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COMMENTARY

Incretin concept revised: The origin of the insulinotropic function of glucagon-like peptide-1 – the gut, the islets or both?

Incretins comprise a pair of gut hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which are secreted in response to food ingestion and enhance glucose-dependent insulin secretion from pancreatic β-cells. Immediately after secretion, GLP-1 is degraded by dipeptidyl peptidase-4 more rapidly than GIP, and circulating levels of biologically intact GLP-1 are substantially lower than those of biologically intact GIP. Therefore, there has been a debate on how the gut-derived GLP-1 exerts insulinotropic actions. Recent publications have revealed two novel mechanisms by which GLP-1 exerts insulinotropic actions: (i) the gut-derived GLP-1 activates receptors expressed in nodose ganglions, thereby potentiating glucose-dependent insulin secretion through the vagus nerves; and (ii) the pancreatic α-cell-derived GLP-1 activates receptors expressed in β-cells in a paracrine manner. While the relative contributions of the two mechanisms under normal and pathological conditions remain unknown and mechanisms regulating GLP-1 secretion from α-cells need to be investigated, the available data strongly indicate that the effects of GLP-1 on insulin secretion are far more complex than previously believed, and the classical incretin concept regarding GLP-1 should be revised.

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More than 100 years ago, the incretin concept was proposed and was later revised to include incretin-based drugs, including dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists, both of which are currently used in the management of type 2 diabetes worldwide.[1] Inspired by Bayliss and Starling's discovery of secretin, Moore *et al.*[1] hypothesized that gut extracts contain a hormone or hormones that regulate the endocrine pancreas, and showed in 1906 that gut extracts reduce urinary glucose excretion in individuals with diabetes, possibly by stimulating the endocrine pancreas. La Barre[1] succeeded in biochemical purification of the glucose-lowering element from gut extracts in 1929, which he named incretin (INtestine seCRETtion INsulin). Later, studies found that incretin comprises a pair of intestinal hormones, glucose-dependent insulinotropic polypeptide (GIP) and GLP-1, and confirmed that i.v. infusions of GLP-1 and GIP at pharmacological levels enhance glucose-dependent insulin secretion in humans.[1] In addition, studies in mice revealed that both GIP and GLP-1 exert their insulinotropic effects through their specific receptors, the GIP receptor and the GLP-1 receptor (GLP-1R), and that simultaneous genetic ablation of GIP receptor and GLP-1R in mice abolished the potentiation of glucose-induced insulin secretion (GIIS) in response to oral glucose load.[1] Studies in mice also showed the molecular mechanisms regulating secretions of GIP and GLP-1 from the K- and L-cells of the duodenum in response to ingestion of various nutrients.[2] Together, these data confirmed the critical role of GIP and GLP-1 as incretins that mediate the entero-insular axis and elucidated the classical incretin concept (Figure 1). However, there has been debate on how GLP-1 exerts insulinotropic action. GLP-1 is degraded by DPP-4 after its secretion from the gut ($t_{1/2}$ = ~1.5 min) more rapidly than GIP ($t_{1/2}$ = ~5 min).[1] Our group and others observed that circulating preprandial and postprandial concentrations of biologically intact GLP-1 determined by the revised GLP-1 immunoassay are substantially lower (~1–3 pmol/L) than those of biologically intact GIP (~10–100 nmol/L) determined by a similar immunoassay.[1,3] As GLP-1R and GIP receptor have similar EC_{50} values for cyclic adenosine monophosphate production (10–100 pmol/L), it is still unclear how the gut-derived GLP-1 exerts insulinotropic actions in response to ingestion of various nutrients.

A first model was developed by Waget *et al.*,[4] who showed that selective DPP-4 inhibition in the gut by administering low doses of the DPP-4 inhibitor, sitagliptin, improved glucose tolerance and insulin secretion with a concomitant increase in vagus nerve activity in normal diet-fed mice subjected to oral glucose load. That study suggested that the gut-derived GLP-1 activates GLP-1R expressed in nodose ganglion neurons, thereby potentiating GIIS through the vagus nerves, and that local DPP-4 inhibition and the subsequent increase of biologically intact GLP-1 locally in the gut is sufficient to improve glucose tolerance and insulin secretion in response to oral glucose ingestion. Consistent with this model, Smith *et al.*[5] showed that β-cell-specific GLP-1R deletion had little effect on glucose tolerance and insulin secretion in response to oral glucose load in the presence or absence of the

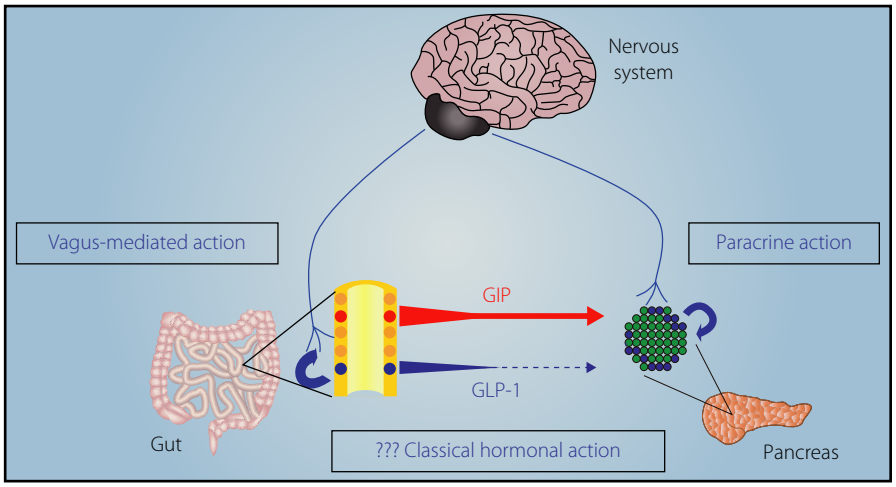

Figure 1 | Models of glucagon-like peptide-1 (GLP-1) insulinotropic actions. The classical incretin concept suggests that glucose-dependent insulinotropic polypeptide (GIP; red arrow) and GLP-1 (blue arrows) secreted from the gut travel through the portal vein and the liver, eventually reaching the pancreatic β-cells, where they bind to their specific receptors, GIP receptor and GLP-1 receptor, to enhance glucose-induced insulin secretion. Because of the rapid inactivation of GLP-1 by dipeptidyl peptidase-4, the circulating levels of biologically intact GLP-1 are extremely low compared with those of GIP. Recent studies using genetically engineered mice clearly show two novel mechanisms for GLP-1 to exert insulinotropic actions: (i) the gut-derived GLP-1 activates its receptor expressed in nodose ganglions, thereby potentiating glucose-induced insulin secretion through the vagus nerves (vagus-mediated action); and (ii) the pancreatic α-cell-derived GLP-1 activates its receptor expressed in β-cells in a paracrine manner (paracrine action). While the relative contributions of the two mechanisms under normal and pathological conditions remain unknown, the available data strongly indicate that mechanisms of GLP-1 on insulin secretion are far more complex than previously expected, and the classical incretin concept regarding GLP-1 (??? classical hormonal action) needs to be revised.

DPP-4 inhibitor, vildagliptin. Iida *et al.*[6] also showed that the DPP-4 inhibitor anagliptin improved oral, but not intraperitoneal, glucose tolerance. Following up these observations, Mulvihill *et al.*[7] recently reported that genetic ablation of enterocyte DPP-4, which accounts for a substantial amount of gut DPP-4, did not produce alterations in GLP-1 levels and glucose tolerance in response to oral glucose load. However, they found that endothelial DPP-4 contributed 25–50% of soluble plasma DPP-4 activities and GLP-1 degradation, and that endothelial DPP-4 ablation improved glucose tolerance in high-fat diet-fed mice, but not in normal diet-fed mice after an oral glucose load. Importantly, low-dose sitagliptin, used in the Waget study,[4] no longer improved glucose tolerance and insulin secretion in endothelial DPP-4-ablated mice on high-fat diets, suggesting that endothelial, but not enterocyte, DPP-4 plays a critical role in regulation of local GLP-1R activation and the subsequent insulinotropic effects in insulin-resistant conditions often observed in type 2 diabetes patients. However, high-dose sitagliptin, which presumably inhibits systemic DPP-4, improved glucose tolerance and insulin secretion in endothelial DPP-4-ablated mice on high-fat diets, suggesting that DPP-4 sources other than endothelial cells also play a role in the GLP-1-mediated insulinotropic actions.

Another model came from the study by Ellingsgaard *et al.*,[8] in which GLP-1 was shown to be secreted from pancreatic α-cells and to activate GLP-1R on β-cells, thereby exerting insulinotropic actions in a paracrine manner. For many years, it was postulated that GLP-1 is produced from preproglucagon peptide through proteolytic processing catalyzed by prohormone convertase (PC)1/3 expressed in the L-cells, and that GLP-1 is not produced in the α-cells expressing PC2, which generates glucagon from the same preproglucagon peptide. Interestingly, Ellingsgaard showed that α-cells express PC1/3 in response to muscle-derived interleukin-6, as well as fat-derived interleukin-6, thereby producing GLP-1.[8] Following up these observations, Chambers *et al.*[9] recently reported a critical role of the α-cell-derived GLP-1 in glucose homeostasis. Chambers *et al.*[9] generated genetically engineered preproglucagon gene-deficient mice with the tissue-specific reactivable preproglucagon gene. They showed that the GLP-1R antagonist exendin(9-39)amide deteriorated post-challenge glucose excursion in normal diet-fed mice with the preproglucagon gene reactivated in the pancreas, suggesting that the α-cell-derived GLP-1 plays a critical role in the maintenance of postprandial glucose homeostasis. Surprisingly, they showed that exendin(9-39)amide had little effect on post-challenge glucose excursion in normal diet-fed mice with the preproglucagon gene reactivated in the gut, suggesting that the gut-derived GLP-1 is dispensable for the maintenance of postprandial glucose homeostasis in physiological conditions. They also showed that exendin(9-39)amide had little effect on post-challenge glucose excursion in normal diet-fed mice deficient in GLP-1R in neuronal and glial cells, suggesting that

the vagus-mediated GLP-1 actions are physiologically dispensable in postprandial glucose homeostasis. Another recent study independently clarified an important role of the α-cell-derived GLP-1 in glucose homeostasis. Traub *et al.*[10] studied α-cell-specific GLP-1 deficiency by establishing mice with genetic ablation of PC1/3 in α-cells. These mice can address the role of the α-cell-derived GLP-1 more specifically, as the abovementioned mice with the preproglucagon gene reactivated in the gut are deficient in not only GLP-1 but also glucagon, which substantially affects insulin secretion and glucose tolerance. Using the α-cell-specific GLP-1 deficient mice, they showed that intraperitoneal glucose tolerance and insulin secretion were unaffected in these mice on normal diets, but impaired when these mice were subjected to metabolic stress, such as high-fat diets and β-cell toxin streptozotocin. Interestingly, they showed that GIIS was severely impaired in isolated islets from the same α-cell-ablated mice, and that GLP-1 supplementation significantly restored GIIS from these islets. These results together show an important role of the α-cell-derived GLP-1 in GIIS, especially under metabolic stress. Unfortunately, as they did not examine oral glucose tolerance in the α-cell-specific GLP-1-deficient mice, relative contributions of the gut-derived GLP-1 and the α-cell-derived GLP-1 in postprandial insulin secretion and glucose excursion remain unknown, and this should be evaluated under normal and various pathological conditions. In addition, it is largely unknown what triggers GLP-1 secretion from α-cells other than interleukin-6.

A series of experiments using various genetically manipulated mouse models showed that the physiological mechanisms underlying the effects of GLP-1 on insulin secretion are far more complex than previously expected, and the classical incretin concept with regard to GLP-1 needs to be revised. The vagus-mediated insulinotropic action of the gut-derived GLP-1 and that of the α-derived GLP-1 seemingly contribute to maintenance of normal glucose homeostasis with varying degrees depending on different metabolic conditions in mice. While the current findings await evaluation in humans, an obvious question is why Nature developed such a complicated system to regulate insulin secretion and glucose homeostasis. Further analysis is clearly required to fully elucidate the incretin system.

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