Insulin Regulation of Phosphoenolpyruvate Carboxykinase-C Gene Transcription: The Role of Sterol Regulatory Element-Binding Protein 1c

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The effect of insulin on the regulation of phosphoenolpyruvate carboxykinase C (PEPCK-C) gene transcription, while pivotal for control of carbohydrate metabolism, constitutes only a small part of its overall action in cellular processes. Transcription of the PEPCK-C gene is the target for a number of pathways involved in the signal transduction initiated by insulin, and these processes involve an array of transcription factors and co-regulatory proteins that either alone or in concert bind to a subset of sites in the gene promoter to regulate its expression. This review will focus on a specific transcription factor, sterol regulatory element-binding protein 1c (SREBP-1c), and its role in the control of PEPCK-C gene transcription.

Key words: insulin regulation, phosphoenolpyruvate carboxykinase-c, PEPCK-C, sterol regulatory element binding protein 1c, SREBP-1c

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INTRODUCTION

Phosphoenolpyruvate carboxykinase (PEPCK) (EC.4.1.1.32) was discovered by Merton F. Utter and Kiyoshi Kurahashi in 1953,[1] when they isolated it from chicken liver mitochondria and demonstrated its involvement in what was then termed the Wood-Werkman pathway (the process of CO_2 fixation in animal tissues). There are two isozymes of PEPCK, a mitochondrial (PEPCK-M) and a cytosolic (PEPCK-C) form. PEPCK is now considered a key enzyme in both gluconeogenesis and glyceroneogenesis, and catalyzes the following reaction:

$$Oxaloacetate + GTP \leftrightarrow Phosphoenolpyruvate + CO_2 + GDP$$

GENE STRUCTURE

The rat PEPCK-C gene is 6 kb in length and has 9 introns.[2] PEPCK-C resides on chromosome 2 in the mouse, on chromosome 3 in the rat, and on chromosome 20 in humans.[3-5] PEPCK-C and PEPCK-M are encoded by separate nuclear genes. All vertebrate species studied to date have both isoforms of PEPCK but express them at different levels. For example, most mammals have similar activities of both PEPCK-C and PEPCK-M in their livers, while the rat and mouse have 90% PEPCK-C and birds have 100% PEPCK-M. The metabolic significance of this distribution of the isoforms of PEPCK-C has been discussed in detail in a previous review.[6]

PROCESSING AND REGULATION OF PEPCK-C mRNA

The half-life of PEPCK-C mRNA is 30 minutes[7] and the half-life of the protein is 6 to 8 hours.[8] When the transcription of the gene for PEPCK-C was stimulated by the administration of Bt2cAMP, a number of RNA species were detected that were shorter than the mature, 2.8-kb mRNA.[7] These RNA species ranged from 6.6 to 0.2 kb in length. Hatzoglou et al.[9] proposed that these are precursors of the mature PEPCK-C mRNA that are processed in an organized manner, starting from the 5′ to the 3′ end. The precursors accumulate in the nucleus because the rate of transcription of the gene for PEPCK-C is more rapid than the processing and/or transport of nascent RNA from the nucleus to the cytoplasm for subsequent translation.[9] Alteration in the synthesis rate of PEPCK-C closely parallels the concentra-

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tion of its mRNA. This acute regulation insures that changes in the activity of PEPCK-C in the liver can be rapidly induced by altering the concentration of the enzyme RNA. This is critical, since there are no known allosteric regulators of PEPCK-C.

METABOLIC ROLE OF PEPCK-C IN MAMMALIAN TISSUES

Liver and Kidney

While PEPCK-C is involved in gluconeogenesis in the liver and kidney, it has several other proposed metabolic roles, such as glyceroneogenesis in liver and adipose tissue and the control of acid/base balance in the kidney. During periods of chronic metabolic acidosis, renal PEPCK-C is involved in maintaining acid-base balance by its involvement in gluconeogenesis from glutamine.[10,11] PEPCK-C is found in the proximal tubules of the kidney cortex (not in the kidney medulla), and its gene expression is induced by metabolic acidosis.[10,12] Gluconeogenesis therefore removes the α-ketoglutarate produced when glutamine in the kidney cortex is metabolized during acidosis.

Adipose and Other Tissues

There is significant PEPCK-C activity in white[13] and brown[14] adipose tissue. Ballard et al.[13] proposed that the function of this enzyme in white adipose tissue is to provide 3-glycerophosphate for triglyceride synthesis during starvation, a pathway later termed glyceroneogenesis. Glyceroneogenesis is required for the re-esterification of free fatty acids to sustain triglyceride synthesis even during periods of net lipolysis.[15,16] There is also considerable activity of PEPCK-C in brown adipose tissue, where it has been shown to be involved in glyceroneogenesis.[17]

The metabolic role of PEPCK-C in the mammary gland is unclear, but it mostly likely participates in either gluconeogenesis or glyceroneogenesis, since the rate of synthesis of both glucose and triglyceride is high during active milk production. Recent research by Mithieux et al.[18] has shown that PEPCK-C in the small intestine is involved in gluconeogenesis, and the investigators suggested that this process provides lactate for local use by the mucosal cells of the small intestine.[19]

TRANSCRIPTIONAL REGULATION OF PEPCK-C GENE EXPRESSION

Organization of the PEPCK-C Gene Promoter

The PEPCK-C gene promoter can be arbitrarily divided into four regions (I–IV) (Figure 1). Region 1 encompasses a TATA box, crucial for basal transcription, and a cAMP regulatory element (CRE) through which cAMP exerts its stimulatory effect on PEPCK-C gene transcription.[20-25] Other transcription factors that

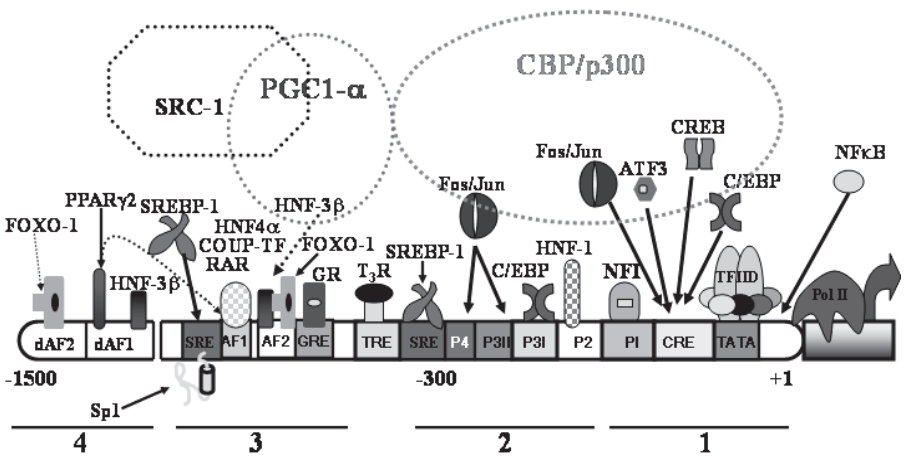

Figure 1. Transcription factor binding site on the phosphoenolpyruvate carboxykinase C (PEPCK-C) gene promoter. The location of regulatory elements in the PEPCK-C gene promoter (–1100 to +73 bp) and the transcription factors known to bind to these sites are shown. The elements are: CRE, cAMP regulatory element; GRU, glucocorticoid regulatory unit; IRE, insulin regulatory element; IRS, insulin regulatory sequence; PPARE, peroxisome proliferator-activated receptor element; SRE, sterol regulatory element; TRE, thyroid hormone response element. The transacting factors are: AF, accessory factor; CBP, CREB binding protein; C/EBP, CAAT/enhancer binding protein; COUP-TF, chicken ovalbumin upstream promoter transcription factor; CREB, CRE binding protein; DBP, D binding protein; GR, glucocorticoid receptor; HNF-1, hepatic nuclear factor-1; NF-1, nuclear factor one; PGC-1, PPAR gamma coactivator 1; PPAR, peroxisome proliferator-activated receptor; RAR, retinoid acid receptor; RXR, retinoic acid nuclear receptor; SRC-1, sterol receptor coactivator 1; SREBP, sterol regulatory element-binding protein; and TR, thyroid hormone receptor.

bind this region include NF1, CREB, CREM, C/EBP, Fos/Jun, ATF-3, and AT-4.[26-29]

Region II of the gene promoter is important for tissue-specific regulation,[30] has an HNF-1 binding domain, and is required for renal specific expression of the PEPCK-C gene[30,31] and a regulatory element that interacts with members of the C/EBP family of transcription factors called P3(1),[32] which is required for liver-specific transcription of the gene. Region III encodes a glucocorticoid regulatory unit (GRU), which contains two binding sites for the glucocorticoid receptors (GR1 and GR2),[33-35] two accessory factor binding sites (AF1 and AF2), a putative insulin regulatory sequence (IRS), hepatic nuclear factor-3B (HNF-3B) and 4A, chicken ovalbumin upstream promoter transcription factor (COUP-TF) binding element, and peroxisome proliferator-activated receptor (PPARγ2) sites.[36] In addition, two SREBP-1 binding sites located in both regions II and III allow insulin regulation of PEPCK-C gene expression.[37] Region IV contains regulatory elements that are involved in the adipose tissue-specific expression of the PEPCK-C gene. There is a PPARγ2 binding site at –990, which is required for the expression of the gene for PEPCK-C in white and brown adipose tissue.[38,39]

MECHANISM OF INSULIN REGULATION OF PEPCK-C GENE TRANSCRIPTION

The mechanisms responsible for the tissue-specific and hormonal regulation of transcription of the gene for PEPCK-C have been extensively studied previously.[6] As mentioned above, the transcription factors involved in cAMP and glucocorticoid regulation of PEPCK-C gene transcription have also been extensively studied. However, the mechanism by which insulin dominantly represses PEPCK-C gene transcription has proven to be more elusive. Granner et al.[40,42] have identified two putative insulin response sequence (IRS) on the PEPCK-C gene promoter. The core IRS maps between −413 to −407 on the promoter and overlaps the AF2 binding sites in the GRU. Deletion of the IRS blocks the ability of insulin to inhibit PEPCK-C gene transcription by only 50% in transfected hepatoma cells, suggesting the existence of a second IRS for insulin regulation of PEPCK-C gene transcription.[40,42] Recently, Doung et al.[41] demonstrated, using chromatin immunoprecipitation (ChIP) analysis, that insulin markedly represses, while cAMP and glucocorticoids enhance, the occupancy of CBP and RNA polymerase II (pol II) on the PEPCK-C gene promoter.[41] They suggest that the PEPCK-C gene promoter is constitutively occupied by accessory factors that coordinately recruit activating factors in response to hormones such as dexamethasone.[41] Glucocorticoids, in concert with CBP, the glucocorticoid receptor, and pol II, stimulate transcription from the PEPCK-C gene promoter. When both glucocorticoids and insulin are present simultaneously, the site occupancy by CBP and pol II is lost, even in the presence of ligand-bound GR, which remains anchored to the PEPCK-C gene promoter.[40-41,43]

Another key transcription factor, C/EBPβ, can orchestrate the recruitment of CBP to drive transcription from the PEPCK-C gene promoter.[41,44] C/EBPβ has two isoforms that are expressed depending on the dietary and hormonal states of the animal. C/EBPβ-C1 is the inhibitory form that represses PEPCK-C gene transcription (also called LIP), while C/EBPβ-B1 (also called LAP) stimulates PEPCK-C gene transcription.[38,45] Insulin increases the concentration of C/EBPβ-C1 in a pathway that involves PI3K signaling.[41] As the C/EBPβ-C1 level increases, C/EBPβ-B1, the activating counterpart of PEPCK-C gene transcription, is competed away, abolishing any further assembly of CBP-pol II complex on the PEPCK-C gene promoter and thereby inhibiting PEPCK-C gene transcription in an insulin-dependent manner.[41] However, the insulin-mediated repression of PEPCK-C gene transcription by C/EBPβ-C1 is not immediate, requiring 60 to 120 minutes to alter gene expression.[41]

Recently, Forkhead (FKHR) has been suggested as a mediator of insulin-regulated transcription of the gene for PEPCK-C.[46-51] It has been proposed that insulin activates protein kinase B (PKB) phosphorylation of FKHR, resulting in the nuclear exclusion of the transcriptional regulator with a concomitant decrease in gene transcription.[49-52] However, using different methods, several laboratories have reported that FKHR is not implicated in the regulation of all genes that are inhibited by insulin.[48,49,51] For example, adenoviral expression of FKHR in insulin-insensitive kidney cells is sufficient to restore insulin inhibition of G6Pase, but has no effect on PEPCK-C gene transcription.[48,49] Yeagley et al.[51] have studied the contribution of FKHR and the IREs of the PEPCK-C and IGFBP-1 promoters to inhibition by insulin. They demonstrated that FKHR can interact with the IRE in the IGFBP-1 gene promoter, and that insulin inhibits basal IGFBP-1 gene expression by disrupting this interaction. Conversely, the PEPCK-C IRE binds FKHR, but this interaction is not important for insulin-mediated inhibition of the PEPCK-C gene expression.[51] In summary, FKHR inhibits G6Pase and IGFBP-1 gene transcription in an insulin-dependent manner, but has no effect on insulin-mediated PEPCK-C gene transcription.[46-51]

PROBLEMS WITH OUR CURRENT UNDERSTANDING OF THE MECHANISM OF THE INSULIN REGULATION OF PEPCK-C GENE TRANSCRIPTION

Insulin is known to be a dominant negative regulator of PEPCK-C gene transcription due to its ability to

completely and rapidly suppress PEPCK-C gene expression. Despite the strong effect of insulin on PEPCK-C gene transcription, delineating the insulin responsive element in the promoter has been difficult. Segments of the PEPCK-C gene promoter ranging from –1800 to +73 linked to reporter genes were stably or transiently transfected in hepatoma cells. The ability of insulin to block the inductive effect of cAMP on PEPCK-C gene transcription has been tested, but no inhibitory effect of insulin could be detected.[53,54] Granner et al.[55,56] have described a putative IRS responsible for insulin sensitivity on the PEPCK-C gene promoter. As mentioned above, the proposed IRS lies within the glucocorticoid regulatory unit and overlaps the AF2 binding site.

Using transient transfection of DNA segment containing the IRS linked to a reporter gene, O'Brien et al.[56] found that a mutation in the core IRS sequence in the PEPCK-C gene promoter resulted in only a 50% reduction of insulin repression of PEPCK-C gene transcription. Subsequently, this IRS in the PEPCK-C gene promoter was intensively studied using cells in culture transfected with chimeric genes containing a modified PEPCK-C gene. Genes containing the PEPCK-C gene promoter with a deletion of the IRS or a mutation of the element were introduced into transgenic mice. Transcription from these modified promoters in the liver of the mice was inhibited by insulin to the same extent as the wild-type promoter in control mice carrying the endogenous PEPCK-C gene.[57] Thus, the putative IRS is not solely responsible for the insulin-mediated inhibition of the PEPCK-C gene transcription. If not the IRS, then what sites are involved? The PEPCK-C gene promoter contains a SREBP-1 binding site. SREBP-1c is a transcription factor that is known to positively control gene transcription for enzymes involved in lipid metabolism. Previous studies demonstrated that SREBP-1 is also involved in regulating genes that are involved in carbohydrate metabolism, such as G6Pase and PEPCK.[37,58]

SREBP-1C AND THE CONTROL OF GENE TRANSCRIPTION BY INSULIN

The overexpression of SREBP-1c in cultured preadipocytes results in the differentiation of these cells into mature adipocytes.[59,60] In addition, insulin strongly activates SREBP-1c gene expression, whereas glucagon opposes the effect of insulin on SREBP-1c expression.[63] Insulin also induced an increase in the precursor form of SREBP-1c and a concomitant increase in the mature form of the protein in the nucleus within 2 hours after insulin addition.[64] Finally, Shimomura et al.[65] reported that diabetes decreased the concentration of SREBP-1c mRNA in the liver of rats to barely detectable levels; there was no change in the hepatic concentration of mRNA for SREBP-1a. These findings are consistent with the known effects of insulin and glucagon (acting via cAMP) on the regulation of gene expression in the liver.

Shimano et al.[61] constructed transgenic mice in which a 2.5-kb segment of the PEPCK-C gene promoter was used to drive transcription of the structural genes for both SREBP-1a and SREBP-1c. These mice express both isoforms of SREBP in the liver and develop a number of metabolic defects, including a fatty liver. SREBP-1c was notably less active than SREBP-1a in altering lipid metabolism in these mice. Based on our studies, we would not predict that a chimeric gene driven by the PEPCK-C gene promoter would express poorly in the livers of the mice because SREBP-1c so markedly inhibits transcription from this promoter in isolated hepatocytes. However, the transgenic mice were fed a low-carbohydrate diet to stimulate transcription from the PEPCK-C gene promoter,[61] and this may have partly modified the negative effect of SREBP-1c on transcription. Alternatively, the site of integration of the transgene in the mouse genome may have influenced transcription from the promoter in the liver.

SREBP-1C REGULATES TRANSCRIPTION OF PEPCK-C AND OTHER GENES INVOLVED IN CARBOHYDRATE METABOLISM

Evidence is accumulating that SREBP-1c is involved in the regulation of carbohydrate metabolism. As mentioned earlier, this most likely occurs via a reciprocal response to the hormones that control the genes involved in these two processes, i.e., insulin and glucagon. Foretz et al.[66] reported that SREBP-1c is a mediator of insulin action on the expression of the gene for glucokinase in isolated hepatocytes in which the gene for SREBP-1c had been introduced by adenoviral infection. In these studies, SREBP-1c greatly stimulated the levels of mRNA for glucokinase in hepatocytes, while dnSREBP-1c had the predicted effect of markedly reducing the concentration of glucokinase mRNA. The concentration of mRNA for S14 and hepatic fatty acid synthase was also increased by the introduction of SREBP-1c into hepatocytes.[66] Interestingly, the effect of insulin on SREBP-1c gene expression, as well as its downstream effects on target genes, is dependent on PI3-kinase-PBK/Akt pathway.[64]

Current literature strongly implicates an interaction between SREBP-1c and CBP, the transcriptional coactivator that is critical in coordinating the cAMP stimulation of PEPCK-C gene transcription.[29] SREBP-1c blocks the stimulation of PKA-induced transcription from the PEPCK-C gene promoter by CBP and dnSREBP-1c and overcomes the inhibition of PKA-induced transcription by both E1A and NF1c. Both E1A and NF1c have been shown to inhibit PEPCK-C gene

transcription by acting via CBP.[29] Ericsson and Edwards[62] reported that CBP was required for the sterol/SREBP-1a-regulated increase in transcription from the promoters of the HMG-CoA synthase and HMG-CoA reductase genes in transient transfection assays. E1A blocked the effect of SREBP-1a, but this inhibition could be overcome by the coexpression of CBP. These authors concluded that SREBP bound to the promoter DNA of the target gene and then to the amino-terminal domain (amino acids 1 through 451) of CBP. A similar interaction between SREBP-1c, the PEPCK-C gene promoter, and CBP has been suggested.

There are data demonstrating that SREBP-1c is involved in insulin's control of PEPCK-C gene transcription in the liver.[37,58] If this regulation of PEPCK-C gene transcription also extends to adipose tissue, the insulin activation of SREBP-1c would be predicted to control the rate of glyceroneogenesis in that tissue. As mentioned above, the overexpression of SREBP-1c in adipose tissue caused lipodystrophy in transgenic mice. This could be due in part to a lower level of PEPCK-C activity in that tissue, resulting in a decreased rate of glyceroneogenesis during fasting. This would be expected to cause a reduced rate of fatty acid re-esterification to triglyceride.[16,67] In support of this concept is the finding that the ablation of expression of the gene for PEPCK-C in the adipose tissue resulted in profound lipodystrophy in mice due to an inhibition of glyceroneogenesis.[38] It thus seems likely that by controlling the level of PEPCK-C gene expression in both the liver and the adipose tissue, SREBP-1c plays a key role in the integration of lipid and carbohydrate metabolism. Thus, SREBP-1c is a likely target for diseases such as type 2 diabetes and other insulin-resistant states.

MECHANISM OF SREBP-1C INHIBITION OF PEPCK-C GENE TRANSCRIPTION

Any discussion of the mechanism of SREBP-1c inhibition of PEPCK-C gene transcription starts with a consideration of the models developed to explain the action of this transcription factor on the better studied genes involved in cholesterol synthesis. Wang et al.[68] have reported that the promoter of the gene for the LDL receptor, a gene that has several SREs called repeats 1 through 3, also contains an SP1-binding site adjacent to the SRE repeat 3. Based on these findings, they suggested that SREBP-2 interacts with SP1 to control transcription of the gene for the LDL receptor. Later studies by Sanchez et al.[69] demonstrated that these two transcription factors act synergistically and communicate with each other when bound to the DNA. Based on their studies,[59] they proposed a model for the coordinated control of transcription of the LDL receptor gene by sterols. When cellular levels of sterols fall, the mature form of SREBP is translocated to the nucleus, where it binds to SRE repeat 2 and interacts with SP1. As a result, it increases the binding of SP1 to its adjacent binding site, stimulating gene transcription. The interaction between SREBP and SP1 on the LDL receptor gene promoter is unstable, but is favored by the high levels of SREBP in the nucleus that occur when there is a chronically low concentration of sterols in the cell; this sustains the increased rates of gene transcription. When the intracellular concentration of sterols increases due to high LDL receptor activity, the flux of SREBP into the nucleus is markedly decreased, resulting in a dissociation of DNA-bound SREBP and subsequent SREBP degradation. This would result in the predicted decrease in transcription of the LDL receptor gene.

The rapid reversibility of gene transcription to reflect the concentration of sterols in the cell is an attractive feature of this model. Bennett and Osborne[70] have demonstrated that sterol depletion resulted in the recruitment of two additional transcription factors, the cAMP regulatory element binding protein (CREB) and nuclear factor-Y (NF-Y), to the promoter of the gene for HMG-CoA reductase. Interestingly, the recruitment of SREBP to the LDL receptor gene promoter is specific to SP1, while the HMG-CoA reductase gene promoter involves an interaction of SREBP with CREB. Like SP1, both NF-Y and CREB bind to SREBP without binding to DNA.[70] The interaction of SREBP with specific transcription factors is accompanied by increased activation of histone H3 (not histone H4). This is due to the N-terminal activation domain of SREBPs that interacts with the co-activators CBP and P300; these proteins have intrinsic histone acyltransferase activity and are capable of inducing chromatin remodeling.[62] Naar et al.[71] have purified a multiprotein component from isolated nuclei, which includes CBP, binds to the SRE of the LDL receptor gene promoter, and is able to mediate high levels of synergistic activation by SREBP/SP1 on chromatin templates.

Our work has shown that the PEPCK-C gene promoter, like the promoter for the LDL receptor gene, contains two functional SREs, one centered at –322 and the other at –590. Interestingly, the SRE in the PEPCK-C gene promoter at –590 differs from the consensus SRE in the LDL receptor gene promoter by a single base at –582. This seemingly subtle difference, when introduced into the native PEPCK-C gene promoter, converts the SRE into a positive rather than a negative regulatory element. This is due to a critical alteration caused by this mutation in an SP1 binding site that is present on the opposite strand of DNA to the SRE. This alteration also increases the binding of SREBP-1c to the SRE. In a cellular transfection assay using the consensus SRE from the LDL gene promoter, SREBP-1c markedly stimulates

transcription from the PEPCK-C gene promoter. Current evidence shows that the SRE in the PEPCK-C gene promoter does not bind SREBP-1c as firmly in the presence of low concentrations of SP1 and is thus not as effective an inhibitor of PEPCK-C gene transcription.

USA (upstream stimulatory activity) is required to get enhanced binding, furthermore HMG-1, a component of USA, is specifically required for high affinity binding of SREBP-1 to the PEPCK-C gene promoter (Chakravarty and Hanson, unpublished observations). In addition, we have demonstrated that as the concentration of SREBP-1c increases in the nucleus due to the increase in the levels of insulin in the liver, SREBP-1c competes with SP1 for binding to the SRE at –590, resulting in an inhibition of PEPCK-C gene transcription. As the level of insulin falls and SREBP-1c is degraded, SP1 interacts with other transcription factors to stimulate gene expression. This scheme has parallels with the model proposed for the effect of SREBP on transcription from the LDL receptor gene promoter proposed by Bennett and Osborne,[70] except that the one base pair mutation in the SRE in the PEPCK-C gene promoter at –582 results in inhibition of gene transcription rather than stimulation. This is an example of the duplication of a sequence for a regulatory element in a gene that codes for a protein that is critical in the pathway of gluconeogenesis, but with a subtle mutation that alters the function of an SP1 site on the opposite strand of the DNA.

This is a major variation in the organization of the PEPCK-C gene promoter compared with the LDL receptor gene promoter, since the SP1 site is not adjacent and on the same strand of the DNA. This minor alteration of the SRE in the PEPCK-C gene results in the reciprocal regulation by SREBP of a gene that is important in gluconeogenesis (decreasing its transcription) compared with the increase it induces in the expression of genes such fatty acid synthase, HMG CoA reductase, or acetyl CoA carboxylase, enzymes that are critical for fatty acid and cholesterol synthesis.

The presence of two functional SREs in the PEPCK-C gene promoter leads to the question of the role of each in the control of PEPCK-C gene transcription. Only one of the sites binds SP1 (–590), while the other (–322) is adjacent to the thyroid hormone regulatory element 72 and to the P4 site that binds fos/jun heterodimers.[21,28] An individual mutation of the SRE at –590 results in a marked increase (14-fold) in PKA-induced transcription from the modified PEPCK-C gene promoter compared with the native promoter. This suggests that the removal of this site overcomes an inhibition of transcription caused by the binding of SREBP-1. Mutation of the SRE at –590 does not prevent the inhibition of transcription by SREBP-1c, supporting the important role of the SRE at –322. Mutation of the two sites results in complete loss of the inhibitory effect of SREBP-1c on PKA-stimulated transcription from the PEPCK-C gene promoter.[37] The single base pair mutation of the SRE at –590 also causes SREBP-1c to stimulate basal transcription from the PEPCK-C gene promoter rather than inhibit it.[37] Supporting work from Yamamoto et al.[58] also demonstrates that PEPCK-C and Glc-6-Pase mRNA levels are down-regulated as a result of the overexpression of both SREBP-1a and SREBP-1c. In addition to the inhibitory role of SREBP-1c on PEPCK-C, transcriptional suppression of gluconeogenic genes can also be attained due to the interference of HNF-4a on the PGC-1 recruitment to the transcriptosome in the presence of SREBP-1.[58]

PHYSIOLOGICAL ROLE OF SREBPs IN THE CONTROL OF CARBOHYDRATE METABOLISM

The biological function of the SREBPs has been largely restricted to the control of transcription of genes coding for proteins involved in lipid metabolism. Liang et al.[73] have stressed the key role of SREBP as "activators of the complete program of cholesterol and fatty acid synthesis in the liver." This subject has also been extensively studied using a number of lines of genetically modified mice. A central feature of the biological role of SREBP-1 is its acute activation by insulin. This activation suggests that SREBP-1 could play a critical role in coordinating the control of carbohydrates, as well as lipid metabolism. Thus, the demonstration that SREBP-1c, when introduced into hepatoma cells[72] or the livers of mice with an adenoviral vector,[63,64,66] inhibits transcription of the gene for PEPCK-C, further supports this concept of reciprocal regulation of the two opposing metabolic pathways. However, deletion of the individual genes for SREBP in mice has not provided a clear picture of whether individual members of this family of transcription factors are involved in the control of carbohydrate metabolism. For example, the deletion of the gene for SREBP-1c does not alter the level of PEPCK-C gene transcription in the liver.[73] Also, overexpression of the gene for SREBP-1 in the livers of mice using the PEPCK-C gene promoter does not result in the predicted negative feedback on transcription by SREBP-1. However, deleting the genes for either SREBP-1a or SREBP-2 is lethal to mice embryos, negating any further analysis of the metabolic phenotype of these mice.

We have also demonstrated that all three isoforms of SREBP can inhibit transcription from the PEPCK-C gene promoter.[37] It is possible that in the livers of mice with a deletion in the gene for a specific isoform of SREBP, one of the other members of the family can function to insure the continued regulation of PEPCK-C gene transcription. In this regard, Liang et al.[73] reported

that SREBP-1a and/or SREBP-2 could partially substitute for SREBP-1c in permitting the insulin-mediated induction of acetyl CoA carboxylase and fatty acid synthase in the livers of mice lacking the gene for SREBP-1c. This effect was gene specific, since there was no increase in the level of hepatic mRNA caused by insulin for other lipogenic enzymes normally regulated by SREBP-1c, such as glucose-6-phosphate dehydrogenase and NADP malate dehydrogenase. Also, the deletion of the gene for SREBP-1c resulted in a 2.88-fold increase in the concentration of PEPCK-C mRNA in the livers of these mice.[73]

The redundant action of the three isoforms of SREBP on the transcription of the gene for PEPCK-C complicates a simple interpretation of its physiological role in the insulin-mediated control of gene transcription. However, the total concentration of all three isoforms has been manipulated in mice that contain a partial disruption of the gene for Site-1 protease (S1P). These mice were generated by floxing the S1P allele and breeding the mice with animals that were heterozygous for a chimeric gene consisting of the Cre recombinase structural gene driven by the interferon-inducible MX1 promoter.[73,74] Interferon treatment of these mice resulted in an approximate 30% reduction in the concentration of hepatic mRNA for SREBP-1a, an 80% to 90% reduction in the level of mRNA for SREBP-1c, and a 50% reduction ion mRNA for SREBP-2.[73,74] When these mice were fasted for 12 hours and then re-fed a diet high in carbohydrate, the decrease in PEPCK-C mRNA in their livers was markedly attenuated.[73] This is the only available evidence demonstrating the effect of a global decrease in SREBP isoforms in the liver on PEPCK-C gene transcription in an intact animal, and it provides evidence that supports a role this protein in regulating carbohydrate metabolism. Finally, preliminary data on the control of transcription from the glucose-6-phosphatase gene promoter demonstrate that it is inhibited by co-transfection with SREBP-1c into hepatoma cells in culture (Duna Massillon, unpublished results). Thus, the transcription of two genes that are critical for gluconeogenesis can be strongly affected by SREBP-1.

CONCLUSIONS

Mechanistic details of the nuclear regulation of the PEPCK-C gene promoter by insulin remains elusive. However, our research has demonstrated that SREBP-1, in concert with other transcription factors, can markedly inhibit the transcription of the PEPCK-C gene. SREBP-1, along with other transcription factors, regulates PEPCK-C gene transcription in a combinatorial fashion, as demonstrated by the by Naar et al.[75] SREBP-1 docks on the PEPCK-C gene promoter assisted by accessory factors. Binding of SREBP-1 helps to recruit other protein complexes that include mediators, co-activators (CBP, and PGC-1), and other unknown transcription factors. Simultaneously, SP1 is competed off the PEPCK-C SRE site at –590, leaving the control sequence open for SREBP-1 binding. The multi-protein complex formed by SREBP-1 and other proteins changes the conformation of the DNA, de-stabilizing the RNA polymerase II transcriptosome complex. This leads to the inhibition of the PKA-stimulated transcription of the gene for PEPCK-C. The inhibition of the PEPCK-C gene transcription is rapid and transient, responding to the reciprocal control of insulin and glucagon, whose levels are acutely regulated by the diet.

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