SREBP, sterol-regulatory-element-binding protein.

Abstract

Effects of insulin on the expression of liver-specific genes are part of the adaptive mechanisms aimed at maintaining energy homeostasis in mammals. When the diet is rich in carbohydrates, secreted insulin stimulates the expression of genes for enzymes involved in glucose utilization (glucokinase, L-type pyruvate kinase and lipogenic enzymes) and inhibits genes for enzymes involved in glucose production (phosphoenolpyruvate carboxykinase). The mechanisms by which insulin controls the expression of these genes have been poorly understood. Recently, the transcription factor sterol-regulatory-element-binding protein 1c has been proposed as a key mediator of insulin transcriptional effects. Here we review the evidence that has led to this proposal and the consequences for our understanding of insulin effects in physiological or pathological conditions.

Introduction

Glucose homeostasis is a major requirement for survival in mammals because this substrate is used continuously at a high rate by organs such as the...
brain (120 g/day), blood cells and renal medulla. This requires continuous adaptations of metabolic pathways because glucose intake during the meals does not temporally match glucose output. Insulin has a major role in these adaptations. When a meal that contains carbohydrate is absorbed, insulin is secreted and induces several metabolic events aimed at decreasing endogenous glucose production by the liver (glycogenolysis and gluconeogenesis) and increasing glucose uptake and storage in the form of glycogen in the liver and muscle. If glucose is delivered into the portal vein in large quantities and once the glycogen stores are replete, glucose can even be converted into lipids (lipogenesis) in adipose tissue. Insulin exerts its effects by rapidly modulating the activity of specific proteins (enzymes and transporters) but also on a longer-term basis through changes in their quantity. This can be achieved by modulating their transcription rate and also post-transcriptional steps such as mRNA half-life or translation efficiency.

In the liver, the transcriptional effects of insulin on genes involved in glucose metabolism have been well characterized. Insulin induces the transcription of enzymes of the glycolytic pathway (gluokinase (GK), L-type pyruvate kinase (L-PK), lipogenic pathway, fatty-acid synthase (FAS), acetyl-CoA carboxylase (ACC), ATP-citrate lyase and glucose-6-phosphate dehydrogenase) but inhibits the expression of a key enzyme of the gluconeogenic pathway, phosphoenolpyruvate carboxykinase (PEPCK) [1–4]. Among the genes mentioned above, some are exclusively dependent on insulin for their transcriptional modulation; this is so for GK and PEPCK [2–4], whereas others, such as L-PK and lipogenic enzymes, are dependent on high concentrations of both insulin and glucose [1]. In addition, glucose must be at least partly metabolized to achieve its stimulatory effect [1].

Important progress has been made in the identification of the partners involved in the events after the binding of insulin to its receptor. In contrast, the factors involved in the transcriptional effects of insulin were largely unknown until recently, although insulin response elements have been identified in some genes. Here we review the recent findings on a transcription factor, sterol-regulatory-element-binding protein 1c (SREBP-1c), that could mediate some of the transcriptional effects of insulin on the hepatic genes involved in glucose metabolism.

What is the relationship between SREBP-1c and the hepatic transcriptional effects of insulin?

SREBP-1c belongs to a family of transcription factors originally involved in the regulation of genes by the cellular availability of cholesterol [5]. Three members of the SREBP family have been described. SREBP-1a and SREBP-1c are encoded by a single gene through the use of alternative transcription start sites; they differ in the first exon [6]: the first exon of SREBP-1a is composed of 42 amino acid residues, of which 12 are acidic, whereas the first exon of SREBP-1c is composed of 24 residues, of which only 6 are acidic. This leads to a higher transactivation capacity of SREBP-1a than that of SREBP-1c. SREBP-1c is expressed in liver, adipose tissue, adrenal gland, muscle and brain; SREBP-1a is expressed in the spleen, intestine and hepatoma and adipose cell lines. In rat adipocytes, SREBP-1c was independently described as a factor involved in adipocyte determination and differentiation and was therefore called ADD1 [7]. The third member of the family, SREBP-2, is derived from a different gene. SREBPs are synthesized as a precursor form bound to the endoplasmic reticulum and nuclear membranes. They all have a common structure: (1) an N-terminal fragment of 480 residues that is in fact a transcription factor of the basic domain-helix–loop–helix leucine zipper family, (2) a region of 80 residues containing two transmembrane domains separated by 31 residues that are in the lumen of the endoplasmic reticulum, and (3) a regulatory C-terminal domain of 590 residues. Brown and Goldstein [5,6] have elegantly unravelled the mechanisms by which the transcriptionally active fragment of SREBP-2 and SREBP-1a is liberated. When the concentration of cholesterol decreases in the membranes, the precursor form of SREBP-2 and SREBP-1a is cleaved by a complex mechanism involving two proteolytic cleavages and a protein 'sensor' for cholesterol concentration. The mature form migrates to within the nucleus, where it binds as homodimers to sterol response elements (SREs), 5'-ATCACCCAC-3', on the promoter of genes involved in cholesterol uptake (such as the low-density-lipoprotein receptor) or in cholesterol synthesis (such as cytoplasmic hydroxymethylglutaryl-CoA synthase or hydroxymethylglutaryl-CoA reductase). SREBP factors are able to bind both to SREs and E-boxes (5'-CANNTG-3') owing to the presence of a tyrosine residue in place.
Insulin Action

of arginine in their basic domain [8]. Finally, the transcription rate of SREBP-1a and SREBP-2 is increased under conditions of cellular cholesterol depletion.

In contrast with SREBP-2 and SREBP-1a, the expression and nuclear abundance of SREBP-1c are not increased under conditions of low availability of cholesterol. A nutritional protocol aimed at increasing the demand for cholesterol induces in the hamster liver a clear-cut increase in the expression and nuclear abundance of SREBP-2, as expected, whereas SREBP-1c expression and nuclear abundance are decreased somewhat [9].

In fact, SREBP-1c expression and transcriptional effects seem to be related to carbohydrate and lipid metabolism. In mouse liver, the expression of SREBP-1c and the presence of its mature form in nuclei are higher in carbohydrate-refed animals than in starved animals [10]. The expression of SREBP-1c is controlled positively by insulin and glucagon [13]. This effect was clearly transcriptional, as shown by run-on assays. Glucagon, which antagonizes the action of insulin on many metabolic hepatic processes, opposes the effects of insulin on SREBP-1c expression via its second messenger cAMP [13].

At the protein level, insulin induced an increase in the precursor form of SREBP-1c and a concomitant increase in the nuclear mature form, which was detectable 2 h after the addition of insulin [14]. An obvious question was then whether insulin, like cholesterol for SREBP-2 and SREBP-1a, was inducing not only the synthesis but also the proteolytic cleavage of SREBP-1c. The experiments that we have performed tend to suggest that this is not true and that the proteolytic cleavage could be constitutive [14]. However, the interpretation of the results is not straightforward because insulin also increases the synthesis of SREBP-1c. Definitive proof would require the insulin-independent expression of a tagged SREBP-1c in primary cultured hepatocytes.

The effect of insulin on SREBP-1c was corroborated by studies in vitro showing that SREBP-1c expression and nuclear abundance was low in the liver of diabetic rats and increased markedly after an insulin treatment [15]. The effects on the SREBP-1c transcript were highly specific in that no changes in levels of the mRNA species encoding SREBP-1a or SREBP-2 were detected.

More recently an effect of a high glucose concentration on SREBP-1c expression was described in a hepatocyte cell line transformed with a temperature-sensitive strain of simian virus 40 [16]. However, this effect was not specific for SREBP-1c, because SREBP-1a was also affected. We have never observed such an effect of glucose in primary cultured hepatocytes. In addition, the fact that diabetic hyperglycaemic rats do not express SREBP-1c suggests that, at least in the liver, insulin rather than glucose is the primary effector of SREBP-1c expression [15].

**SREBP-1c expression in the liver is regulated positively by insulin and negatively by glucagon**

The decisive argument for the involvement of SREBP-1c in insulin signalling came from studies on primary cultured hepatocytes in which we demonstrated that insulin was able to strongly activate SREBP-1c expression at concentrations compatible with an effect through the insulin receptor [13]. This effect was clearly transcriptional, as shown by run-on assays. Glucagon, which antagonizes the action of insulin on many metabolic hepatic processes, opposes the effects of insulin on SREBP-1c expression via its second messenger cAMP [13].

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**SREBP-1c is the mediator of insulin action on hepatic glycolytic and lipogenic gene expression**

The next step was to assess whether SREBP-1c is indeed involved in the effects of insulin on hepatic gene expression. We used a dominant-negative form of SREBP-1c consisting of the N-terminal fragment of SREBP-1c (residues 1–403), which is readily directed to the nucleus but contains an alanine mutation at residue 320 [17]. This mutation abolishes the binding of SREBP-1c to both SREs and E-boxes but still permits dimerization, leading to a decreased availability of endogenous SREBP-1c. This dominant-negative form was cloned into an adenovirus vector, which has the advantage of allowing the transfection of more than 90% of primary hepatocytes in culture with a high expression of the transgene. Overexpression of this dominant-negative form of SREBP-1c in hepatocytes counteracts the stimulatory effect of
insulin on GK expression [18]. Conversely, overexpression of a dominant-positive form of SREBP-1c corresponding to the mature nuclear form of SREBP-1c induces GK expression in the absence of insulin [18].

Similar results were obtained for genes that are dependent on high concentrations of both glucose and insulin, namely the genes encoding L-PK, FAS, ACC and S14 [13,18]. The importance of SREBP-1c for the expression of this class of genes was also demonstrated in vivo by showing that their induction by a high-carbohydrate diet was precluded in SREBP-1 knock-out mice [19]. However, one could argue that the effect of SREBP-1c on these genes is indirect. Indeed, because they are dependent on glucose metabolism and thus on the presence of an active GK for their full expression, the effect of SREBP-1c could be secondary to the induction of GK. Several arguments suggest that this is not so. First, the stimulatory effect of SREBP-1c could be observed in the absence of any change in the concentration of glucose 6-phosphate, the product of GK activity [18]. Secondly, several studies have shown a direct effect of SREBP-1c on the promoter of these genes [11,17]. It was also reported recently that glucose and insulin were acting on the promoter of FAS and S14 through distinct DNA response elements and that SREBP-1c was accounting for the effect of insulin [20]. Finally, we have recently been able to link the different lipogenic capacities of liver and adipose tissue in various species to the expression of SREBP-1c [21]. Because adipose tissue lacks GK activity, this is a strong argument for a direct effect of SREBP-1c on the expression of lipogenic enzyme genes.

Thus, in summary, SREBP-1c could account for the positive effect of insulin observed on glycolytic and lipogenic gene expression [22].

How does insulin stimulate SREBP-1c transcriptional activity?

As described above, insulin stimulates the transcription of SREBP-1c, leading ultimately to an increased content of the nuclear mature form [13,14]. As shown by studies with inhibitors of various branches of the insulin signalling pathway, the effect of insulin on SREBP-1c expression and synthesis in primary hepatocytes involves mainly the phosphoinositide 3-kinase pathway [14]. Moreover, acute activation of protein kinase B/Akt (PKB/Akt) is sufficient to induce the accumulation of SREBP-1c mRNA in primary hepatocytes [23]. This effect could be mediated through insulin receptor substrate 1 because animal models with a decreased expression of insulin receptor substrate 2 are still able to express SREBP-1c strongly [24]. SREBP-1c itself does not seem to be involved in the stimulation of its own expression by insulin, because in mice in which the wild-type SREBP-1c gene has been replaced by a gene encoding a non-functional form of SREBP-1c without modifying the promoter sequence, the SREBP-1c promoter still responds to carbohydrate refeeding [19].

A number of arguments suggest that insulin could also stimulate the mature form of SREBP-1c. When the mature form of SREBP-1c is overexpressed in 3T3-L1 adipocytes, its transcriptional activity is further enhanced by insulin [11]. It has been suggested that in cell lines this mechanism involves a phosphorylation process by mitogen-activated protein (MAP) kinase [25], although in primary cultured hepatocytes inhibitors of the MAP kinase pathway do not antagonize the effect of insulin on target gene expression [14,26]. However, this does not exclude the possibility that insulin can affect SREBP-1c transcriptional activity through pathways that do not involve MAP kinase, such as the phosphoinositide 3-kinase/PKB/Akt pathway.

SREBP-1c and glucose homeostasis

The liver has a central role in glucose homeostasis. It can produce glucose from glycogen stores or through the gluconeogenic pathway when glucose is not provided by the diet. It is also the first organ to handle glucose arising from intestinal absorption through the portal vein for glycogen storage and lipid synthesis. Hepatic GK has a key role in glucose metabolism, as underlined by the anomalies associated with GK mutations and by the consequences of tissue-specific knock-out [27]. One could then expect that a decreased SREBP-1c activity should lead to defects in glucose homeostasis. Conversely, a forced expression of SREBP-1c in the liver could bypass the need for a normal insulin concentration. In recent studies we have analysed the consequences of overexpressing SREBP-1c in the liver of hyperglycaemic diabetic mice by using adenoviral transduction. There was a remarkably rapid and effective hypoglycaemic effect of SREBP-1c expression in this animal model, suggesting that SREBP-1c does indeed have a pivotal role in glucose metabolism that might involve not only increased hepatic utilization of glucose but also decreased glucose pro-

Further questions
At this stage, several crucial questions remain unanswered.

1. The mechanisms by which insulin increases SREBP-1c transcription or transcriptional activity are not understood.

2. Insulin also has repressive effects on gene transcription, for instance on the gene encoding PEPCK. Could SREBP-1c be also involved in this phenomenon? Insulin-dependent GK gene induction and PEPCK gene repression follow similar kinetics; in both cases, PKB/Akt could be involved [26]. However, a direct effect of SREBP-1c expression remains to be demonstrated.

3. In addition to liver and adipose tissue, muscles are also responsive to insulin. SREBP-1c is expressed in this tissue. However, because muscle is not a lipogenic tissue, a potential role of SREBP-1c in this tissue is unclear in that the regulation of its expression in muscle has not been studied (is it also sensitive to insulin?). It has been suggested that the first part of the lipogenic pathway, namely as far as the ACC step, could be important in muscles owing to the generation of malonyl-CoA, an inhibitor of fatty acid oxidation [28]. Increased glucose availability would then decrease fatty acid oxidation in oxidative muscles by increasing the concentration of malonyl-CoA. In the liver, ACC expression is clearly regulated by SREBP-1c. However, it is not known whether, in muscles, ACC, which is encoded by a different gene from the liver isoform, could also respond on a long-term basis to the nutritional environment through SREBP-1c activation. If this is so, then SREBP-1c could be involved in the modulation of the relative utilization of glucose and fatty acids.

4. The pancreatic β-cell is another interesting tissue in this respect. Indeed, on the one hand, the generation of malonyl-CoA after a glucose stimulus has been implicated as one of the mechanisms allowing a full response of insulin secretion through the accumulation of long-chain fatty-acyl-CoA esters [29]. On the other hand, abnormal accumulation of lipids in the β-cells of diabetic Zucker (fa/fa) rats through an enhanced expression of lipogenic enzymes seems to be deleterious to insulin secretion capacity (phenomenon of lipotoxicity) and an enhanced expression of SREBP-1c has been implicated in this situation [30]. Clearly, the role of SREBP-1c and the regulation of its expression in the β-cell (by insulin?) are important issues in understanding some of the anomalies of insulin secretion.

In conclusion, SREBP-1c, which was first related to the regulation of cholesterol metabolism, is now clearly seen as having a pivotal role in glucose and lipid metabolism and in the action of insulin. This suggests that SREBP-1c itself or regulatory factors might be involved in pathologies such as type 2 diabetes, obesity and more generally insulin resistance syndromes.

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References
Abstract

Insulin regulates the expression of more than 150 genes, indicating that this is a major action of this hormone. At least eight distinct consensus insulin response sequence (IRSs) have been defined through which insulin can regulate gene transcription. These include the serum response element, the activator protein 1 ('AP-1') motif, the Ets motif, the E-box motif and the thyroid transcription factor 2 ('TTF-2') motif. All of these IRSs mediate stimulatory effects of insulin on gene transcription. In contrast, an element with the consensus sequence T(G/A)TTT(T/G)-(G/T), which we refer to as the phosphoenolpyruvate carboxykinase (PEPCK)-like motif, mediates the inhibitory effect of insulin on transcription of the genes encoding PEPCK, insulin-like-growth-factor-binding protein 1 (IGFBP-1), tyrosine aminotransferase and the glucose-6-phosphatase (G6Pase) catalytic subunit. The forkhead transcription factor FKHR has recently been shown to bind this PEPCK-like IRS motif and a model has been proposed in which insulin inhibits gene transcription by stimulating the phosphorylation and nuclear export of FKHR. Our results suggest that this model is consistent with the action of insulin on transcription of the gene encoding IGFBP-1 but not that of the G6Pase catalytic subunit. Thus, even though the IRSs in both promoters seem identical, they are functionally distinct. In addition, in the G6Pase catalytic subunit promoter, hepatocyte nuclear factor 1 ('HNF-1'), acts as an accessory factor to enhance the effect of insulin mediated through the IRS.

Introduction

Insulin regulates metabolism by altering the quantities of critical proteins and by altering the activity or location of pre-existing enzyme molecules. The mechanisms through which insulin mediates the latter effects have been studied extensively for many years [1–3]. Similarly, over the past 10 years there have also been considerable advances in the understanding of several aspects of insulin-regulated gene expression [4,5].

Insulin has been shown to regulate the expression of specific genes by affecting transcription, mRNA stability or mRNA translation [4,5]. Little is known about the regulation of mRNA