggerated hepatic gluconeogenesis. Third, FFAs stimulate hepatic gluconeogenesis in vivo (15, 16, 19, 20, 22, 23). Fourth, FFAs can stimulate transcription of the PEPCK gene in adipocytes, although this effect induced by a specific FFA was not observed in a hepatoma cell line (see Ref. 59 for review). Fifth, FFA can activate p38 in cardiac myocytes and endothelial cells (46, 47). Sixth, p38 has recently been shown to mediate glucagon-induced transcription of hepatic gluconeogenic genes (36). In this study, using isolated primary hepatocytes, we methodically studied the role of FFA on activation of p38 and expression of key gluconeogenic genes. Our study has identified p38 as a critical mediator of FFA regulation of gluconeogenic gene transcription. However, the mechanism by which FFA stimulates p38 activity remains largely undetermined.

Several orphan G protein-coupled receptors, including GPR40, -41, -43, and -120, have recently been identified as receptors for FFA with different length and saturation (60–65). All these receptors are coupled to Gq protein and dependent upon Ca^{2+} mobilization (60, 61, 63, 64). FFA-induced phosphorylation of p38 in hepatocytes was not influenced by the inhibition of Ca^{2+} mobilization in our study (data not shown). Therefore, FFA activation of p38 is unlikely mediated by these GPRs. Our results suggest that PKCδ is one of the upstream components of FFA-induced activation of p38 in hepatocytes. This observation is consistent with previous reports that PKCδ is an activator of p38 (66, 67). However, the signaling components beyond PKCδ remain unknown.
Our results also show another interesting aspect of the regulation of PECK and G6Pase genes. Although the expression of both PECK and G6Pase genes are stimulated by FFA in this study, only the expression of the PECK gene is mediated by p38, whereas the FFA induction of the G6Pase gene is independent of p38. It is well known that both PECK and G6Pase are rate-limiting enzymes of gluconeogenesis. They share many common features in their gene transcription. For example, their transcriptions are both stimulated by AMP-producing hormones such as glucagon but suppressed by insulin (3, 68). However, there are some distinctions in the regulation of these two genes. For example, 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 (69). Because PECK is the earliest rate-limiting enzyme in the process of gluconeogenesis, it may play a more important role in the regulation of gluconeogenesis. Our results appear to support this notion, because gluconeogenesis is blocked by the inhibition of p38, although only the expression of the PECK gene is suppressed by the inhibition of p38. This notion is also strongly supported by the previous study that the deletion of the PECK gene causes early death of newborn mice due to severe hypoglycemia (70).

In summary, our results in this study demonstrate that both mid- and long-chain FFA (saturated or unsaturated) can stimulate transcription of hepatic gluconeogenic genes. This stimulation is dependent upon p38, PGC-1α, and CREB (Fig. 11). In addition to FFA, glucagon is another established and principle stimulator of hepatic gluconeogenesis. Plasma levels of both FFA and glucagon are increased during fasting and in diabetes (reviewed in Ref. 71). Our previous (36) and current studies show that p38 plays a critical role in regulation of hepatic gluconeogenesis induced by either glucagon or FFA. Therefore, future studies on the signaling pathways by which glucagon and FFA activate p38 may shed new light into understanding of glucose homeostasis and provide new intervening targets for the prevention and treatment of diabetes.

Acknowledgments—We thank Drs. Sheila Collins, Jamie Bonner, and Jacques Robidoux for their instrumental advice and critical reading of the manuscript. We also thank Drs. Bruce Spiegelman, Charles Vinson, and Jiahua Shao for providing some of the constructs used in this study.

REFERENCES

1. Magnusson, I., Rothman, D. L., Katz, L. D., Shulman, R. G., and Shulman, G. I. (1992) J. Clin. Invest. 90, 1323–1327
2. Cline, G. W., Rothman, D. L., Magnusson, I., Katz, L. D., and Shulman, G. I. (1994) J. Clin. Invest. 94, 2369–2376
3. Barthel, A., and Schnoll, D. (2003) Am. J. Physiol. 285, E685–E692
4. Boden, G. (2003) Life Sci. 72, 977–988
5. Blaak, E. E. (2003) Proc. Nutr. Soc. 62, 753–760
6. Balderweg, S. E., Golay, A., Natali, A., Balkau, B., Del Prato, S., and Coppack, S. W. (2000) Eur. J. Clin. Invest. 30, 45–52
7. Bolinder, J., Kerekhoff, D. A., Moberg, E., Hagsstrom-Toft, E., and Arner, P. (2000) Diabetes 49, 797–802
8. Coppack, S. W., Evans, R. D., Fisher, R. M., Frym, K. N., Gibbons, G. F., Humphreys, S. M., Kirk, M. L., Potts, J. L., and Hockaday, T. D. (1992) Metabolism 41, 264–272
9. Boden, G. (1997) Diabetes 46, 3–10
10. Henry, R. R. (1995) J. Clin. Invest. 95, 1427–1428
11. Kelley, D. E., and Mandarino, L. J. (2000) Diabetes 49, 677–683
12. Lewis, G. F., Carpentier, A., Adeli, K., and Giacca, A. (2002) Endoc. Rev. 23, 201–229
13. Wyne, K. L. (2003) Am. J. Med. 115, Suppl. 8A, 295–365
14. Boden, G. (2005) Curr. Diab. Rep. 5, 167–170
15. Bevilacqua, S., Bonadonna, R., Buzigoli, G., Boni, C., Ciociaro, D., Macari, F., Giorio, M. A., and Ferrannini, E. (1987) Metabolism 36, 502–506
16. Boden, G., Chen, X., Ruiz, J., White, J. V., and Rossetti, L. (1994) J. Clin. Invest. 93, 2438–2446
17. Lam, T. K., van de Werve, G., and Giacca, A. (2003) Am. J. Physiol. 284, E281–E290
18. Lam, T. K., Yoshii, H., Abder, C. A., Bogdanovic, E., Lam, L., Fantus, I. G., and Giacca, A. (2002) Am. J. Physiol. 283, E682–E691
19. Lewis, G. F., Vranic, M., Harlem, P., and Giacca, A. (1997) Diabetes 46, 1111–1119
20. Rebrin, K., Steil, G. M., Mittelman, S. D., and Bergman, R. N. (1996) J. Clin. Invest. 98, 741–749
21. Saloranta, C., Koivisto, V., Widen, E., Falholt, K., DeFronzo, R. A., Harkonen, M., and Groop, L. (1993) Am. J. Physiol. 264, E599–E605
22. Sindler, D. K., Chu, C. A., Rohlie, M., Neal, D. W., Swift, L. L., and Cherrington, A. D. (1997) Diabetes 46, 187–196
23. Wiesenthal, S. R., Sandhu, H., McCull, R. T., Thorpe, V., Yoshi, H., Polonsky, K., Shi, Z. Q., Lewis, G. F., Mari, A., and Giacca, A. (1999) Diabetes 48, 766–774
24. Iglesias, M. A., Ye, J. M., Frangioudakis, G., Saha, A. K., Tomas, E., Ruderman, N. B., Cooney, G. J., and Kraegen, E. W. (2002) Diabetes 51, 2886–2894
25. Oakes, N. D., Cooney, G. J., Camilleri, S., Chisholm, D. J., and Kraegen, E. W. (1997) Diabetes 46, 1768–1774
26. Lam, T. K., Carpentier, A., Lewis, G. F., van de Werve, G., Fantus, I. G., and Giacca, A. (2003) Am. J. Physiol. 284, E863–E873
27. Antras-Ferry, J., Le Bigot, G., Robin, P., Robin, D., and Forest, C. (1994) Biochem. Biophys. Res. Commun. 203, 385–391
28. Bizeau, M. E., and Hazel, J. R. (1999) J. Nutr. Biochem. 10, 709–715
29. Cao, W., Medvedev, A. V., Daniel, K. W., and Collins, S. (2001) J. Biol. Chem. 276, 27077–27082
30. Cao, W., Daniel, K. W., Robidoux, J., Puijserver, P., Medvedev, A. V., Bai,
**FFAs, p38 MAPK, and Hepatic Gluconeogenesis**

X. Floering, L. M., Spiegelman, B. M., and S. C. (2004) *Mol. Cell. Biol.* **24**, 3057–3067

31. Rolli, M., Kotlyarov, A., Sakamoto, K. M., Gaestel, M., and Neininger, A. (1999) *J. Biol. Chem.* **274**, 19559–19564

52. Bhat, N. R., Feinstein, D. L., Shen, Q., and Bhat, A. N. (2002) *J. Biol. Chem.* **277**, 29584–29592

53. Butler, M. P., Hanly, J. A., and Moynagh, P. N. (2005) *J. Biol. Chem.* **280**, 27759–27768

54. Gao, Z., Zhang, X., Zuberi, A., Hwang, D., Quon, M. J., Lefevre, M., and Ye, J. (2004) *Mol. Endocrinol.* **18**, 2024–2034

55. Kobayashi, M., Mutharasan, R. K., Feng, J., Roberts, M. F., and Lomasney, J. W. (2004) *Biochemistry* **43**, 7522–7533

56. Eitel, K., Staiger, H., Rieger, J., Mischak, H., Brandhorst, H., Brendel, M. D., Berset, R. G., Haring, H. U., and Kellerer, M. (2003) *Diabetes* **52**, 991–997

57. Gum, R. J., Gaede, L. L., Heindel, M. A., Waring, J. F., Trevillyan, J. M., and Ulrich, R. G. (2003) *Mol. Endocrinol.* **17**, 1131–1143

58. Carlson, C. J., Kotsorci, S., Sciotti, R. J., Poccard, G. B., and Rondinone, C. M. (2003) *Diabetes* **52**, 634–641

59. Tordjman, J., Khazen, W., Antoine, B., Chauvet, G., Quette, J., Fouque, F., Beale, E. G., Benelli, C., and Forest, C. (2003) *Biochimie (Paris)* **85**, 1213–1218

60. Itoh, Y., Kawamata, Y., Harada, M., Kobayashi, M., Fujii, R., Fukusumi, S., Ogi, K., Hosoya, M., Tanaka, Y., Ueijima, H., Tanaka, H., Maruyama, M., Satoh, R., Okubo, S., Kizawa, H., Komatsu, H., Matsunuma, F., Noguchi, Y., Shinohara, T., Hinuma, S., Fujisawa, Y., and Fujino, M. (2003) *Nature* **422**, 173–176

61. Briscoe, C. P., Tadayyon, M., Andrews, J. L., Benson, W. G., Chambers, J. K., Ellert, M. M., Ellis, C., Elshourbagy, N. A., Goetz, A. S., Minnick, D. T., Murdock, P. R., Sauls, H. R., Jr., Shabon, U., Spinage, L. D., Strum, J. C., Szekeres, P. G., Tan, K. B., Way, J. M., Ignar, D. M., Wilson, S., and Muir, A. I. (2003) *J. Biol. Chem.* **278**, 11303–11311

62. Le Poul, E., Loison, C., Struyf, S., Springael, J. Y., Lannoy, V., Decobecc, M. E., Brezillon, S., Dupriez, V., Vassart, G., Van Damme, J., Parmentier, M., and Detheux, M. (2003) *J. Biol. Chem.* **278**, 25481–25489

63. Brown, A. J., Gouldsworth, S. M., Barnes, A. A., Ellert, M. M., Tcheang, L., Daniels, D., Muir, A. L., Wigginsworth, M. J., Kinghorn, L., Fraser, N. J., Pike, N. B., Strum, J. C., Steplewski, K. M., Murdock, P. R., Hilder, J. C., Marshall, F. H., Szekeres, P. G., Wilson, S., Ignar, D. M., Foord, S. M., Wise, A., and Dowell, S. J. (2003) *J. Biol. Chem.* **278**, 11312–11319

64. Hirasawa, A., Tsumaya, K., Awaji, T., Katsuma, S., Adachi, T., Yamada, M., Sugimoto, Y., Miyazaki, S., and Tsujimoto, G. (2005) *Nature* **431**, 90–94

65. Katsuma, S., Hatae, N., Yano, T., Ruike, Y., Shinohara, T., Hinuma, S., Fujisawa, Y., and Fujino, M. (2003) *Nature* **422**, 173–176

66. Igarashi, M., Wakasaki, H., Takahara, N., Ishii, H., Jiang, Z. Y., Yamauchi, A., Kimura, M., Hirasawa, A., Tsumaya, K., Awaji, T., Katsuma, S., Adachi, T., Yamada, M., Sugimoto, Y., Miyazaki, S., and Tsujimoto, G. (2005) *Nature* **431**, 90–94

67. Katsuma, S., Hatae, N., Yano, T., Ruike, Y., Kimura, M., Hirasawa, A., and Tsujimoto, G. (2005) *J. Biol. Chem.* **280**, 19507–19515

68. Igarashi, M., Wakasaki, H., Takahara, N., Ishii, H., Jiang, Z. Y., Yamauchi, T., Kuboki, K., Meier, M., Rhodes, C. J., and King, G. L. (1999) *J. Clin. Invest.* **103**, 185–195

69. Yamaguchi, I., Igarashi, M., Hirata, A., Sugae, N., Tsuichi, H., Jimbu, Y., Tominaga, M., and Kato, T. (2004) *Arterioscler. Thromb. Vasc. Biol.* **24**, 2095–2101

70. Hanson, R. W., and Reshef, L. (1997) *Annu. Rev. Biochem.* **66**, 581–611

71. Lam, T. K., Pacic, A., Gutierrez-Juarez, R., Obici, S., Bryan, J., Aguilar-Bryan, L., Schwartz, G. J., and Rossetti, L. (2005) *Nat. Med.* **11**, 320–327

72. Hakimi, P., Johnson, M. T., Yang, J., Lepage, D. F., Conlon, R. A., Kalhan, S. C., Reshef, L., Tilghman, S. M., and Hanson, R. W. (2005) *Nutr. Metab. (Lond.)* **2**, 33

73. Accili, D. (2004) *Diabetes* **53**, 1633–1642