A New Activator of Hepatocyte CaMKII in Fasting and Type 2 Diabetes

Lale Ozcan and Ira Tabas

Diabetes 2018;67:1742–1744 | https://doi.org/10.2337/dbi18-0026

COMMENTARY

Positive regulation of hepatic glucose production (HGP) and stimulation of glycogenolysis during fasting are critical processes to prevent hypoglycemia. A key hormone that carries out this function is glucagon, which is secreted by pancreatic α-cells in response to fasting and acts on its receptor on hepatocytes to promote HGP and glycogenolysis (1). However, as often happens, processes that are critical in physiology can promote disease processes in nonphysiological settings. As such, glucagon-induced HGP is a major contributor to excessive HGP and hyperglycemia in obesity and type 2 diabetes (T2D) (2). In this issue of *Diabetes*, Wang et al. (3) provide evidence that another circulating factor that is increased in fasting and obesity/T2D—prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)—also promotes HGP in both fasting and obese, insulin-resistant mice.

Previous work has elucidated a number of pathways through which activation of the hepatocyte glucagon receptor (GcgR), which is a G-protein–coupled receptor (GPCR), promotes HGP and glycogenolysis in both fasting and obesity. Specifically, adenylyl cyclase/cyclic AMP-mediated protein kinase A (PKA) activation by G proteins of the GcgR controls the transcriptional regulation of HGP and glycogenolysis through at least three distinct but complementary processes (Fig. 1): phosphorylation of CREB, which results in recruitment of its coactivators, CBP and p300; deactivation of a kinase called SIK2; and activation of inositol 3-phosphate receptors (IP3Rs), leading to release of endoplasmic reticulum calcium into the cytosol and subsequent activation of the phosphatase calcineurin and the kinase Ca^{2+}/calmodulin-activated protein kinase IIγ (CaMKIIγ) (4,5). Deactivation of SIK2 and activation of calcineurin promote nuclear translocation of the transcription factor CRTC2, which is a CREB coactivator that induces the expression of a key HGP-promoting transcription factor, PGC-1α (6). Activation of CaMKIIγ activates the kinase P38α, which phosphorylates the transcription factor FoxO1 on specific serine and threonine, leading to FoxO1 nuclear translocation (5). Nuclear FoxO1, in concert with PGC-1α, induces key genes involved in HGP and glycogenolysis, notably *G6pc* and *Pck1* (7). During feeding, insulin-mediated activation of the kinase AKT promotes nuclear exclusion of CRTC2 and FoxO1 and thereby appropriately dampens HGP and glycogenolysis (7,8). Moreover, in the context that glucagon action on hepatocytes is elevated in obesity and T2D, both the CRTC2 and FoxO1 pathways are activated in obesity/T2D and contribute substantially to pathological hyperglycemia in this setting (4,5).

The findings of Wang et al. (3) suggest that another GPCR pathway in hepatocytes also contributes to protective HGP during fasting and pathologically excessive HGP in obesity/T2D. Previous work had shown that $PGF_{2\alpha}$, which is formed from arachidonic acid via cyclooxygenase, is elevated in hepatocytes of both fasting and obese, insulin-resistant mice and that a $PGF_{2\alpha}$ metabolite in the urine is increased in T2D patients (9,10). Interestingly, aberrant production of another cyclooxygenase product, PGI2, in fasting and insulin resistance was shown to enhance HGP by activating the cAMP/PKA/CREB pathway and to block insulin-mediated Akt phosphorylation by increasing the Akt inhibitor Tribbles homolog 3 (Trb3) (9). Using cultured hepatocytes and fasting and obese mice, Wang et al. (3) now show that interaction of $PGF_{2\alpha}$ with its receptor (F-prostanoid [FP] receptor) on hepatocytes activates the same CaMKIIγ/P38/FoxO1 pathway elucidated previously for glucagon/GcgR (Fig. 1). Based on inhibitor experiments in cultured hepatocytes, the authors suggest that the proximal step of increasing cytosolic calcium is mediated by activation of phospholipase C. Glucose response to insulin was also improved in both fasting lean mice and obese mice that lacked the FP receptor in hepatocytes. Although the mechanism of insulin sensitization in mice lacking the hepatocyte FP receptor was not addressed, it may involve enhanced insulin-mediated Akt activation in hepatocytes through

Department of Medicine, Columbia University Irving Medical Center, New York, NY
Corresponding author: Ira Tabas, iat1@columbia.edu.

See accompanying article, p. 1748.

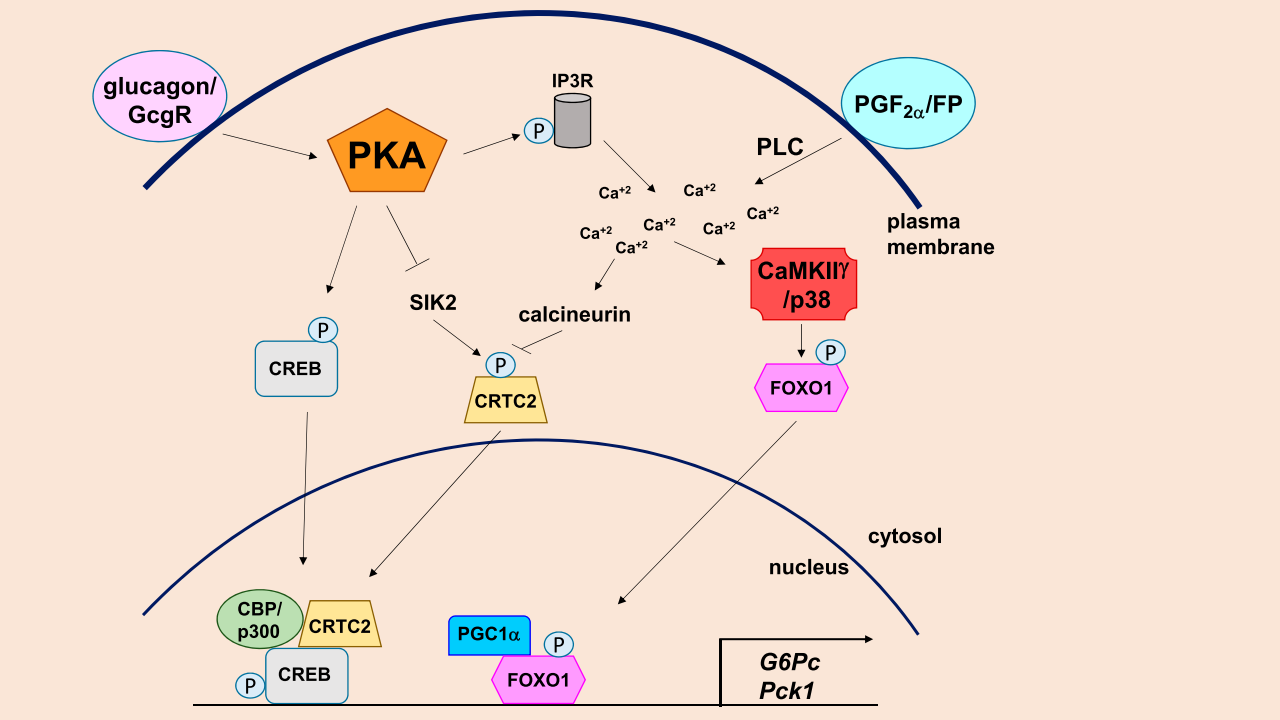

Figure 1—Pathways through which glucagon, acting through GcgR, and $PGF_{2\alpha}$, acting through FP, increase the expression of genes involved in HGP, notably *G6pc* and *Pck1*. CBP, CREB-binding protein; P, phosphate; PLC, phospholipase C.

suppression of a previously described CaMKII/P38/Trb3 pathway (11).

The key question to emerge from this study is how the $PGF_{2\alpha}$/FP receptor pathway is integrated with the glucagon/GcgR pathway in both physiology (fasting) and pathophysiology (T2D). The pathways may directly interact with each other, e.g., activation of GcgR may affect FP receptor signaling and/or vice versa; they may be separate but complementary; or they may be redundant. As a first step, this question could be addressed by assaying GcgR and FP receptor activity and their common downstream pathway in hepatocytes incubated with each hormone alone versus both together. If the two pathways turn out to have complementary roles, it would be interesting to determine if they affect different stages of fasting, e.g., glycogenolysis in the earliest stage followed by HGP in later stages. In this context, inhibition of CaMKIIγ/p38 or deletion of hepatic FoxO1 regulates both glycogenolysis and gluconeogenesis (5,12), whereas the study by Wang et al. (3) reported that FP receptor signaling regulates gluconeogenic gene expression without affecting hepatic glycogen levels. Given that the mRNA of the glycogenolytic enzyme glucose-6-phosphatase was lower in FP-deficient versus control mice, it is possible that one or more other downstream mediators of the $PGF_{2\alpha}$/FP pathway overrides *G6Pc*–mediated glycogenolysis during fasting. Finally, there are other pathways that boost HGP in fasting, e.g., white adipose tissue–derived asprosin, which also activates PKA in hepatocytes (13). Therefore, elucidating the specific roles and integration of these multiple activators of HGP in fasting and T2D represents an important future goal in this area of research.

What might be the translational implications of the study by Wang et al. (3)? Although treatment of T2D subjects with high-dose aspirin, which inhibits cyclooxygenase and prostaglandin synthesis, was shown to improve fasting and postprandial hyperglycemia, the beneficial effect was attributed to inhibition of IKKβ activity as opposed to an effect on cyclooxygenase (14). In another arena, there have been several phase 2 clinical trials testing glucagon receptor antagonists in T2D, and although these drugs very effectively improve hyperglycemia, their use has been stymied by elevations of plasma LDL cholesterol (15). However, studies in diabetic mice have suggested that targeting mediators downstream of the GcgR may capture the benefits of GcgR blockade while avoiding the LDL-raising effect. For example, genetic silencing or drug-mediated inhibition of CaMKII or the P38 effector MAPKAPK2 (MK2) lowers blood glucose and improves insulin sensitivity without increasing plasma cholesterol in obese mice (11,16). Whether or not FP receptor blockade would be as efficacious but safer than GcgR blockade remains to be seen. Finally, the leading cause of death associated with T2D is cardiovascular disease. In this context, studies in mice have shown that silencing of the FP receptor decreases atherosclerosis and protects against lipopolysaccharide-induced tachycardia (17,18), and silencing of CaMKII or MK2 suppresses atherosclerosis and heart failure (19–21).

Thus, leveraging the knowledge of these pathways may suggest an integrated therapeutic approach to cardiometabolic disease.

Duality of Interest. L.O. is a scientific advisor and I.T. is a co-founder of Tabomedex Biosciences, Inc., which is developing allosteric MK2 inhibitors as a treatment for T2D. No other potential conflicts of interest relevant to this article were reported.

References

1. Ramnanan CJ, Edgerton DS, Kraft G, Cherrington AD. Physiologic action of glucagon on liver glucose metabolism. Diabetes Obes Metab 2011;13(Suppl. 1):118–125

2. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. J Clin Invest 2012;122:4–12

3. Wang Y, Yan S, Xiao B, et al. Prostaglandin $F_{2\alpha}$ facilitates hepatic glucose production through CaMKIIγ/p38/FOXO1 signaling pathway in fasting and obesity. Diabetes 2018;67:1748–1760

4. Wang Y, Li G, Goode J, et al. Inositol-1,4,5-trisphosphate receptor regulates hepatic gluconeogenesis in fasting and diabetes. Nature 2012;485:128–132

5. Ozcan L, Wong CCL, Li G, et al. Calcium signaling through CaMKII regulates hepatic glucose production in fasting and obesity. Cell Metab 2012;15:739–751

6. Koo SH, Flechner L, Qi L, et al. The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature 2005;437:1109–1111

7. Lin HV, Accili D. Hormonal regulation of hepatic glucose production in health and disease. Cell Metab 2011;14:9–19

8. Dentin R, Liu Y, Koo SH, et al. Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. Nature 2007;449:366–369 PubMed

9. Yan S, Zhang Q, Zhong X, et al. I prostanoid receptor-mediated inflammatory pathway promotes hepatic gluconeogenesis through activation of PKA and inhibition of AKT. Diabetes 2014;63:2911–2923

10. Helmersson J, Vessby B, Larsson A, Basu S. Association of type 2 diabetes with cyclooxygenase-mediated inflammation and oxidative stress in an elderly population. Circulation 2004;109:1729–1734

11. Ozcan L, Cristina de Souza J, Harari AA, Backs J, Olson EN, Tabas I. Activation of calcium/calmodulin-dependent protein kinase II in obesity mediates suppression of hepatic insulin signaling. Cell Metab 2013;18:803–815

12. Matsumoto M, Pocai A, Rossetti L, Depinho RA, Accili D. Impaired regulation of hepatic glucose production in mice lacking the forkhead transcription factor Foxo1 in liver. Cell Metab 2007;6:208–216

13. Romere C, Duerrschmid C, Bournat J, et al. Asprosin, a fasting-induced glucogenic protein hormone. Cell 2016;165:566–579

14. Hundal RS, Petersen KF, Mayerson AB, et al. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest 2002;109:1321–1326

15. Scheen AJ, Paquot N, Lefèbvre PJ. Investigational glucagon receptor antagonists in Phase I and II clinical trials for diabetes. Expert Opin Investig Drugs 2017;26:1373–1389

16. Ozcan L, Xu X, Deng SX, et al. Treatment of obese insulin-resistant mice with an allosteric MAPKAPK2/3 inhibitor lowers blood glucose and improves insulin sensitivity. Diabetes 2015;64:3396–3405

17. Takayama K, Yuhki K, Ono K, et al. Thromboxane A2 and prostaglandin F2alpha mediate inflammatory tachycardia. Nat Med 2005;11:562–566

18. Yu Y, Lucitt MB, Stubbe J, et al. Prostaglandin F2alpha elevates blood pressure and promotes atherosclerosis. Proc Natl Acad Sci U S A 2009;106:7985–7990

19. Jagavelu K, Tietge UJ, Gaestel M, Drexler H, Schieffer B, Bavendiek U. Systemic deficiency of the MAP kinase-activated protein kinase 2 reduces atherosclerosis in hypercholesterolemic mice. Circ Res 2007;101:1104–1112

20. Shiroto K, Otani H, Yamamoto F, Huang CK, Maulik N, Das DK. MK2-/- gene knockout mouse hearts carry anti-apoptotic signal and are resistant to ischemia reperfusion injury. J Mol Cell Cardiol 2005;38:93–97

21. Doran AC, Ozcan L, Cai B, et al. CAMKIIγ suppresses an efferocytosis pathway in macrophages and promotes atherosclerotic plaque necrosis. J Clin Invest 2017;127:4075–4089