REVISITING GLUCAGON ACTION IN DIABETES: IS IT ALL BAD?

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ABSTRACT

Traditionally, the islet hormone glucagon has been considered as a counterbalance to insulin, preventing hypoglycemia by promoting glucose release from the liver. This model is compatible both with clinical studies demonstrating that one of the initial endocrine responses to insulin-induced hypoglycemia is a rise in glucagon and with *in vitro* work demonstrating that glucagon signaling activates glycogenolysis in hepatocytes. This model has been extended to implicate glucagon in diabetogenesis, positing that the increased secretion of glucagon acts as a primary driver of hyperglycemia. However, recent work suggests an alternative set of actions for glucagon, including stimulation of insulin secretion and enhancement of hepatic insulin action. These recent findings align with the results of clinical trials using novel drugs that activate the glucagon receptor as part of a multireceptor mechanism of action. Taken together, it appears that glucagon has distinct actions in the fed and fasted states, and glucagon receptor agonism has potential as a therapeutic approach to the treatment of diabetes.

INTRODUCTION

Glucagon was discovered in 1923, a year after people with diabetes were first treated using insulin. Insulin was extracted from an animal pancreas, and early preparations caused a paradoxical rise in blood glucose before the onset of glucose lowering. This effect was the result of a second factor in the extracts that could be separated by improved chemical purification of the insulin, and the hyperglycemic factor was named descriptively, using a shortened version of glucose-agonist (1). Glucagon was first purified in the early 1950s, and the 30 amino-acid sequence was determined in 1957—two years after the sequence of

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insulin was reported. Sutherland and colleagues (2) demonstrated that glucagon acts on hepatocytes to stimulate glycogenolysis. In the 1940s, de Duve, et al showed glucagon to be a product of islet α-cells (3). Insulin and glucagon were the first two hormones to be measured directly in circulating plasma using the revolutionary immunoassay technique described in 1960 (4,5). Signaling by glucagon through its receptor on hepatocytes was fundamental to the discovery of cyclic AMP and G-proteins as second messengers (2,6). The two islet peptides, produced in distinct but neighboring cells in the endocrine pancreas, have been fundamental to understanding principles of endocrinology, regulation of normal fuel metabolism, and pathophysiology of diabetes. Furthermore, work related to their biology contributed to seven Nobel Prizes.

The discovery of glucagon was based on its glycogenolytic/glucogenic properties. Based on this function, its conceptualization in metabolic physiology has been as an antagonist to insulin (7). Early physiology experiments demonstrated that glucagon could counteract the effect of insulin treatment to reduce blood glucose, and glucagon preparations were made to treat insulin-induced hypoglycemia in patients with diabetes; this practice remains common to the present day. However, in animals or humans with insulinopenic diabetes, administration of glucagon provokes severe hyperglycemia, regardless of the initial level of blood glucose. In the setting of insulinopenia, which increases lipolysis and generation of fatty acids, glucagon administration also increases hepatic ketogenesis (8). A role for glucagon in diabetic ketoacidosis was supported by studies in which somatostatin was given to suppress glucagon, and reduce ketogenesis, in subjects with type 1 diabetes (T1DM) (9). That ketogenesis does not occur as frequently in persons with type 2 diabetes (T2DM) and residual insulin production, led to the inference that hepatic metabolism, measured as either blood glucose or ketone bodies, was determined by the portal venous ratio of insulin and glucagon concentrations. And since several studies suggested that plasma glucagon levels tend to be higher in persons with diabetes than controls, a model of diabetes including pathogenic abnormalities of both islet hormones, the bihormonal hypothesis, was proposed in the 1970s (10,11). The general outlines of this hypothesis (e.g., too much glucagon and too little insulin) remain in common usage to explain diabetes and its treatment.

While originally identified as a glucogenic factor, one of the earliest physiologic actions of glucagon was the stimulation of insulin secretion. In a series of careful experiments, Samols and Marks demonstrated that glucagon stimulated a rise in circulating insulin that was out of proportion to, and preceded, the increase in plasma glucose (12,13). This

observation, along with studies demonstrating an increase in plasma glucagon concentrations after meals, was advanced as an explanation for the incretin effect—the greater rise of insulin secretion after oral compared to intravenous glucose. While this work was passed over and forgotten for several decades, the insulinotropic effects of glucagon were applied as a diagnostic test to distinguish between diabetic subjects with severe and relative insulin deficiencies through the 1970s (14).

Glucagon is secreted promptly in response to insulin-induced hypoglycemia, with plasma concentrations rising in most people when blood glucose is <70 mg/dl; hyperglucagonemia in this circumstance is inversely proportional to the glucose nadir. Glucagon is generally suppressed by hyperglycemia induced by IV glucose infusion with minimal changes in response to oral glucose loads. However, in response to the ingestion of protein, amino acids or mixed nutrient meals glucagon concentrations rise, in concert with insulin. Glucagon levels increase slightly with fasting and have been proposed to maintain glycemia when no nutrients are available from the GI tract. However, mice with genetic deletions of the glucagon receptor or proglucagon have normal or only slightly reduced fasting glycemia and respond similarly to extended food deprivation as wild-type controls (15,16). In fact, the most prominent phenotype of mouse models with abolished glucagon signaling is hyperaminoacidemia and α-cell hyperplasia (15); mice with global or hepatic glucagon receptor (GCGR) deletions have massive elevations of proglucagon peptides in their circulation. Thus, despite some established pharmacologic effects, and suggestive evidence for a role in diabetes, the physiologic role of glucagon remains poorly defined.

RESEARCH ADVANCES

Over the past five years, a number of published papers support a new twist on glucagon action. Several groups, including ours, reported evidence that glucagon acts locally in the islet, through a paracrine mechanism, to regulate insulin secretion. This work includes studies in human and murine islets using GCGR antagonists (17), acute suppression of glucagon secretion in an α-cell DREADD model (18), and pancreatic perifusion in mice with deletion of receptors for glucagon (19). These studies each demonstrate important insulinotropic action of glucagon that is independent of peptide in the circulation and contained within the islet (20). In contrast to previous work using infusions of exogenous glucagon, the loss of function approaches applied in these complementary studies support α-cell-to-β-cell communication as a physiologic mechanism.

Our first study in this area demonstrated that glucagon stimulates insulin secretion by activating β-cell GCGR and GLP-1 receptors (GLP-1R) (21). In mice with a genetic deletion of *Gcg* and absence of any proglucagon products, islets studied *ex vivo* showed severe impairment in the insulin response to either glucose or amino acids, although stimulation by exogenous glucagon and GLP-1 was normal. These results were replicated in mouse islets treated with GLP-1R and GCGR antagonists, and in islets from mice with genetic deletion of both of these receptors. In the absence of stimulation by proglucagon glucagon-derived peptides, mouse β-cells had blunted rises in intracellular cAMP and calcium following glucose stimulation. The proximate signaling defect was cAMP generation as *Gcg* knockout mice had impaired insulin responses to isobutyl methyl xanthine, an inhibitor of cAMP degradation, but normal insulin secretion when treated with forskolin, which bypasses activity of the GLP-1R/CGCR and directly stimulates cAMP production. Importantly, our findings in mouse islets were replicated in human islets that demonstrated impaired basal and stimulated insulin secretion when treated with GLP-1R/GCGR antagonists. Finally, we observed that α-to-β cell communication was important for metabolic compensation in that, compared to controls, mice with a double β-cell GLP-1R/GCGR knockout developed diabetes when fed a high fat diet for eight weeks with development of obesity. Overall, the paper describing this work reported dual receptor regulation of β-cells by glucagon, the maintenance of β-cell tone through the generation of cAMP, and its essentiality for nutrient stimulated insulin secretion and compensation of metabolic stress.

In two follow-on studies, we examined α-to-β cell communication to greater depth *in vivo*. In the first of these, we observed that glucagon had different effects based on the state of fasting/feeding (22). Specifically, glucagon administered to fasting mice with blood glucose concentrations ~75 mg/dl caused hyperglycemia and a minimal increase of circulating insulin, while fed mice with glucose levels of ~150 mg/dl increased insulin secretion threefold and reduced glycemia. These results yielded two conclusions: (a) the β-cell stimulatory action of glucagon is dependent on elevated levels of blood glucose, similar to other endogenous insulin secretagogues like GLP-1 and glucose dependent insulinotropic polypeptide (GIP); and (b) glucagon has two entirely distinct actions on glucose metabolism that are context specific (i.e., the hyperglycemia inducing effects, mediated principally by actions in the liver, to protect basal glycemia, and the insulinotropic effects that reduce blood glucose, which operate in response to different homeostatic challenges). In particular, these results advance the role of glucagon in the prandial

state, actions mostly overshadowed by the hepatic, counter-regulatory effects that have been the predominant focus of research into glucagon. In a second set of experiments, we focused on glucagon physiology in response to meal ingestion (23). It has been known for decades that an increase in circulating amino acids stimulates α-cells (7), and more recent work indicated that the incretin GIP also acts as a glucagon secretagogue. We observed that there is strong synergy between amino acids and GIP that amplifies glucagon release, and this leads to substantial increases in portal glucagon concentrations after mixed nutrient meals. Moreover, disrupting this synergism by genetically deleting the GIP receptor from α-cells causes hyperglycemia after meals. Together, these two studies support a role for glucagon in the concert of factors regulating efficient disposition of ingested nutrients.

Since our research group's goals are always directed toward translation, we moved to extend the physiologic effects we had observed in mice to humans. In a proof-of-concept study (24), we infused healthy men and women with glucagon after an overnight fast or during experimental hyperglycemia; the glucose levels at the onset of glucagon administration were ~85 and 150 mg/dl on the two experiments. Our results in this group of human subjects paralleled what we saw in mice: during fasting, glucagon stimulated a brisk rise in hepatic glucose production, with a delayed insulin response that followed an increase in blood glucose. In contrast, when given under a setting of blood glucose elevation comparable to what occurs after eating, glucagon had a prompt action on the β-cell, with insulin secretion increasing to rates ~threefold of what was measured in the fasting experiment. An unexpected secondary finding from this study was a significant decrease in insulin clearance when glucagon was administered; an effect that would add to insulinotropism and accentuate delivery of insulin to peripheral tissues in the prandial state. Based on these results, we believe the disparate effects of glucagon on glucose metabolism in the fasting and fed states are shared among mammals and indicate that secretion of glucagon after meals serves a core physiologic function in humans as well.

Distinct from our work on glucose regulation by glucagon, but in keeping with the process of revisiting established views of its functions, are some experiments on the role of glucagon in ketogenesis (25). It is broadly held that glucagon is essential for ketogenesis during fasting, physiology that has been extended to a central pathogenic role in diabetic ketoacidosis. Moreover, with the broad usage of SGLT2 inhibitors for the treatment of diabetes, rare cases of ketoacidosis with these medications have been attributed to their tendency to raise plasma glucagon levels (26). We tested the necessity of glucagon to generate ketosis in

the settings of fasting and dapagliflozin treatment using pharmacologic and genetic abrogation of glucagon signaling. In these studies, we demonstrated that glucagon does not contribute to increases in circulating β-hydroxybutyrate after a 16-hour fast or SGLT2 blockade. Rather, our findings indicate that ketosis in response to fasting or SGLT2i is driven by increased fatty acids, the key substrate for ketone body production. Only in the circumstance of profound reduction in insulin action (streptozotocin destruction of β-cells and an insulin receptor antagonizing antibody) did we see an effect of glucagon contributing to ketosis. These findings challenge a long-held conception of a core action of glucagon, albeit one based primarily on studies with cultured hepatocytes (8), that was extended to adverse clinical syndromes. Our findings need to be extended to studies in humans, but they mitigate to some extent concerns about the application of glucagon activity to pharmacologic compounds.

PHYSIOLOGICAL AND CLINICAL IMPLICATIONS

Despite research in the field of glucagon research that spans more than 70 years, recent work by our group and others has led to reconsideration of what had been considered established physiology. The demonstration of α-to-β cell communication as an active process in pancreatic islets has focused attention on glucagon actions to regulate glycemia after meals as well as during the fasting state. Our findings on meal-stimulated glucagon secretion through an amino acid/GIP synergism, insulinotropism through the GLP-1/GCGR, and a role of α-to-β cell communication in glucose tolerance lend credence to this shift in focus. Consistent with prandial effects of glucagon is our recent observation on its effect to reduce insulin clearance, and we have preliminary data supporting coordinated regulation of hepatic insulin sensitivity by glucagon and insulin, both of which are increased after meals. Moreover, while regulation of hepatic glucose production has been the signature role attributed to glucagon, it now appears that this is not the only, or even the major, action on glucose metabolism. Finally, some glucagon actions such as ketogenesis may have been overstated, while others like amino acid metabolism may be underappreciated. It is notable that a strict interpretation of the findings from mice with deletion of the glucagon receptor is hyperaminoacidemia rather than major effects on glucose or lipid metabolism is the primary phenotype. We have recently embarked on studies to parse and prioritize hepatic actions of glucagon based on signaling immediately downstream of the glucagon receptor.

The glucagon receptor has been a target for drug development since the early 1980s. However, for most of that time, the goal was to antagonize the glucagon receptor and reduce hepatic glucose production, an effect posited to improve glycemic control in persons with T2DM. In fact, a number of small-molecule GCGR inhibitors have been developed and advanced into clinical trials, only to fail because of unfavorable side effect profiles. In recent years, drug developers have taken a different tack with the addition of glucagon receptor activation to multi-receptor agonist (MRA) peptides. The rationale for this approach started with the serendipitous observation that a GLP-1/glucagon co-agonist caused greater weight loss and lower blood glucose than a peptide with solely GLP-1 agonism (27). This strategy has led to the development of a number of GLP-1/glucagon co-agonists as well as a GLP-1/GIP/glucagon triagonist that is the most potent weight loss medication yet described (28–30). The initial rationale for these exciting new compounds was that GLP-1R agonism would promote glycemic lowering and counteract any effects of glucagon to raise blood glucose, while GCGR agonism would accentuate satiety effects of GLP-1 and add an energy expenditure component for more weight loss. These mechanisms have not yet been proven, and our recent data suggest that increased insulin action (i.e., greater secretion, less clearance, and more hepatic insulin sensitivity) may also contribute to the clinical benefits of MRAs. We are currently conducting studies to determine the role of α-to-β cell communication in states of insulin resistance and the balance of glucagon activity between hepatic and β-cell effects in persons with diabetes.

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DISCUSSION

Liedtke, Durham: Wonderful talk. I regret that we didn't overlap more since I left. My question is regarding drugs like semaglutide. Can you explain a little bit about why some GLP-1R agonists are more potent at weight loss versus others earlier drugs that are not as powerful? What is the reason for that? How does that go, how much do you implicate the signaling in the brain, and what is known about that from your end?

D'Alessio, Durham: In just the last few weeks, the Lasker Award was given to people that worked on the fundamentals of the GLP drugs for obesity, but most of the development around these drugs has been for diabetes. With the early drugs, people lost about 4 kg, which was good because a lot of diabetes drugs made people gain weight. As the pharmacokinetics have changed, mostly by increasing the drugs' half-life so they last longer, these big weight losses have resulted. Has that really been documented? No, but that's what we see—the shorter-acting ones have the least effect and the longer-acting ones have the most. We also find that these drugs affect receptors in different ways. I can't tell you that all three receptors are playing a role in the huge effects of retatrutide; it may be that it's particularly good at GLP or one of the others. The biochemistry there hasn't been worked out, and that's very important. These are brain drugs, right? We got into this because these drugs seem to stimulate insulin, but they work in the brain and affect satiety, hedonics, and hepatic glucose production. They probably affect islet function as well so I think that's another kind of revelation. We thought these drugs were going to work in the periphery, and it turns out they're very potent in the brain.