Daniel J. Drucker

Deciphering Metabolic Messages From the Gut Drives Therapeutic Innovation: The 2014 Banting Lecture

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The Banting Medal for Scientific Achievement is the highest scientific award of the American Diabetes Association (ADA). Given in memory of Sir Frederick Banting, one of the key investigators in the discovery of insulin, the Banting Medal is awarded annually for scientific excellence, recognizing significant long-term contributions to the understanding, treatment, or prevention of diabetes. Daniel J. Drucker, MD, of the Department of Medicine, Mount Sinai Hospital and the Lunenfeld-Tanenbaum Research Institute in Toronto, Ontario, Canada, received the prestigious award at the ADA's 74th Scientific Sessions, 13–17 June 2014, in San Francisco, California. He presented the Banting Lecture, "Deciphering Metabolic Messages From the Gut Drives Therapeutic Innovation," on Sunday, 15 June 2014.

Gut peptides convey nutrient-regulated signals to the enteric nervous system and to distal organs, acting as circulating hormones secreted in the basal and postprandial state. Here I provide an overview of the actions of glucagon-like peptide (GLP)-1 and GLP-2, the two major enteroendocrine L-cell peptides. The endogenous physiological actions of GLP-1 have been delineated using antagonists and *Glp1r*$^{-/-}$ mice and include the control of islet hormone secretion in a glucose-dependent manner, leading to improvement of fasting and postprandial glucose homeostasis. GLP-1 receptors (GLP-1Rs) are also widely distributed in multiple extrapancreatic organs, providing a mechanistic explanation for the nonglycemic actions attributed to GLP-1. The multiple metabolic actions of GLP-1 enable reduction of glycemia and body weight in diabetic and obese subjects, providing the opportunity to reduce glycemia in human subjects with diabetes with a low risk of hypoglycemia. GLP-2 plays a key role in the control of energy absorption and in the integrity of the intestinal mucosa, and a GLP-2R agonist, teduglutide, is now used for augmentation of energy absorption in parenteral nutrition–dependent subjects with short bowel syndrome. GLP-1 and GLP-2 are both cleaved by dipeptidyl peptidase-4 (DPP-4); hence, inhibition of DPP-4 activity enables yet another pathway for potentiation of incretin action and the therapy for type 2 diabetes. Here I review our 30-year experience with the elucidation of gut hormone action and, wherever possible, highlight therapeutic implications of our preclinical studies and future opportunities for incretin research.

My journey of discovery that emanated from our work on the glucagon gene and the glucagon-like peptides (GLPs) is partly due to serendipity, yet also reflects the wisdom, mentorship, and guidance of key individuals. Specifically, Gerard Burrow, who, as my elective supervisor in 1976, suggested that I try endocrinology research as a medical student and resident and instilled in me his infectious enthusiasm while providing wonderful mentorship. Charles Hollenberg, our physician-in-chief and chair of medicine at the University of Toronto, encouraged me to consider and pursue endocrinology as a career despite numerous outstanding young endocrinologists already active on the faculty. Finally, Joel Habener was kind enough to take me into his laboratory in 1984 to learn molecular biology, despite the fact that my scientific background and laboratory skills were marginal and I was clearly a risky proposition as a postdoctoral fellow.

Department of Medicine, Mount Sinai Hospital and the Lunenfeld-Tanenbaum Research Institute, University of Toronto, Toronto, Ontario, Canada

Corresponding author: Daniel J. Drucker, drucker@lunenfeld.ca.

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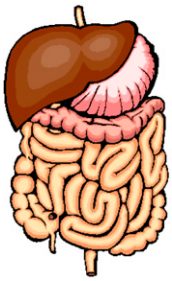

Figure 1—Spectrum of gut hormones produced by enteroendocrine cells. The early classical view of unihormonal enteroendocrine cells has given rise to the concept that L cells, like all enteroendocrine cells, are plurihormonal. Hence, L cells not only express GLP-1, GLP-2, oxyntomodulin, and glicentin but may also produce GIP, neurotensin, peptide YY, and cholecystokinin. Additional diversity is generated by proteolytic cleavage of peptide hormones, which generates truncated yet bioactive peptides with differential receptor selectivity (30).

The area of endocrinology that I have been privileged to study is the gastrointestinal endocrine system (Fig. 1). The enteroendocrine system encompasses dozens of specialized endocrine cells that synthesize and secrete numerous peptide hormones with amazing effects that control immune function, appetite, gut motility, and nutrient digestion, absorption, and disposal and that constantly refine our metabolism, energy balance, and body weight.

THE PROGLUCAGON-DERIVED PEPTIDES

When I started working as a postdoctoral fellow in 1984, I set about pondering the structure of the proglucagon cDNAs and genes. As we scrutinized the open reading frame, one could visualize the sequence of 29 amino acid glucagon and two additional putative peptides with unknown function, designated GLPs, due to their considerable amino acid identity with glucagon. In one of my first experiments, we demonstrated that the proglucagon cDNA and prohormone could be expressed in transfected pituitary or islet cells, leading to liberation of GLP-1 and GLP-2 (1). At the same time, Svetlana Mojsov et al. (2), in the Habener laboratory, demonstrated the presence of immunoreactive GLP-1 and GLP-2 peptides in the rat intestine. These studies preceded the definitive molecular identification of the family of mammalian prohormone convertases that we now know play a key role in defining the identity of proglucagon-producing islet α-cells and L cells.

DELINEATION OF THE ACTIONS OF GLP-1

Toward the end of 1984, we initiated experiments examining the putative action of GLP-1. These simple studies, carried out with my colleague Jacques Philippe, involved incubating various forms of GLP-1, including GLP-1(1-37), (7-37), or (7-36), with islet insulinoma cells. Remarkably, GLP-1(7-37) potently increased cAMP formation, insulin secretion, and insulin gene expression in β-cells (3). At the same time, Jens Holst et al. (4) in Copenhagen, Svetlana Mojsov et al. (5) in Boston, and Steve Bloom and colleagues (6) in London demonstrated that GLP-1(7-36) amide and GLP-1(7-37) were robust activators of insulin secretion in vivo. At the time, in 1985–1986, most of us did not envision that this little peptide might one day have a therapeutic role in the treatment of diabetes. However, Joel Habener certainly had that vision and filed the first patent describing the use of GLP-1 as a therapy to treat type 2 diabetes. That was probably the first time I directly encountered the concept that a series of scientific observations could enable a filed patent. I was fortunate to have trained in an environment where recognition and development of intellectual property was viewed as a potential positive byproduct of our laboratory science.

Today, more than 30 years later, the scientific community actively contemplates multiple approaches to harness the therapeutic potential of GLP-1. As a result of pioneering efforts of multiple colleagues, the first GLP-1 receptor (GLP-1R) agonist, exenatide, was approved for clinical use in April 2005, followed by approval of the first dipeptidyl peptidase-4 (DPP-4) inhibitor, sitagliptin, in October 2006 (7). The tantalizing search for potent stimulators of GLP-1 secretion continues; however, our challenge is to improve on the unexpected actions of metformin, which not only inhibits hepatic glucose production and stimulates GLP-1 secretion but in murine studies also potentiates incretin action at the β-cell (8). It seems unlikely that we can meaningfully improve on the selectivity and clinical potency of the current generation of highly selective potent DPP-4 inhibitors (DPP-4i). In contrast, the ongoing development of structurally distinct GLP-1R agonists with unique pharmacokinetic and pharmacodynamics properties suggests that newer agents may improve the tolerability and efficacy of the first-generation GLP-1R agonists. A holy grail continues to be the identification of small-molecule GLP-1R activators, orally bioavailable GLP-1R agonists, or allosteric activators enabling activation of the GLP-1R without the need for injections.

We must applaud the vision of John Eng et al. (9), who isolated a GLP-1–related lizard venom salivary gland peptide from a poisonous *Heloderma suspectum* (Gila monster). In our laboratory, we pondered why the lizard would produce a peptide in its salivary gland related to GLP-1. We thought there were at least two possibilities: either exendin-4 was actually lizard GLP-1, or reptiles, specifically lizards, had evolved with a gene encoding exendin-4 related to, but distinct from, native GLP-1. To answer this question, Eugene Chen and I (10), in our laboratory, made cDNA libraries from the lizard intestine and salivary gland. We demonstrated that the lizard has a separate exendin-4 gene as well as two distinct

proglucagon genes that encode lizard GLP-1. At that time, before the widespread availability of human genome sequence, we were hoping to isolate a mammalian exendin-4 gene. Laurie Baggio, in our laboratory, tried very hard to clone exendin-4 from human cDNA and genomic libraries; however, we were not successful for the painfully simple reason that mice, rats, and humans simply do not have an exendin-4 gene. Laurie subsequently made transgenic mice continuously expressing exendin-4, enabling analysis of whether its metabolic actions are sustained in vivo (11).

PHYSIOLOGY OF GLP-1 ACTION: AN INITIAL AND SUSTAINED FOCUS ON β-CELLS

As the pharmacological potential of GLP-1 emerged, we were simultaneously interested in understanding the physiological importance of endogenous GLP-1. Although the GLP-1R antagonist exendin (9-39) had been identified, it was a weak partial agonist and inverse agonist (12); hence, achieving selective and complete GLP-1R blockade in chronic studies with this antagonist was challenging. Louise Scrocchi initiated a project, with the collaborative assistance of Alex Joyner and colleagues (13), to inactivate the *Glp1r* in mice. Two unexpected conclusions emerged from her initial studies. Although we were gratified to observe impaired oral glucose tolerance and defective insulin secretion in *Glp1r*$^{-/-}$ mice, the findings of abnormal fasting glycemia and impaired intraperitoneal glucose tolerance were unexpected (13). However, subsequent studies have demonstrated that basal GLP-1R signaling is essential for control of glucagon and insulin secretion in rodents and humans (14). Indeed, our initial concepts of GLP-1 as a postprandial regulator of insulin secretion have now been extended to encompass a role for basal GLP-1 in the control of glycemia, even in the interprandial or fasting state.

The relative importance of the β-cell versus the α-cell versus the central nervous system (CNS) as a target for the glucoregulatory actions of endogenous GLP-1 continues to be a subject of debate. Yazhou Li, Ben Lamont, and colleagues (15) set out to tackle this issue by creating a mouse enabling transgenic rescue of human GLP-1R signaling selectively in β-cells of *Glp1r*$^{-/-}$ mice. Islets from Pdx1-hGLP-1R:*Glp1r*$^{-/-}$ mice exhibited restoration of "normal" levels of hGLP-1R expression and action in β-cells, as judged by cAMP, protein, and gene expression responses to GLP-1 in isolated islets (15). Remarkably, the abnormal glucose tolerance phenotype of *Glp1r*$^{-/-}$ mice was completely normalized by selective rescue of hGLP-1R expression in β-cells. Hence, despite the importance of neural GLP-1R signaling networks for aspects of GLP-1 action relevant to control of appetite and body weight, the β-cell, and not the brain, is essential for GLP-1R–dependent glucoregulation.

GLP-1 CONTROLS β-CELL PROLIFERATION AND SURVIVAL

Subsequent studies led us to examine actions of GLP-1 in β-cells beyond regulation of insulin secretion. Yazhou Li et al. (16) injured β-cells with streptozotocin (STZ) in mice treated with or without exendin-4 for 10 days and then observed the mice without additional interventions for 3 weeks. To our surprise, mice that received STZ and transient administration of exendin-4 exhibited lower glucose and higher insulin levels 3 weeks after the last dose of exendin-4 (16). Li et al. (16) demonstrated that exendin-4 reduced β-cell apoptosis in mice, findings extended by Philippe Halban, who demonstrated in collaborative studies that exendin-4 directly reduced apoptosis in isolated purified populations of rat β-cells exposed to a cytokine cocktail. Remarkably, *Glp1r*$^{-/-}$ mice exhibited enhanced sensitivity to β-cell injury, with higher levels of β-cell apoptosis and more severe hyperglycemia after administration of STZ (16). Collectively, these findings, together with studies from the Brubaker laboratory (17), established the pharmacological and physiological importance of GLP-1R signaling for cell survival, a paradigm replicated in studies of GLP-1R^{+} neurons in the CNS (18).

A simultaneous set of experiments, pioneered by Doris Stoffers working with Susan Bonner-Weir and colleagues (19) in Boston, examined whether GLP-1R agonists stimulated β-cell proliferation and expanded β-cell mass. Indeed, in the experimental context of mild hyperglycemia or insulin resistance, GLP-1R agonists potently increased the number of proliferating β-cells and increased islet and β-cell mass (20,21). The results of numerous preclinical studies fostered hope that GLP-1R agonists would exert disease-modifying effects in human studies, leading to preservation or enhancement of β-cell function. Nevertheless, evidence for a similar durable protective effect of GLP-1R agonists in human subjects remains scant (22), and we have learned that older human β-cells from individuals with diabetes exhibit a reduced capacity for proliferation relative to young rodent β-cells (22).

A complementary physiological question that arose from these studies was whether endogenous incretin receptor signaling is critical for the adaptive response to hyperglycemia and insulin resistance. The answer depends on the specific experimental model under study. Louise Scrocchi et al. (23) examined the importance of endogenous *Glp1r* signaling for the robust expansion of β-cell mass that occurs in leptin-deficient *ob/ob* mice. Remarkably, fasting glucose, glucose tolerance, and the extent of islet hyperplasia were identical in *ob/ob*:*Glp1r*$^{+/+}$ versus *ob/ob*:*Glp1r*$^{-/-}$ mice. In contrast, Tanya Hansotia et al. (24) found that *Gipr*$^{-/-}$ or *Glp1r*$^{-/-}$ mice exhibited significant defects in the expansion of β-cell mass and impaired upregulation of insulin biosynthesis and secretion after several months of high-fat feeding. Hence, under some circumstances, endogenous incretin receptor signaling is essential for the adaptive response to hyperglycemia and insulin resistance.

The actions of GLP-1R agonists to expand β-cell mass, increase insulin secretion, and yet sustain β-cell survival made us wonder whether GLP-1R signaling engaged pathways regulating endoplasmic reticulum (ER) stress.

Bernardo Yusta et al. (25) found that *db/db* mice exhibited a significant induction of molecular markers of ER stress in their β-cells, yet concomitant treatment with exendin-4 reduced splicing of XBP-1 and decreased expression of the pivotal transcription factor CHOP, while simultaneously increasing levels of insulin and the prosurvival factor *Irs2*. Yusta et al. demonstrated that glucose-dependent insulinotropic polypeptide (GIP) or exendin-4 attenuated molecular features of ER stress in isolated β-cells. By inducing a complex molecular cascade involving induction of ATF-4, CHOP, and GADD34, activation of incretin receptor signaling attenuated the phosphorylation of eIF2-α, relieving the translational repression of insulin synthesis that might otherwise occur in β-cells undergoing ER stress (25). Hence, GLP-1R agonists uniquely augment β-cell function, simultaneously increasing the expression of molecules required for differentiated β-cell function and enhancing insulin biosynthesis and secretion while triggering pathways ensuring the survival and/or proliferation of stressed β-cells.

GLP-1 AND NEURAL PATHWAYS

Most studies of GLP-1 action use pharmacological administration of GLP-1R agonists, resulting in circulating levels that are 2- to 10-fold higher than levels observed for native GLP-1. An ongoing question asks whether the classical actions of native GLP-1 reflect its endocrine actions as a circulating hormone or whether recruitment of CNS GLP-1R circuits mediates some of the actions of endogenous GLP-1. Circulating levels of GLP-1 are higher in the portal than in the systemic circulation, and elegant studies by Remy Burcelin et al. (26) demonstrated the existence of a gut-brain GLP-1R signaling system that triggers glucose disposal in mice independent of classical GLP-1 action on the β-cell. Remarkably, it still remains unclear whether native GLP-1 exerts some of its metabolic actions by communicating with the brain via ascending neural pathways, or in part, by direct access of GLP-1Rs in different regions of the CNS.

With the emergence of high molecular weight (HMW) GLP-1R agonists for the treatment of diabetes, we asked whether larger GLP-1R agonists inhibited gastric emptying and induced satiety and weight loss, reflecting engagement of CNS GLP-1Rs. Our laboratory used four structurally unique HMW GLP-1R agonists, CJC1131, CJC1134, albiglutide, and CNTO736 (20,21,27,28), to study the biology of large GLP-1R proteins. Remarkably, despite a presumed inability to rapidly and efficiently penetrate the brain, HMW GLP-1R agonists displayed the full range of actions exhibited by smaller peptide agonists, including activation of neuronal c-Fos expression, inhibition of gastric emptying, and reduction of food intake, ultimately resulting in weight loss with chronic administration. The findings of weight loss have subsequently been confirmed in clinical studies examining HMW GLP-1R agonists, yet the mechanisms and pathways remain enigmatic and require more careful elucidation.

DPP-4

Our laboratory has been interested in the biology of DPP-4, starting at a comparatively early juncture. We were motivated to study the biology of DPP-4i and their role in controlling glucose homeostasis by William Bachovchin, a pioneering chemist with a long track record of innovation in the chemistry and biology of serine proteases. Although we never published a single paper with Bill, our collaboration led to a series of nine patents describing the use of DPP-4i for the treatment of diabetes. Coincidentally, around the same time, we were approached by Nicolai Wagtmann and Didier Marguet, who had inactivated the *Dpp4* gene to study the immune system in mice. Remarkably, they were unable to obtain compelling evidence for an immune-related phenotype in $Dpp4^{-/-}$ mice. In contrast, metabolic studies demonstrated that $Dpp4^{-/-}$ mice are healthy and exhibit increased levels of intact GLP-1 and GIP, augmentation of insulin secretion, and improved glucose homeostasis (29). At the time, it was unclear whether DPP-4 could be safely targeted for the treatment of diabetes because of its alleged primacy as a key regulator of immune function. The robust metabolic phenotype of otherwise healthy $Dpp4^{-/-}$ mice provided important validation of DPP-4 as a potential target that could be selectively and safely inhibited for therapeutic potentiation of incretin action.

Remarkably, despite the astonishing success of DPP-4i in the clinic, understanding the pleiotropic metabolic actions of these drugs remains challenging, partly due to the potential complexity of their substrates (30). We subclassify DPP-4 substrates as pharmacological or physiological (30). Our laboratory defines a physiological DPP-4 substrate as one whose relative levels of intact versus cleaved peptide are significantly different in an animal or human treated with a DPP-4i, or in an animal with genetic inactivation of *Dpp4*. In contrast, a pharmacological substrate is cleaved by DPP-4 ex vivo, but evidence for cleavage of the endogenous peptide in vivo may be lacking (30). Given the low circulating and tissue levels of most DPP-4 substrates and the technical challenge inherent in quantifying intact versus cleaved peptides, it seems likely that some DPP-4 substrates currently classified as pharmacological may one day also be found to be cleaved by DPP-4 in vivo (30).

Tanya Hansotia asked a simple, clinically relevant question, namely, "How do DPP-4i lower blood glucose?" Hansotia et al. (31) administered oral glucose in separate experiments with four structurally distinct DPP4i to mice and found that DPP-4i lowered blood glucose in wild-type mice and in mice without the GIP receptor (GIPR) or GLP-1R. However, none of the DPP-4i lowered glucose or stimulated insulin secretion in a mouse with inactivation of both the *Glp1r* and the *Gipr*, the double incretin receptor knockout (DIRKO) mouse (31). These findings (Fig. 2) unequivocally demonstrated that despite the potential glucoregulatory pharmacological substrates of DPP-4 (30), the

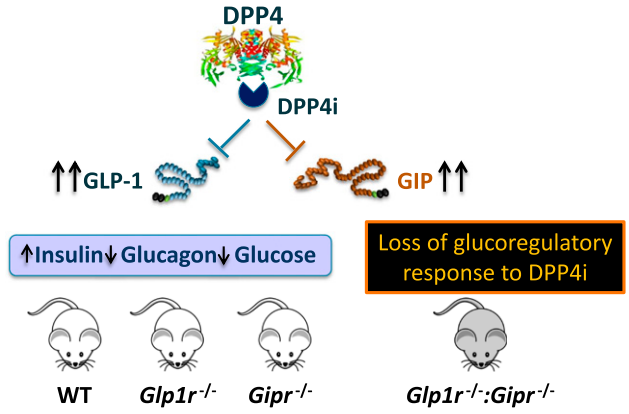

Figure 2—Mechanisms through which DPP-4i lower blood glucose. DPP-4i prevent the degradation of GLP-1 and GIP, thereby augmenting signaling through their cognate receptors (GLP-1R and GIPR), resulting in stimulation of insulin and inhibition of glucagon secretion. DPP-4i continue to lower glucose in single incretin receptor knockout mice (*Glp1r*$^{-/-}$ and *Gipr*$^{-/-}$) but no longer exert any glucose-lowering activity in mice with combined genetic disruption of both incretin receptors (*Glp1r*$^{-/-}$:*Gipr*$^{-/-}$) (31,32). WT, wild-type.

two classical incretin receptors account for the glucose lowering in response to inhibition of DPP-4.

Because this was an acute experiment in normoglycemic mice, Grace Flock et al. (32) treated wild-type high-fat–fed diabetic or DIRKO mice with vildagliptin for 8 weeks. Flock et al. observed that DIRKO mice no longer exhibited a glucose-lowering or insulin-stimulatory response to chronic vildagliptin therapy (32). Hence, although numerous substrates have been invoked as potentially important for DPP-4–dependent glucoregulation, our studies using acute or chronic DPP-4 inhibition in normoglycemic and diabetic mice definitively demonstrate that the GLP-1Rs and GIPRs mediate the glucoregulatory responses to DPP-4i (31,32).

To extend our understanding of the biology of DPP-4 beyond glucose control, Adriano Maida et al. (33) examined how DPP-4 controlled β-cell survival. They administered STZ to normal or DIRKO mice. The DPP-4i sitagliptin potently reduced β-cell apoptosis in wild-type mice but was unable to reduce β-cell apoptosis in DIRKO mice (33). Maida et al. (8) went on to show that DPP-4i also exhibit an unexpectedly favorable glucoregulatory interaction with the biguanide metformin. Maida et al. demonstrated that metformin acutely increased GLP-1 secretion and acted as an incretin sensitizer, both directly in islet cells in vitro and in mice. Metformin increased the expression of the GLP-1Rs and GIPRs in mouse islets, actions requiring the transcription factor peroxisome proliferator–activated receptor-α (8). Hence, combining DPP-4 inhibitors with metformin for the treatment of diabetes represents a logical approach to therapy through unexpected mechanistic interactions; however, the importance and contribution of the metformin-incretin axis for glucose reduction in humans with diabetes has not yet been unambiguously defined.

GLP-2: A NOVEL THERAPY FOR SHORT BOWEL SYNDROME

Although our laboratory focuses predominantly on gut hormones exerting metabolic actions, our longstanding interest in the products of the proglucagon gene led us to discover the actions of the second major GLP, designated GLP-2. The GLP-2 sequence was revealed in the early 1980s by the cloning of the proglucagon cDNAs and genes; for many years, however, we had no idea whether GLP-2 exhibited biological activity.

Ying Lee, my first postdoctoral fellow, generated a transgenic mouse expressing the oncoprotein SV40 T antigen under the control of the rat proglucagon promoter. This transgene was expressed in islets and gut endocrine cells, and these glucagon promoter T antigen (GLUTag) mice consistently developed glucagon-immunopositive endocrine tumors in the colon (34). To generate a stable immortalized gut endocrine cell line from these tumors, we injected tumor fragments subcutaneously in nude mice. We noticed that whenever we passaged a glucagon-producing tumor subcutaneously in nude mice, we detected marked enlargement of the small bowel and hypoplasia of glucagon-producing α-cells in the pancreatic islets (35). We reproduced the findings of small-bowel growth using two different islet glucagon-producing cell lines (InR1-G9 and RIN1056A) and a small bowel–derived glucagon-producing gut endocrine cell line (STC-1) (36).

We postulated, as had many others before us, that one or more proglucagon-derived peptides exhibited bowel growth factor activity. Accordingly, we injected individual synthetic peptides derived from proglucagon into mice and assessed small-bowel growth. Excitingly, glicentin, the 69 amino acid protein containing the sequence of glucagon and extensions at the amino and carboxy termini, induced small-bowel growth. However, GLP-2, the 33 amino acid GLP located immediately adjacent to GLP-1 in proglucagon, was even more potent than glicentin (36). Remarkably, this simple experiment, assessing small-bowel weight after administration of a peptide hormone, was the first description of the biological activity of GLP-2 and led to multiple patent applications. After considerable clinical testing, a GLP-2R agonist, teduglutide, was approved for the treatment of short bowel syndrome (37).

NONGLYCEMIC ACTIONS OF GLP-1: FOCUS ON THE CARDIOVASCULAR SYSTEM

Although most of the effort devoted to the study of incretin-based therapies has focused on mechanisms related to glucose control, GLP-1R agonists and DPP-4i both exert a large number of nonglycemic actions, many with clinical relevance (14). Among the nonglycemic targets attracting the most attention, the cardiovascular system has been a major focus of our recent studies. We were fortunate to collaborate with Mansoor Husain and his laboratory, demonstrating that *Glp1r*$^{-/-}$ mice exhibited defective ventricular function after insulin or epinephrine administration (38). In subsequent studies, Kiwon Ban

et al. (39) demonstrated that native GLP-1 and GLP-1(9-36) both improved ventricular function in ischemic ventricles ex vivo, through non–GLP-1R–dependent mechanisms of action, likely through regulation of blood flow. Hossein Noyan-Ashraf et al. (40) examined the cardioprotective mechanisms of action of liraglutide in normoglycemic and diabetic mice with ligation of the left anterior descending coronary artery. Remarkably, liraglutide produced a cardioprotective gene and protein expression profile in the normal and ischemic mouse heart, reduced infarct size, and induced a significant increase in survival (40). Cardioprotective effects of liraglutide were also demonstrated in a mouse model of high-fat feeding obesity-related cardiomyopathy. Liraglutide reduced cardiac inflammation and ER stress and improved ventricular function, findings reversed by treatment of mice with an inhibitor of AMP-activated protein kinase (41).

In complementary studies, Meghan Sauvé et al. (42) showed that nondiabetic *Dpp4*$^{-/-}$ mice exhibited a cardioprotective phenotype after left anterior descending coronary artery ligation. Sauvé et al. subsequently treated diabetic mice with sitagliptin or metformin for several weeks and then assessed the response to ischemic myocardial injury. Remarkably, sitagliptin and metformin both produced a cardioprotective gene and protein expression program in the mouse heart and improved survival in diabetic mice with myocardial infarction. Sauvé et al. asked whether direct inhibition of DPP-4 in ischemic hearts ex vivo was sufficient to improve recovery of left ventricular–developed pressure. Although the intracoronary artery infusion of sitagliptin had no direct effect on ventricular function, treatment of mice with sitagliptin in vivo subsequently improved recovery of developed pressure in ischemic hearts ex vivo. Hence, sitagliptin (a prototype DPP-4i) does not exert direct cardioprotective actions and likely mediates its effects on the heart through one or more cardioactive substrates, including GLP-1 (42).

Our laboratory has also undertaken studies to examine the actions of GLP-1R agonists and DPP-4i on nonglycemic actions (Fig. 3) relevant to cardiovascular disease, including effects on inflammation (43), lipid metabolism (44), and blood pressure (45). The surprising demonstration that *Glp1r* expression is localized to the atrium, and not the ventricles (45), has prompted reexamination of how GLP-1R agonists exert their cardioprotective actions. To address this question, John Ussher et al. (46) generated mice with cardiomyocyte (CM)-specific inactivation of the *Glp1r* using conditional expression of Cre recombinase under the control of the myosin heavy chain promoter. *Glp1r*$^{CM-/-}$ mice exhibit ~90% reduction of atrial *Glp1r* expression and normal cardiac structure and function (46). Levels of several genes and proteins in the ventricle, and to a greater extent in the atria, were expressed at different levels in RNA isolated from *Glp1r*$^{CM-/-}$ hearts; however, the responses to experimental myocardial infarction or doxorubicin-induced cardiomyopathy were comparable in *Glp1r*$^{CM-/-}$ versus *Glp1r*$^{CM+/+}$ mice. Surprisingly, exogenous administration of liraglutide for 1 week still produced robust cardioprotection in *Glp1r*$^{CM-/-}$ mice, with decreased infarct size and significantly increased survival compared with saline-treated controls (46). These findings demonstrate that the cardioprotective actions of GLP-1R agonists are not

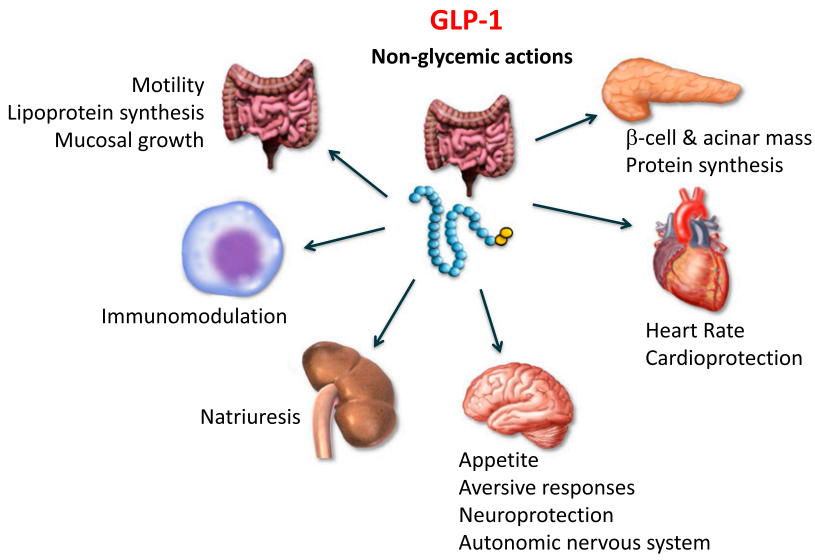

Figure 3—Nonglycemic actions of GLP-1 on organs that express a functional GLP-1R. GLP-1R agonists produce multiple nonglycemic actions through indirect and direct actions. Here, we highlight organs and cell types shown to directly express the canonical GLP-1R, which, when activated, produces nonglycemic actions, as outlined. Readers wishing a more detailed overview of these actions are referred to several recent reviews (14,30,59,60).

mediated by CM GLP-1Rs. Ussher et al. next examined whether loss of the atrial GLP-1R perturbed the acute control of heart rate in *Glp1r*$^{CM-/-}$ mice, mindful of previous experiments done in our collaboration with Joel Elmquist and Hiroshi Yamamoto and colleagues (47,48) that identified the importance of sympathetic nervous system activation for the GLP-1R–dependent increase in heart rate (HR) in rodents. Ussher et al. found that liraglutide acutely increased HR in *Glp1r*$^{CM-/-}$ mice, likely due to preservation of GLP-1R–dependent circuits controlling sympathetic and parasympathetic tone and HR. Furthermore, food intake, a known stimulus for GLP-1 secretion and HR, increased the HR to a comparable extent in *Glp1r*$^{CM-/-}$ versus *Glp1r*$^{CM+/+}$ mice. In contrast, the basal HR was significantly lower in *Glp1r*$^{CM-/-}$ mice (46). Hence, the atrial CM GLP-1R is not critical for pharmacological or physiological cardioprotection or the response to cardiomyopathic injury but is essential for the normal control of HR in mice.

NONGLYCEMIC PANCREATIC ACTIONS OF INCRETIN HORMONES

A major challenge for the GLP-1 field has been the elucidation of the cellular localization of GLP-1R expression in different organs, including the exocrine pancreas. We observed that multiple anti-sera, widely used to detect GLP-1R–immunoreactive proteins, exhibit problems with sensitivity and, to a greater extent, specificity (49), findings confirmed independently by others (50). For example, we were unable to detect RNA transcripts corresponding to the full-length *Glp1r* mRNA transcript in mouse hepatocytes or macrophages or cardiac ventricles, despite multiple reports claiming GLP-1R expression in these cell types using incompletely validated anti-sera or RT-PCR analyses of small cDNA fragments of the *Glp1r*. We suspect that some of the "direct" mechanisms attributed to GLP-1R agonists in cell types, including hepatocytes, macrophages, ventricular CMs, and renal tubular cells, among others, may be incorrect.

Although GLP-1R expression has been localized to pancreatic acinar and ductal cells and to cells within foci of pancreatic intraepithelial neoplasia and pancreatic cancers, controversy surrounds the precise localization of GLP-1R expression in pancreas of rodents and humans (51). Furthermore, clinical reports linking the use of GLP-1R agonists and, to a lesser extent, DPP-4i, to pancreatitis in humans have fostered a great deal of research into potential underlying mechanisms; yet, mechanisms linking GLP-1R signaling or DPP-4 inhibition to development of pancreatitis remain elusive (52). We have been unable to elucidate a pathway linking activation of the pancreatic GLP-1R to increased inflammation; unexpectedly, Jackie Koehler et al. (53) observed that GLP-1R agonists or DPP-4i have no effect in experimental models of pancreatitis or produce pancreatic gene expression profiles strongly suggestive of reduced inflammation.

Equally provocative are reports that GLP-1R agonists enhance cell proliferation in the exocrine pancreas. Our laboratory has studied the mouse pancreas for several decades, and we have assessed whether GLP-1R agonists promote the proliferation or survival of human pancreatic cancer cell lines (54). Koehler et al. (54) did not detect proliferative or cytoprotective actions of GLP-1R agonists in human pancreatic cancer cells, which express functional GLP-1Rs. Nevertheless, we have observed a small but significant increase in the total mass of the pancreas after treatment with GLP-1R agonists (21,53,55). We knew from the earlier work of Lamont et al. (15) in our laboratory that insulin was not sufficient to promote GLP-1R–dependent expansion of pancreatic mass, because *Glp1r*$^{-/-}$ mice with selective restoration of the human GLP-1R in β-cells did not exhibit increased pancreatic mass after treatment with GLP-1R agonists.

Koehler et al. (56) examined how GLP-1R activation increases the mass of the pancreas. Although GLP-1R agonists selectively increased the mass of the exocrine pancreas, we could not detect an increase in DNA content, or increased cell proliferation in the mouse pancreas at multiple time points during concurrent administration of exendin-4 or liraglutide. Similarly, examination of pancreatic wet weight and dry weight revealed that edema or water content was reduced in the pancreas of exendin-4–treated mice. In contrast, protein content was increased and the DNA-to-protein ratio was significantly reduced in mice treated with GLP-1R agonists.

Consistent with these findings, rapamycin, an inhibitor of mammalian target of rapamycin (mTOR), completely abrogated the GLP-1R agonist–mediated induction of pancreatic protein synthesis and eliminated the increase in pancreatic mass in vivo. Analysis of the protein expression profile in the mouse pancreas using tandem mass spectroscopy allowed us to deduce the identity of multiple proteins preferentially induced by GLP-1R agonists, including a subset of proteins important for protein translation. In contrast, we did not detect increases in abundance or activity of amylase or lipase (56). Whether these observations in mice are applicable to primates or humans remains unclear, and further work is needed to determine whether GLP-1 induces pancreatic protein synthesis in other species. Although clinical trial data support a modest link between the use of GLP-1R agonists and a small increased risk of pancreatitis, the results of recent cardiovascular outcome studies have failed to conclusively link the use of DPP-4i with excess pancreatitis. Furthermore, the incidence of medullary thyroid cancer and pancreatic cancer is too low for meaningful conclusions to be drawn about putative risks from incretin therapies using data from individual cardiovascular outcome studies.

THE FUTURE OF INCRETIN-BASED SCIENCE

The first 30 years of incretin-based science have witnessed an explosive growth in our understanding of how GLP-1 controls glucose homeostasis and body weight. Nevertheless,

Table 1—The future of incretin-based therapies

Role of GLP-1 in obesity?
GLP-1 in prediabetes?
GLP-1 in type 1 diabetes?
GLP-1 and pediatric indications in type 1 diabetes and T2D?
GLP-1 action in neuroprotection and neurodegeneration
Therapeutic potential of GLP-1 in diseases of inflammation?
GLP-1 and fatty liver disease—therapeutic potential and MOA?
Cardiovascular indications and MOA?
GLP-1, oxidative stress, and microvascular diseases in nerves, eyes, and kidney?
Coagonists? Triagonists? Insulin combinations?
Safety of GLP-1 therapies—inflammation, cancer, and cardiovascular events?

MOA, mechanism of action; T2D, type 2 diabetes.

the widespread distribution of extrapancreatic GLP-1Rs has been accompanied by delineation of multiple nonglycemic actions of incretin hormones (Fig. 3), many of which may have considerable clinical relevance (Table 1). The therapeutic potential of GLP-1–based therapies may now be extended to the treatment of obesity, raising new scientific questions relevant to mechanisms of action in the CNS and introducing additional questions about long-term safety in a nondiabetic population. The observations that small peptide GLP-1R agonists, such as liraglutide, directly penetrate the arcuate nucleus through mechanisms requiring a functional GLP-1R raises intriguing questions surrounding the use of higher MW GLP-1R agonists for the induction of weight loss in diabetic and/or obese subjects (57). GLP-1R agonists may also be useful in subjects with type 1 diabetes, reducing glucagon secretion and gastric emptying while controlling body weight without incurring a greater risk of hypoglycemia. Evidence from preclinical studies demonstrates that activation of GLP-1R signaling may be neuroprotective (14,18), and a randomized controlled clinical trial of exenatide in human subjects with Parkinson disease (58) provides support for the neuroprotective potential of GLP-1R signaling in human subjects.

Although the GLP-1R is widely expressed in the peripheral immune system, the mechanisms through which GLP-1R signaling attenuates inflammation in multiple tissues require additional study (14,22). Similarly, GLP-1R agonists reduced liver fat in preclinical and human studies, yet the available evidence does not support a direct role for GLP-1R signaling in hepatocytes (49); hence, the mechanisms linking activation of the GLP-1R to improvement of lipid metabolism in hepatocytes remain enigmatic. Equally elusive are the mechanisms through which GLP-1R agonists protect the heart, most notably in preclinical studies (59). The findings that atrial, but not ventricular CMs express the GLP-1R (45), coupled with experiments demonstrating that GLP-1R agonists still produce cardioprotection despite attenuation of CM GLP-1R signaling (46), highlights major gaps in our knowledge of the direct and indirect actions of GLP-1R agonists on the heart.

Rapid progress continues to emanate from efforts to develop more potent GLP-1R agonists, including coagonists, triagonists, and chimeric molecules enabling delivery of steroid hormones to GLP-1R$^+$ cell types. The incretin field has not been without controversy, enveloping topics spanning inflammation (60), medullary thyroid cancer, pancreatitis, pancreatic cancer, tachycardia, and the potential development of pancreatic endocrine tumors. In each instance, rigorous basic and clinical science has helped illuminate data that are correct and reproducible versus findings that may be limited in significance to specific models and species or that do not hold up to widespread scrutiny. Ongoing cardiovascular outcome studies examining the cardiovascular safety of DPP-4i and GLP-1R agonists in tens of thousands of subjects will greatly expand our knowledge of the safety and risk-versus-benefit considerations of incretin-based therapies. Our laboratory has been privileged to work with large numbers of scientists and clinicians in the academic and pharmaceutical sectors, allowing us to play a small role in unraveling the mysteries of incretin biology, and for this opportunity, we are immensely grateful.

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