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Antagonistic Effects of Insulin Signaling and Glucagon Signaling on Controlling Hepatic Gluconeogenic Gene Expression

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Abstract

With the worldwide epidemics of obesity and diabetes, there is an urgent need for action at the global and national levels to prevent and to treat these metabolic disorders. Inappropriate hepatic glucose production is the major cause of hyperglycemia in obese and diabetic patients. In this mini-review, we summarize the antagonistic effects of insulin signaling and glucagon signaling on controlling gene expression related to hepatic glucose production through CREB co-activators and FOXO1. In the fasted state, phosphorylation of CREB at S133 recruits CBP/P300 and CRTC2 to CREB, leading to the formation of CREB co-activators complex. CREB and P300 also up-regulate FOXO1 gene expression. CREB co-activators together with FOXO1 drive gluconeogenic gene expression to maintain euglycemia. In the fed state, insulin suppresses gluconeogenic gene expression through the phosphorylation of CBP, which results in the disassembly of CREB-CBP-CRTC2 complex, furthermore, phosphorylation of CRTC2 and FOXO1 by insulin excludes CRTC2 and FOXO1 from nucleus and promotes their degradation in cytoplasm.

Keywords: Glucagon; Gene expression; Metabolic disorder

The Epidemics of Obesity and Diabetes Cause Serious Health Problems

Over the past three decades, the number of children with obesity has more than doubled, and children obese rate at age of 6-11 years has increased from 7% in 1980 to 18% in 2010 in the United States, accounting for one third of all children [1,2]. The World Health Organization estimates that nearly 43 million children under the age of five are obese worldwide [3].

The obesity epidemic, over nutrition and increasing sedentary lifestyles among young people are the major contributors to the increase in type 2 diabetes among children and adolescences. Currently, 215,000 individuals under the age of 20 in the United States have diabetes [4]. Data from the International Diabetes Federation show that diabetes affects at least 382 million individuals worldwide [5]. Hyperglycemia is the hallmark metabolic abnormality of diabetes and is the major health concerns in those with type 2 diabetes mellitus patients. Elevated glucose levels lead to the severe adverse effect of non-enzymatic glycosylation of many cellular proteins that causes them to function improperly or lose function completely [6,7]. These molecular effects are often seen in diabetes patients evident by microvascular tissue damage to the kidney, retina and nerves, which lead to the commonly observed outcomes of end-stage renal failure, cardiovascular disease, loss of visual acuity, and loss of extremities through amputation [8].

Thus, the prime clinical goal in the treatment of type 2 diabetes mellitus patients is to maintain blood glucose levels as close to the normal blood glucose levels as possible so as to reduce the occurrence of these complications.

Opposing Actions of Insulin Signaling and Glucagon Signaling Maintains Normal Blood Glucose Levels

Blood glucose levels are tightly regulated by the opposing actions of insulin signaling and glucagon signaling pathways. In the fed and postprandial states, glucose is absorbed in the gastrointestinal tract and enters the blood circulation. The elevation in blood glucose levels triggers the secretion of insulin from pancreatic β cells. This, in turn, stimulates glucose utilization in peripheral tissues such as muscle and adipose tissue, and suppresses hepatic glucose production [9].

Eventually, blood glucose levels return to the normal defined range due to insulin signaling. In the fasting state, blood glucose levels drop, and glucagon is secreted from α cells of the pancreas. During early fasting, glucagon stimulates hepatic glycogenolysis, in which stored glycogen in the liver is broken down into glucose and released into the bloodstream to maintain euglycemia.

Moreover, glucagon-stimulated hepatic gluconeogenesis plays a dominant role in maintaining euglycemia during prolonged fasting [10]. The maintenance of normal blood glucose levels (70 -110 mg/dL) is essential in the protection against hypoglycemia during fasting, because hypoglycemia is detrimental to the health of an organism due to the fact that glucose is the main and only energy source for neurons and erythrocytes [11].

Glucagon Stimulates Hepatic Gluconeogenic Gene Expression in the Fasted State

In the fasted state, glucagon stimulates hepatic glucose production through the cAMP-PKA (protein kinase A) signaling pathway (Figure 1).

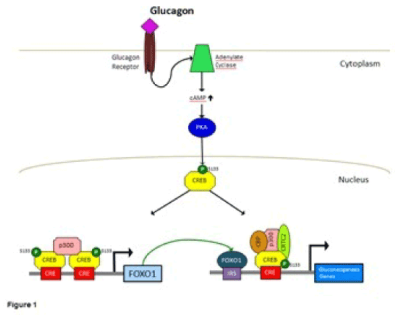

Figure 1: Glucagon Signaling Stimulates Gluconeogenesis in the Liver.

Phosphorylation of CREB at Ser133 leads to the recruitment CBP, P300 and CRTC2 to CREB and the formation of CREB co-activator complex. CREB and P300 also drive Foxo1 gene expression; FOXO1 binds to insulin responsive sequence (IRS) on gluconeogenic gene to fully activate the gluconeogenic program.

Activated PKA by glucagon phosphorylates CREB at Ser133. This event recruits the formation of the CREB (cAMP response-element-binding protein) co-activators complex and initiates the transcription of gluconeogenic genes containing CRE sites (cAMP response elements), including G6pc (glucose-6-phosphatase) and Pck1 (phosphoenolpyruvate carboxykinase1). In addition to PKA mediated CREB phosphorylation, PKA also simulates the dephosphorylation of CRTC2 (CREB regulator transcription coactivator 2) and CBP(CREB binding protein) [12,13]. The salt-inducible kinases (SIKs), especially SIK2, play a critical role in regulating CRTC2 activity. Phosphorylation of CRTC2 by SIK2 excludes CRTC2 from nucleus. However, activated PKA phosphorylates SIK2 at Ser587 and suppresses SIK2 activity, therefore negates its inhibition on CRTC2 [14,15]. Hence, CRTC2 re-localizes into the nucleus. Furthermore, dephosphorylation of CBP also leads to its association with CREB [13,16]. Together, these events result in the formation of the CREB co-activator complex to promote gluconeogenic gene expression and hepatic glucose production.

Recently, we found that fasting led to the marked increase of Foxo1 mRNA andFOXO1 protein levels in the liver [11]. A non-hydrolyzeable cAMP analog-dibutyrlcAMP (Bt-cAMP) activated the cAMP-PKA signaling pathway and also increased Foxo1gene expression, suggesting that the cAMP-PKA pathway may up-regulate Foxo1 gene expression. Using adenoviral shRNAs to deplete co-activators in hepatocytes, we found that depletion of P300 blocked the induction of FOXO1 by Bt-cAMP. Moreover, depletion of CREB abolished the induction of Foxo1 mRNA levels by Bt-cAMP. These data demonstrate that Foxo1 gene expression stimulated by Bt-cAMP is mediated byCREB and P300 [11]. After characterization of the Foxo1 gene promoter, we found thatFoxo1 gene expression is driven by CREB and P300, which bind to tandem CRE sites in the proximal promoter region of Foxo1 gene. In addition, inhibition of P300 histoneacetyl transferase activity decreased hepatic FOXO1 protein levels as well as blood glucose levels. Since FOXO1 also up-regulates gluconeogenic expression by binding to insulin response sequences located in the promoters of G6pc and Pck1, the induction of the Foxo1 gene by cAMP-PKA, thus, fully activates the gluconeogenic program and maintains euglycemia in the fasted state [11].

Suppression of Hepatic Gluconeogenic Gene Expression by Insulin Signaling in the Fed State

Insulin signaling is crucial for the suppression of glucose production in the liver. Mice with liver specific insulin receptor knockout exhibited marked increase of hepaticglucose production and extreme hyperglycemia [17]. In the fed and postprandial states, the suppression of hepatic glucose production by insulin is complicated and involves many transcription factors and signaling mediators that have been reported. The gluconeogenic engine, CREB co-activator complex, first needs to be turned off to reduce endogenous glucose production. When assembled, the CREB co-activators complex upregulates the transcription of hepatic gluconeogenic related genes, such as G6pc and Pck1, and increases hepatic glucose production. To sufficiently control the blood glucose levels in the fed and postprandial states, several mechanisms have been proposed. We have proposed that phosphorylation of CBP at Ser436 by insulin leads to the disassembly of CREB co-activator complex [13]. This phosphorylation event is mediated by aPKC$d\lambda$ (atypical protein kinase C), which is activated by insulin through the PI3K-PDK1 pathway (Figure 2).

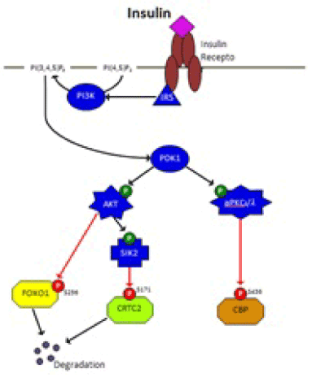

Figure 2: Insulin Signaling Inhibits Glucose Production in the Liver. Insulin mediated phosphorylation of CBP at Ser436 via a PKC disassembles CREB co-activator complex. Phosphorylation of FOXO1 and CRTC2 results in the nuclear exclusion, and degradation of these factors in cytoplasm.

Importantly, a mouse model with a germline-mutation of this CBP phosphorylation site (S436A) exhibits inappropriate activation of gluconeogenesis [16,18]. Interestingly, P300, a closely related protein of CBP, does not have this phosphorylation site. Thus, P300 constitutively maintains basal gluconeogenesis in the liver [16]. In a phosphorylation-competent p300G442S knock-in mouse model that harbors a reconstructed phosphorylation site found in CBP, we found this mutant mouse model displayed hypersensitivity to insulin [19]. These data substantiate the importance of CBP phosphorylation in mediating the suppression of hepatic glucose production by insulin. Second, phosphorylated AKT by insulin through the PI3K-AKT pathway results in the activation of SIK2, which in turn mediates the phosphorylation of CRTC2 at Ser171. Phosphorylated CRTC2 is then excluded from the nucleus and degraded in the cytoplasm [12]. Third, another mechanism relates to the phosphorylation of FOXO1 by insulin. In the absence of insulin, FOXO1 factor is localized within the nucleus. When the PI3K–AKT pathway is activated by insulin, AKT mediates the phosphorylation of FOXO1, and this phosphorylation

event triggers the export of FOXO1 from nucleus to the cytoplasm and promotes its ubiquitinylation and degradation [20,21].

Perspective

Insulin and glucagon actions function in concert at the molecular level to maintain the blood glucose levels in a defined normal range. However, diabetic patients often have elevated serum glucagon levels, which should stimulate gluconeogenic gene expression and excessive production of glucose in the liver. In addition, the impairment of insulin signaling due to insulin resistance in diabetic patients weakens the insulin-mediated suppression of gluconeogenic gene expression, suppression of glucose production in the liver, and uptake of glucose in peripheral tissues. Together, these effects result in the development of hyperglycemia. The elucidation of the signaling cascade and transcriptional activity in both the glucagon and insulin pathways will provide us with valuable information to identify and create more efficient and robust therapeutic compounds to better combat obesity and type 2 diabetes mellitus in children and adolescents. For example, the inhibition of P300 histone acetyltransferase activity may be a target for the treatment of diabetes because inhibition of P300 histone acetyltransferase activity decreases fasting blood glucose levels [11,22].

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