Glucagon as the First Incretin: Objects (in the Rearview Mirror) Are Closer Than They Appear

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Soon after publication of the radioimmunoassay to measure circulating insulin in 1960, several critical observations were made that added new depth to the understanding of metabolic physiology. Among these were several studies confirming a long-held hypothesis that factors released from enteral organs promoted the secretion of insulin and disposal of glucose (1,2). This hypothesis was based on studies in the early 20th century in which intestinal extracts could be demonstrated to reduce circulating blood glucose and alleviate diabetes (3). The term "incretin" was coined in 1932 by the Belgian physiologist Jean La Barre to describe hormones that stimulated the internal (i.e., islet) secretions of the pancreas; it was also suggested these hormones would be useful for the treatment of diabetes (3). Indeed, in the last four decades of the 20th century, considerable research activity was dedicated to the identification and study of incretins, leading to a general consensus that glucose-dependent insulinotropic polypeptide (GIP), discovered in 1970, and glucagon-like peptide 1 (GLP-1), discovered 1983–1987, were the factors that accounted for the incretin effect. GIP and GLP-1 are made and released from intestinal enteroendocrine cells and mediate insulinotropic effects by activating specific family B G-protein–coupled receptors (GPCR), through a mechanism that involves increasing intra-β-cellular cyclic AMP production (4). More than 40 years of work support a role for these peptides as physiological incretins. Moreover, in the past 20 years, they have become the basis for the most effective new therapeutics to treat diabetes.

While GIP and GLP-1 have become synonymous with the incretin concept, recent work suggests that another GPCR ligand may also play a role in amplifying prandial insulin secretion. In the last several years, preclinical (4) as well as human (5) studies suggest that stimulatory actions of glucagon contribute to physiologic insulin secretion. These new observations indicate that glucagon can increase insulin secretion by acting through its cognate receptor, the GCGR, as well as through the GLP-1 receptor. In experiments with transgenic and wild-type mice, glucagon seems to impact β-cell tone and establish the set point for circulating glycemia (6,7). However, while these findings have kindled new interests in glucagon physiology, in actuality they represent a rediscovery of observations made by Samols, Marri, Marks, and colleagues in the mid-1960s. These investigators gave purified glucagon to healthy subjects and noted a rapid rise in plasma insulin suggesting an effect on β-cell secretion (8). This led them to test the hypothesis that glucagon has insulinotropic as well as glycogenolytic properties.

While Marks and colleagues published a series of articles on glucagon as an insulin secretagogue, their most comprehensive study was "Interrelationship of Glucagon, Insulin and Glucose: The Insulinogenic Effect of Glucagon," published in *Diabetes* in December 1966 (9), which serves as the article for this month's Classics in *Diabetes*. The goal of the experiments related in this article was to extend the initial observation that glucagon seemed to promote insulin secretion independent of its effect to raise blood glucose. The authors were able to confirm the findings of their first report by demonstrating that bolus injection of large doses (1 mg) of glucagon elicited a rise of plasma insulin typical of first-phase insulin secretion, and that this occurred prior to the subsequent rise in blood glucose due to hepatic glucose release. In addition, they demonstrated that glucagon had a greater effect to raise insulin during a state of hyperglycemia than at fasting euglycemia. Several patients with diabetes and hyperglycemia had only modest responses to glucagon when studied initially, but after several weeks of glucose-lowering therapy, the insulin response seen after intravenous glucagon was more robust. In several patients with hepatic cirrhosis, glucagon stimulated insulin secretion but did not cause a rise in blood glucose,

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presumably because of meager glycogen stores. The effects of glucagon to stimulate insulin release were dose dependent and greater at higher concentrations of blood glucose. The authors concluded from these results that glucagon stimulated insulin directly and not as a secondary response to hyperglycemia from brisk glycogenolysis. Their findings were corroborated in short order by work from Crockford et al. (10).

In reflecting on the legacy of this article and its times, Dr. Marks recalled:

The paper you cite was not the first that Ellis Samols and I wrote together but was undoubtedly the one that established our careers as serious researchers. I had been led to believe that glucagon might have insulinogenic properties around 1958, when I got free samples of the newly isolated insulin contaminant from Mary Root at Eli Lilly to investigate its use as a treatment for the hypoglycemia produced by insulinomas. These were still considered unspeakably rare, but there was a generous supply of them at the Institute of Neurology where I had recently taken up a training post in clinical biochemistry and introduced a new method for measuring glucose at very low concentrations in blood. To my astonishment, after an initial rise in blood glucose following the injection of glucagon into patients with insulinoma, there was a profound fall to hypoglycemic levels that was much greater than that produced by intravenous glucose. I suspected that this was due to glucagon-stimulated insulin release but could not prove it until I teamed up with Ellis, who had learned the technique of radioimmunoassay from Yalow and Berson while on his honeymoon.

Our friends at the Royal Free Hospital had taken over Ellis's radioimmunoassay laboratory during his sabbatical in Seattle and had rediscovered the incretin concept, which led me and Ellis to assume that "glucagon," especially that originating in the gut, might in some way play a role as mediator of the effect. At the time of our paper in Diabetes*, Ellis and I were in no doubt, as expressed in the penultimate paragraph of that paper, that (pancreatic) glucagon also played a role in the exercise of the incretin effect mainly through a paracrine mechanism.*

We had no idea what stimulated pancreatic glucagon following a meal but thought it was the presence of glucose in the intestine. Fifty-seven years later, I think that pancreatic glucagon secreted by the α-cells may still have a role in the incretin effect, although I have not kept up with the biomedical literature on this topic at all attentively. Ordinarily, however, the negative feedback from endogenous insulin secretion on glucagon secretion appears also to be physiologically important.

Much of the value of this work is the wonder of finding a lost treasure of relevant thought and data, and being reminded that there is often continuity and coherence in scientific observation that extends across decades. For investigators who grew up studying human physiology in settings like clinical research centers, the paper has the added appeal of presaging many of the methods and strategies that became the foundation of modern metabolic research. In addition, the work of Marks and colleagues is both intuitive and reassuring to investigators working in the area of intraislet cell communication and making their own observations on insulinotropic effects of glucagon, e.g., closer than six decades in the rearview mirror would make it seem. Revisiting this article is especially timely given active development of drugs with glucagon activity as components of multireceptor agonists to treat diabetes (11). Moreover, the prescience of some of the conclusions and predictions made by Marks and his collaborators from their findings is remarkable given what has transpired in incretin research since 1966. They predicted a cAMP-mediated effect on glucose-stimulated insulin secretion before GPCR were discovered, identified glucose dependence as a central component of incretin action, and suggested paracrine interactions between islet cells as a mechanism of secretory regulation. Moreover, their observations on the relative potency of a β-cell GPCR ligand in subjects with diabetes before and after glucose-lowering therapy have also been borne out over time. While glucagon stimulation tests were used throughout the 1970s as a clinical tool for identifying patients with insulin-dependent diabetes (12), the potential application of the findings of Samols, Marks, and colleagues to physiology was lost for some time, buried beneath the abundant evidence that glucagon was essential to counterbalance insulin and mitigate hypoglycemia. That glucagon stimulation of β-cells is now an active area of investigation is a tribute to the insight and clarity of the authors of the present Classics in *Diabetes*.

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