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RESEARCH HIGHLIGHT

InsP3R-Ca^{2+} signaling takes center stage in the hormonal regulation of hepatic gluconeogenesis

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During fasting, dephosphorylation-dependent activation of the CREB coactivator CRTC2 by glucagon is crucial for activation of the hepatic gluconeogenic program, but the molecular mechanism by which hormones regulate CRTC2 activation remains unclear. A recent report in *Nature* showed that PKA-dependent phosphorylation of the inositol-1,4,5-trisphosphate receptor (InsP3R) induces Ca^{2+} mobilization, leading to increase in the phosphatase activity of calcineurin and the subsequent dephosphorylation of CRTC2, thereby resulting in the induction of gluconeogenic gene expression. It also showed that insulin-dependent phosphorylation of InsP3R by Akt inhibits Ca^{2+} mobilization and CRTC2 dephosphorylation, resulting in the suppression of gluconeogenesis.

The liver is a central organ responsible for maintaining glucose homeostasis under conditions of a fluctuating food supply through the counter-regulatory actions of the pancreatic hormones glucagon (released during fasting) and insulin (released during feeding). During the initial stages of fasting in the liver, glucagon promotes glycogen breakdown to produce glucose (glycogenolysis). After hepatic glycogen is exhausted by prolonged fasting, the *de novo* synthesis of glucose from non-carbohydrate substrates, i.e., gluconeogenesis, becomes a major contributor to hepatic glucose production by increasing the transcription of gluconeogenic enzymes such as glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) [1, 2]. When nutrients are available after a meal, insulin suppresses glycogenolysis and gluconeogenesis in the liver and initiates glucose uptake in the peripheral tissues, maintaining euglycemia while promoting anabolic reactions and energy storage. Impairment of this hormonal regulation, such as excessive glucagon signaling and insulin resistance, is thought to play a causal role in hyperglycemia in type 2 diabetes [2].

In the fasting liver, glucagon stimulates gene transcription of gluconeogenic enzymes through a signaling pathway involving protein kinase A (PKA), the transcription factor CREB, the CREB coactivator CRTC2 (CREB-regulated transcription coactivator 2), and the CRTC2 kinase SIK2 (salt-inducible kinase 2) [3] (Figure 1). The activation of PKA by glucagon/cyclic AMP (cAMP) induces gluconeogenic genes via dephosphorylation and subsequent nuclear translocation of CRTC2. In the nucleus, CRTC2 coactivates CREB to induce the gluconeogenic coactivator PGC-1α (peroxisome proliferator-activated receptor γ coactivator-1 α), which in turn interacts with the transcription factors HNF-4α (hepatocyte nuclear factor-4 α) and FoxO (forkhead box O) 1, 3a, and 4 [4] to activate the gluconeogenic program. CRTC2 dephosphorylation, a critical step for gluconeogenesis, is promoted in part by PKA-mediated inactivation of SIK2 [3] or by activation of calcium/calmodulin-dependent Ser/Thr phosphatase calcineurin [5]. However, the precise mechanisms by which hormonal and nutritional cues regulate CRTC2 phosphorylation have not been fully elucidated.

Previous studies have suggested that changes in intracellular Ca^{2+} ($[Ca^{2+}]i$) play a role in the regulation of gluconeogenesis (reviewed by Kraus-Friedmann and Feng [6]). This is evidenced by the observations that glucagon and cAMP can increase $[Ca^{2+}]i$, and increased $[Ca^{2+}]i$ promotes gluconeogenesis [7], while $[Ca^{2+}]i$ chelation blocks it [8]. Although these studies highlight the interesting interplay between cAMP signaling and Ca^{2+} signaling in the regulation of gluconeogenesis, the underlying molecular mechanisms remain unclear.

A recent study published in *Nature* [9] has unveiled this mechanism. Using genetic and biochemical approaches, Wang and colleagues showed that glucagon promotes CRTC2 dephospho-

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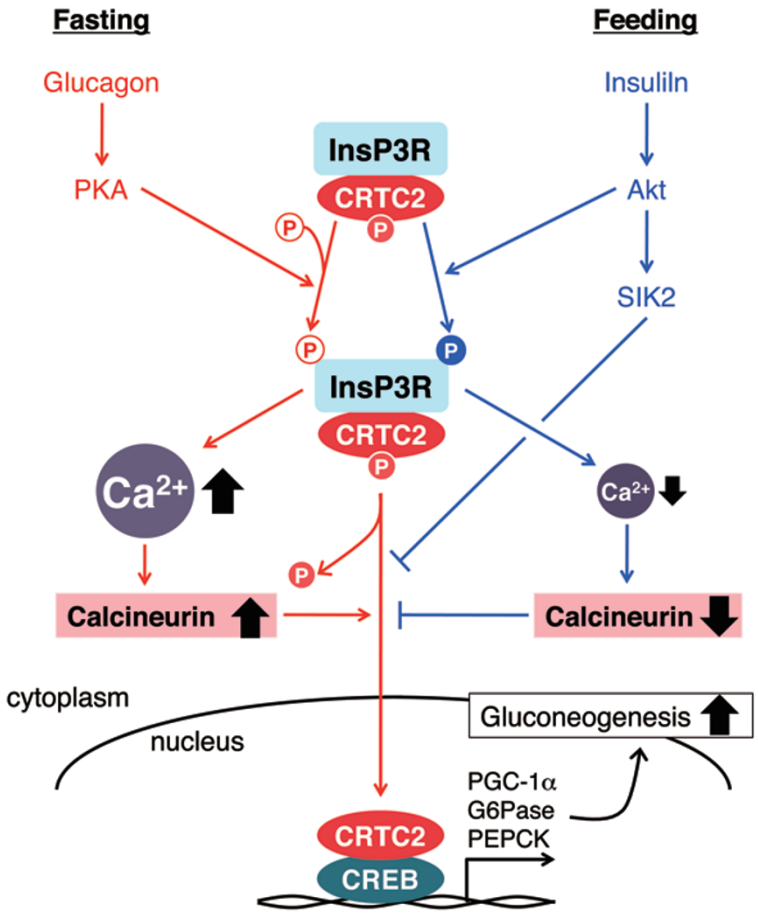

Figure 1 Opposing signaling pathways triggered by glucagon and insulin converge on InsP3Rs and thereby regulate CRTC2-dependent gluconeogenesis in a Ca^{2+}- and calcineurin-dependent manner. During fasting, glucagon activates InsP3Rs via PKA-dependent phosphorylation, leading to the activation of calcineurin and subsequent dephosphorylation of CRTC2, thereby resulting in the induction of gluconeogenic genes. Under feeding conditions, insulin inactivates InsP3Rs via Akt-dependent phosphorylation, leading to the inhibition of calcineurin-dependent dephosphorylation of CRTC2.

rylation in hepatocytes in mice during fasting by triggering an increase in $[Ca^{2+}]i$ that activates calcineurin, leading to the induction of gluconeogenic genes. Glucagon increases $[Ca^{2+}]i$ through the activation of inositol-1,4,5-trisphosphate receptor (InsP3R) Ca^{2+} channels by PKA-dependent phosphorylation (Figure 1). In co-immunoprecipitation analyses in hepatocytes, InsP3R1, one of three InsP3R isoforms, was predominantly associated with the phosphorylated inactive form of CRTC2, while this association was disrupted by glucagon. These findings strongly support the Ca^{2+}-mediated regulation of CRTC2 activity as follows: fasting glucagon→cAMP/PKA→InsP3R→$[Ca^{2+}]i$→calcineurin →nuclear CRTC2 (Figure 1).

After a meal, insulin suppresses hepatic gluconeogenesis in part via phosphorylation of CRTC2 [3]. The authors next investigated whether insulin affects InsP3R-mediated activation of CRTC2 in hepatocytes. They found that insulin induces Akt-dependent serine phosphorylation of Ins3Rs, attenuates glucagon-induced $[Ca^{2+}]i$ mobilization and calcineurin activation, and suppresses glucose output, while these suppressive effects of insulin are completely abolished in hepatocytes overexpressing the mutant form of InsP3R lacking Akt-dependent phosphorylation. Collectively, these results show that Ca^{2+} signaling through InsP3R is critical to the regulation of CRTC2 activity by both glucagon and insulin in response to the feeding conditions (Figure 1). A recent study by Ozcan *et al*. [10] showed that a $[Ca^{2+}]i$-sensing enzyme, CaMKII (calcium/calmodulin-dependent kinase II), is activated in a $[Ca^{2+}]i$- and InsP3R-dependent manner by glucagon in hepatocytes and *in vivo*. CaMKII appears to promote the phosphorylation and nuclear translocation of FoxO1, resulting in the activation of the gluconeogenic program. As shown previously in this lab [11], CRTC2 and FoxO1 are sequentially activated via activator/coactivator exchange during fasting. CRTC2 becomes active during the initial period of fasting, while FoxO1 is activated during prolonged fasting. This functional relay downstream of InsP3R signaling is important to ensure that the gluconeogenic program remains active during the entire fasting period. Although the relative contribution of the two pathways mediated by the gluconeogenic transcriptional regulators CRTC2 and FoxO1 downstream of InsP3Rs is unknown, the activation of both pathways during fasting might warrant a continuous glucose supply to prevent hypoglycemia.

Unrestrained hepatic gluconeogenesis in type 2 diabetes is accounted for excessive glucagon signaling and insulin resistance. Wang and colleagues investigated whether hepatic InsP3R signaling contributes to increases in gluconeogenesis in the setting of diabetes. Both InsP3R and calcineurin activities were upregulated in the liver of obese diabetic mice, leading to the upregulation of the gluconeogenic program. The depletion of InsP3R or calcineurin in the liver by RNA interference improved

diabetes concomitant with the downregulation of the gluconeogenic program. These *in vivo* data suggest that InsP3R is critically involved in increased gluconeogenesis and hyperglycemia in the setting of insulin resistance.

Wang *et al*. have not only unveiled the physiological role of InsP3R-[Ca^{2+}]i signaling in the hormonal regulation of gluconeogenesis but may also have created the possibility of using this signaling as a therapeutic target for the deterioration of hepatic glucose metabolism in the setting of insulin resistance. Since hepatic lipid contents, lipogenic gene expression, and insulin signaling were not observed in this study, it is not clear whether hepatic downregulation of InsP3R or calcineurin in the diabetic liver ameliorates hepatosteatosis and improves hepatic insulin signaling. Previous studies have reported that in the liver of diabetic *ob*/*ob* mice, disruption of [Ca^{2+}]i homeostasis is key to the induction of endoplasmic reticulum stress and insulin resistance [12], suggesting the possibility that the restoration of Ca^{2+} homeostasis improves not only unrestrained hepatic gluconeogenesis but also insulin signaling and hepatosteatosis. Future studies are required to correctly evaluate its potential as a therapeutic target for these disease states.

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