JCI The Journal of Clinical Investigation

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J Clin Invest. 2018;128(2):567-569. https://doi.org/10.1172/JCI99037.

Commentary

Hepatic glucose production (HGP) is a key determinant of glucose homeostasis. Glucagon binding to its cognate seven-transmembrane G_s-coupled receptor in hepatocytes stimulates cAMP production, resulting in increased HGP. In this issue of the *JCI,* Rossi and colleagues tested the hypothesis that activation of hepatic G_i–coupled receptors, which should inhibit cAMP production, would oppose the cAMP-inducing action of glucagon and thereby decrease HGP. Surprisingly, however, the opposite occurred: activation of G_i signaling increased HGP via a novel mechanism, while inhibition of G_i signaling reduced HGP. These results define a new physiologic role for hepatic G_i signaling and identify a potential therapeutic target for HGP regulation.

A double negative: inhibition of hepatic G_i signaling improves glucose homeostasis

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Regulation of hepatic glucose production

Appropriate regulation of hepatic glucose production (HGP) is critical for glucose homeostasis under conditions that range from high glucose demand, such as prolonged fasting, to increased glucose abundance, such as excess dietary carbohydrate intake. HGP is regulated by a complex network of direct and indirect mechanisms (Figure 1) that have been extensively studied in animals and humans (1). Impaired regulation of HGP is an important feature of diabetes and has been attributed to reduced insulin sensitivity and excessive glucagon action. Insulin acts directly on hepatocytes via its tyrosine-kinase receptor to trigger a phosphorylation cascade that inhibits glycogenolysis and gluconeogenesis, thus reducing HGP (Figure 1). Hepatocyte-specific KO of mouse insulin receptors leads to severe glucose intolerance and failure of insulin to suppress HGP (2).

Glucagon activates specific receptors coupled to G_s, the heterotrimeric G protein that stimulates adenylyl cyclase, leading to increased cAMP formation. Increased hepatocyte cAMP activates protein kinase A, initiating a phosphorylation cascade that ultimately leads to increased glycogenolysis, gluconeogenesis, and HGP. Indeed, the roles of cAMP as both a ubiquitous second messenger of hormone action (3) and a downstream effector of G_s (4) were discovered in classic studies on the mechanism of glucagon activation of liver glycogenolysis. Subsequent studies identified the so-called "inhibitory" G protein G_i, which couples to receptors to inhibit adenylyl cyclase. G_i is inactivated by pertussis toxin–catalyzed ADP-ribosylation of a cysteine in the carboxy terminus of its α subunit (5). Three different genes encode subtypes of the G_i-α subunit (6), with $G_{\alpha i}1$ and $G_{\alpha i}3$ being widely expressed and $G_{\alpha i}2$ being ubiquitously expressed. Hepatocytes contain abundant amounts of G_i, but the role of this G protein in HGP regulation has not been well defined.

Elucidating the role of G_i in liver in HGP regulation

In this issue, Rossi and colleagues (7) use in vitro and in vivo murine studies and apply a number of genetic and pharmacologic tools to probe the role of G_i in HGP regulation. On the basis of the classic, cAMP production–inhibiting definition of G_i, the authors hypothesized that activation of G_i in hepatocytes should oppose glucagon action and decrease HGP. Surprisingly, this was not the case.

Rossi and colleagues used viral vectors and a liver-specific promoter to express a designer G_i–coupled receptor (designer receptor exclusively activated by a designer drug [DREADD]) that is only activated by a specific compound devoid of pharmacologic effects elsewhere to study the liver-specific effects of G_i activation and avoid indirect effects on other organs involved in regulating HGP (Figure 1). DREADD activation did indeed inhibit glucagon-stimulated cAMP production and did not alter intracellular Ca^{++} (a $G_{\alpha q}$-mediated effect), consistent with the classic functional definition of G_i. Nonetheless, DREADD activation of G_i in the livers of mice impaired glucose tolerance, activated both glycogenolysis and gluconeogenesis, and potentiated, rather than inhibited, the hyperglycemic effect of glucagon. Interestingly, DREADD activation did not cause insulin resistance.

Pertussis toxin was first termed islet-activating protein because of its stimulatory effect on islets, which was shown to be due to loss of catecholamine inhibition of insulin secretion via G_i-coupled α-adrenergic receptors in β cells (5). Rossi and colleagues were able to study the effect of liver-specific G_i KO by selectively expressing the catalytic subunit of pertussis toxin (S1-PTX) in hepatocytes. The effectiveness of S1-PTX expression was confirmed, as S1-PTX expression abolished the hyperglycemic effect of DREADD activation. Furthermore, G_i KO in the liver improved glucose tolerance in normal chow-fed mice, as well as in mice with high-fat diet–induced insulin resistance.

Overall, the results by Rossi et al. suggest possible physiologic roles for G_i stimulation and G_i inhibition in the liver

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Conflict of interest: A.M. Spiegel holds US-awarded patents on polyclonal antibodies against G proteins that have been licensed nonexclusively by the NIH to several companies.

Reference information: *J Clin Invest*. 2018;128(2):567–569. https://doi.org/10.1172/JCI99037.

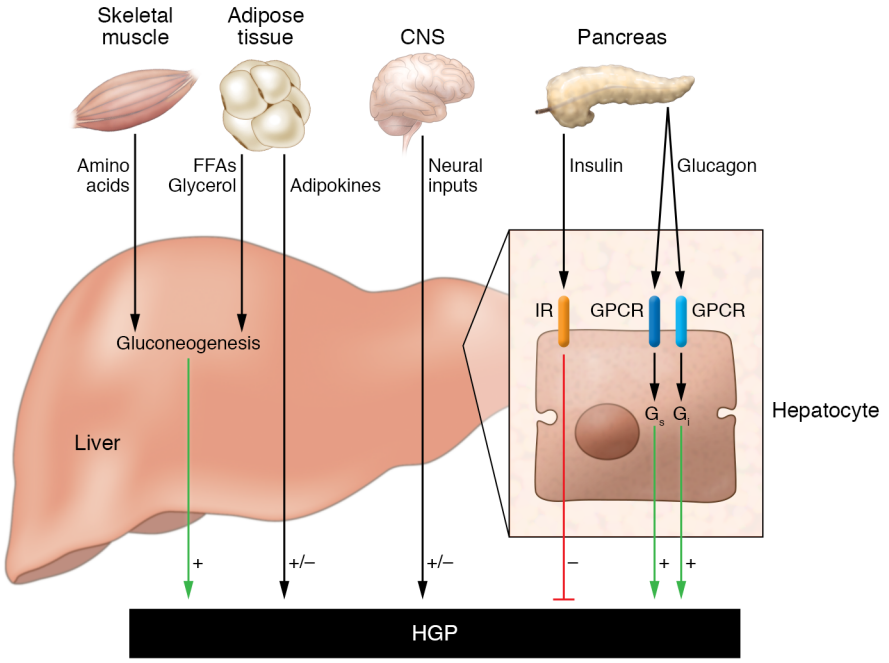

Figure 1. Indirect and direct mechanisms regulate HGP. Free fatty acids (FFAs) and glycerol from adipose cells and amino acids from skeletal muscle provide substrate for liver gluconeogenesis, resulting in increased HGP. HGP is also modulated by adipose-derived cytokines and through neural inputs from the CNS. Additionally, pancreatic islet hormones, insulin, via tyrosine kinase–stimulated phosphorylation of the insulin receptor (IR), and glucagon, via G_s-coupled receptor stimulation of cAMP production, act directly on hepatocytes to decrease and increase HGP, respectively. Hepatocytes also contain other GPCRs that are G_i coupled. In this issue, Rossi and colleagues (7) show, surprisingly, that activation of G_i-coupled receptors increases HGP.

by increasing and decreasing HGP, respectively. However, these results beg the question of which G_i signaling pathway accounts for these effects? Rossi and colleagues addressed this issue by testing the effect of DREADD activation of hepatocyte G_i on various signaling pathways, including MAPK and PI3K pathways. The authors found evidence of JNK activation due to an increased oxygen consumption rate and generation of ROS.

While the methods used by Rossi and colleagues allowed them to define a role for liver G_i in the regulation of HGP, liver-specific expression of DREADD or S1-PTX obviously does not reflect normal physiologic conditions. Endogenous expression of G_i-coupled receptors, including the cannabinoid CB1 receptor and the α_{2A}-adrenergic receptor, in mouse hepatocytes allowed the authors to assess the role of liver G_i under physiologic conditions. The hyperglycemic action of a CB1 receptor agonist was blocked in mice with liver-specific KO of G_i and in animals treated with ROS-scavenging agents or selective JNK inhibitors, supporting the notion of liver G_i signaling in HGP regulation, as elucidated by hepatocyte DREAAD expression. Rossi et al. provided further suggestive evidence of a physiologic role of hepatic G_i signaling in HGP regulation by showing that transcription of $G_{\alpha i}1$- and α_{2A}-adrenergic receptor–encoding genes is increased in livers from mice that have been fasted for 16 hours.

Are the results obtained in mouse liver relevant to human hepatocytes? Rossi and colleagues showed that expression of constitutively activated $G_{\alpha i}$ in human hepatocytes increases HGP via the ROS/JNK signaling pathway identified in mouse hepatocytes. Moreover, examination of gene expression in livers from control subjects compared with expression in patients with nonalcoholic steatohepatitis (NASH) with clear signs of insulin resistance revealed increased transcription of the α_{2A}-adrenergic receptor and the CB1 receptor, both of which are G_i coupled, and markedly decreased transcription of the genes encoding $G\alpha_{i1}$ and $G\alpha_{i3}$ in livers from patients with NASH. Rossi et al. attribute the reduction in RNA levels of $G\alpha_{i1}$ and $G\alpha_{i3}$ to counterregulatory mechanisms caused by enhanced G_i signaling; however, it is unclear why this result is opposite of the increase in $G\alpha_{i1}$ RNA observed in the livers of fasted mice.

Unanswered questions and future directions

The findings of Rossi and colleagues raise a number of important issues that need to be further addressed. Their observation that stimulation of mouse hepatocyte G_i signaling via JNK activation increases HGP without reducing insulin sensitivity is puzzling, as JNK activation has been recognized as a major factor causing insulin resistance (8). Is this discrepancy a function of cell-specific differences in JNK action? Perhaps, but the lack of effect of G_i stimulation on insulin sensitivity observed by Rossi et al. differs from results obtained in studies of mice with hepatocyte-specific KO of the CB1 receptor (9). The effects of hepatocyte-specific KO or overexpression of the CB1 receptor on overall glucose homeostasis are consistent with those seen by Rossi and colleagues; however, these studies also provide evidence of CB1-induced insulin resistance. The reasons for this discrepancy are unclear and deserve further study.

Another question concerns the identity of the signaling pathways downstream of G_i that lead to increased HGP. JNK activation is not the sole mechanism, as hepatic expression of a dominant-negative form of JNK only partially inhibited the increased HGP caused by G_i activation. Comparison of RNA profiles from the livers of mice with and without DREADD stimulation showed differential expression of more than 1,000 genes, including many associated with the unfolded protein stress response. Additionally, genes involved in several other pathways also showed altered expression, suggesting that pathways other than JNK activation should be investigated in future studies.

The specific form(s) and subunits of heterotrimeric G_i ($G_{\alpha i}1$, $G_{\alpha i}2$, and $G_{\alpha i}3$ individually or in combination; the β/γ subunits) involved in the direct stimulation of HGP and the nature of the effector with which G_i interacts to mediate its distal effect remain unclear. Various forms of $G_{\alpha i}$, as well as β/γ subunits, have been

shown to regulate effectors beyond adenylyl cyclase, including K^+ and Ca^{2+} channels and PI3K (5, 10). No phenotypic changes were reported in mice with germline KO of genes encoding either $G_{\alpha i}1$ or $G_{\alpha i}3$, and germline KO of the $G_{\alpha i}2$-encoding gene led to an ulcerative colitis–like disease ascribed to abnormal T cell function (6). Failure to observe overt defects in glucose homeostasis may reflect functional redundancy of $G_{\alpha i}$ genes and/or opposing roles for G_i in the liver and other organs. It is also possible that defects in glucose homeostasis in $G_{\alpha i}$-KO mice remain to be discovered.

Implications for the treatment of diabetes

The enormity of the worldwide diabetes epidemic demands new, more effective forms of therapy. Of the drugs that are currently available to treat diabetes (aside from insulin itself), metformin is the only one that inhibits HGP (1). Other agents (11) act by distinct mechanisms, including insulin sensitization (thiazolidinediones), incretin effects (GLP-1 agonists and DPP-IV inhibitors), and increased renal glucose excretion (SGLT-2 inhibitors). The limited efficacy and side-effect profiles of these drugs have spurred the search for novel therapeutic targets. Recent studies, for example, have identified selective modulators of FOXO1 (12), a key downstream target of insulin action in the liver, and inhibitors of GPR21, an orphan receptor coupled to the G_q phospholipase C pathway (13), as potential therapeutic options. Given their finding that liver G_i KO improves glucose tolerance by reducing HGP, Rossi and colleagues suggest that inhibition of G_i signaling in the liver could represent a novel target for the treatment of diabetes. While theoretically true, developing agents that can selectively inhibit G_i only in liver will represent a formidable challenge, but agents that block hepatic G_i–coupled receptors, e.g., CB1 receptor antagonists, may hold more promise.

The CB1 receptor antagonist rimonabant was approved in Europe for the treatment of obesity on the basis of its ability to suppress appetite. Unfortunately, serious psychiatric side effects led to its withdrawal (14) and the development of peripherally restricted CB1 receptor antagonists (15). While the effects of such antagonists in the liver should improve glucose homeostasis (based on the present work), the ultimate role of CB1 receptor–targeting drugs in treating diabetes will depend on their integrated effect on adipose tissue, skeletal muscle, and the pancreas, where CB1 receptors are also expressed (15).

Acknowledgments

I am grateful to Jeffrey Pessin and Jonathan Backer (Albert Einstein College of Medicine) for their review of this Commentary.

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