Mechanisms underlying glucose-dependent insulino tropic polypeptide and glucagon-like peptide-1 secretion

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INTRODUCTION
Glucose-dependent insulino tropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are gut hormones secreted from specialized enteroendocrine cells within the intestinal epithelium. They are released postprandially and act as circulating markers of food consumption, enabling the body to respond appropriately to food-derived elevations of blood nutrient concentrations. This is crucial for the control of blood glucose concentrations, as co-stimulation of pancreatic β-cells by GIP and GLP-1 approximately doubles the amount of insulin released in response to an elevation in ambient (blood) glucose concentrations. GIP and GLP-1 are hence often termed ‘incretins,’ and underlie the ‘incretin effect’ – a well-documented observation that intravenous glucose infusion at a rate that simulates postprandial blood glucose levels triggers only about half as much insulin release as a matched oral glucose challenge. After the discovery that the insulino tropic effect of GLP-1 is preserved in most people with type 2 diabetes1, GLP-1 mimetics and inhibitors of GLP-1 degradation by dipeptidyl peptidase 4 have been developed and licensed for the treatment of type 2 diabetes2.

Although a wealth of evidence supports the idea that both GLP-1 and GIP underlie the incretin effect, there are important differences in the activity and plasma profiles of the two hormones. GIP, for example, stimulates glucagon secretion from pancreatic α-cells, whereas GLP-1 inhibits α-cell activity. GLP-1, in contrast, has anorexigenic properties, whereas GIP seems to have no effects on food intake. GIP is instead considered pro-adipogenic, based on several observations, including that knockout of the GIP-receptor3 or immunoneutralization of
circulating GIP\textsuperscript{4} are protective against diet-induced obesity in rodents. As the incretin action of GIP seems to be impaired in patients with type 2 diabetes\textsuperscript{1}, it might be postulated that a stimulation of GLP-1 and an inhibition of GIP secretion could be a therapeutic objective in overweight patients with type 2 diabetes. Further understanding the differences between these two hormones should enable a more targeted approach to their exploitation for the treatment of diabetes and obesity. The present review will focus on the physiology of the enteroendocrine cells secreting these two hormones, the GIP-expressing K cells and the proglucagon/GLP-1-expressing L cells.

**LOCATION: OVERLAPPING BUT DISTINCTIVE POPULATIONS OF K- AND L CELLS**

GIP-containing cells are found at highest density in the duodenum in a number of species\textsuperscript{5,7}. GLP-1-containing cells can also be found in the proximal small intestine, but increase in number towards the distal small intestine, and in contrast to K cells are also numerous in the colon and rectum (Figure 1). Based on immunohistochemical staining, so-called LK cells have been described, which were immune-positive for both GIP and GLP-1\textsuperscript{8,9}. In transgenic mice expressing fluorescent markers under the control of either the GIP or the proglucagon promoter, cells producing the ‘other’ incretin have also been observed; that is, GIP in cells labeled by the proglucagon promoter, and GLP-1 in cells labeled by the GIP promoter\textsuperscript{10}; however, these double-positive cells only amounted to \(\sim 10\text{—}20\%\) of the total K- and L cell population. Overall secretory responses from cell populations in vivo or in vitro are thus likely to be dominated by the remaining \(\sim 80\%\) of single-positive cells that produced only GLP-1 or GIP\textsuperscript{9}, and as we describe below, selective stimulation or inhibition of either GIP or GLP-1 secretion is therefore possible. Nevertheless, the observation that K- and L cells additionally produce other hormones, such as CCK\textsuperscript{10}, challenges the traditional classification of enteroendocrine cells according to their expression of one (or sometimes two) specific hormones, and suggests a more plastic expression profile that could be affected by external factors, such as the recent exposure of the intestine to nutrients and other luminal stimulants. Given the relatively rapid turnover of enteroendocrine cells in the small intestine every \(\sim 5\) days\textsuperscript{11}, it seems plausible that recent nutritional availability could result in changes to the overall enteroendocrine cell population within days or weeks. In a recent study to identify the effects of a high-fat diet on mouse L cells, however, we observed a general downregulation of many enteroendocrine cell-specific genes rather than a switch to the preferential production of an alternative hormone\textsuperscript{12}.

**GLUCOSE SENSING: SIMILAR MECHANISMS OPERATE IN BOTH K- AND L CELLS**

Given the importance of both GIP and GLP-1 for the incretin effect, one of the most investigated secretory stimuli of gut hor-

| K/L cell distribution | Enteroneocrine stimulus | EEC Transporter/ion channel | EEC Receptor |
|-----------------------|-------------------------|-----------------------------|-------------|
| L                     | Monosaccharide          | SLC5A1 (SGLT1)              | Tas1R2/3 (?)|
| L                     | Long chain fatty acid   | FFAR1, FFAR4                |            |
| L                     | Monoacyl glycerol, acyl-ethanolamide | GPR119, CB1 | |
| L                     | Amino acid              | BoAT1 (SLC6A19), ATA2 (SLC38A2) | CASR, GPRc6A |
| L                     | Di/tripeptide           | SLC15A1 (PepT1)             | CASR        |
| L                     | Bile acid               | GPBAR1                      |            |
| L                     | Short chain fatty acid  | FFAR2, FFAR3                |            |
| L                     | Bile acid               | GPBAR1                      |            |
| L                     | Indole                  | Kv channels                 |            |
| K                     |                        |                             |            |

Figure 1 | L- and K cell distribution and stimulus detection machinery. The majority of K cells are more proximally located than L cells. Fasting and postprandial glucose-dependent insulinotropic polypeptide (K cells) and glucagon-like peptide-1 (L cells) secretion likely reflect the dynamic gradient of different intestinal stimuli along the gut. Physiological activation of the L- and K cell detection pathways is shown, involving transporters/ion channels (linked to altered cellular electrical activity) and G-protein-coupled receptors, differing between the small intestine and colon. ATA2, amino acid transporter A2 (solute carrier [Slc] Slc38A2); BoAT1, system B(0) neutral amino acid transporter AT1 (Slc6A19); CASR, calcium-sensing receptor; CB1, cannabinoid receptor 1; EEC, enteroendocrine cell; FFAR, free fatty-acid receptor; GPBAR1, G-protein coupled bile-acid receptor 1; GPR119, G-protein coupled receptor 119; GPRC6A, G-protein coupled receptor classC 6A; Kv-channels, voltage gated potassium channels; SGLT1, sodium-coupled glucose transporter 1 (Slc5A1); Tas1R, taste receptor type 1.
mone secretion is glucose. Both K- and L cells in mixed primary intestinal epithelial cultures failed to respond to glucose when the sodium-coupled glucose transporter 1 (SGLT1) was inhibited either pharmacologically or genetically\textsuperscript{13–16}. Indeed, a wealth of in vitro and in vivo data have suggested that the rapid elevations in plasma GIP and GLP-1 concentrations after glucose ingestion are directly linked to the electrogenic uptake of glucose by K- and L cells, resulting in membrane depolarization, voltage-gated calcium entry and enhanced rates of vesicular exocytosis\textsuperscript{17}. More extensive phenotyping of global SGLT1 knockout mice, however, showed differences between the release patterns of GIP and GLP-1, which are likely related to the different locations of K- and L cells along the gastrointestinal tract axis\textsuperscript{18}. In this mouse model, the GIP response to an oral glucose tolerance test was abolished, consistent with the proposed SGLT1-dependent coupling of glucose absorption to GIP secretion in K cells. By contrast, whereas the early GLP-1 response \textasciitilde5 min after a glucose gavage was abrogated in SGLT1 knockout mice or in mice treated with an SGLT1 inhibitor\textsuperscript{13,18}, elevated plasma GLP-1 concentrations were observed at later time-points\textsuperscript{18}. The findings support the idea that the rapid secretion of GLP-1 and GIP from the proximal small intestine after a glucose load is linked to SGLT1-dependent glucose absorption, but suggest that alternative sensory mechanisms operate in the distal gut. Inhibition of glucose absorption in the upper gastrointestinal tract in SGLT1 knockout mice results in a dramatic increase in glucose delivery to the distal gut with its higher density of L cells\textsuperscript{18}, likely underpinning the delayed elevation of GLP-1 levels in these mice. The mechanism by which this glucose load is sensed by the distal ileum and/or colon remains unclear. Candidate pathways include the bacterial fermentation of distally-delivered carbohydrate to metabolites, such as short chain fatty acids that are then sensed by G-protein-coupled receptors, such as GPR43\textsuperscript{19}, or metabolism of the sugar within L cells resulting in the activation of an alternative downstream signaling pathway. Neither of these hypotheses has yet been validated experimentally.

Whereas the SGLT1-dependent pathway is common to K- and L cells, we observed some differences between the responsiveness of the glucose-sensing machinery underlying GIP and GLP-1 secretion from small intestinal primary murine epithelial cultures. Whereas $\alpha$-methyl-glycogenoside, a non-metabolizable SGLT1 substrate, enhanced GLP-1 secretion in the absence of other stimuli\textsuperscript{16}, it only became an effective GIP secretagogue at elevated cyclic adenosine monophosphate (cAMP) levels\textsuperscript{14}. Interestingly, the responsiveness to tolbutamide, an inhibitor of adenosine triphosphate-sensitive potassium ($K_{\text{ATP}}$) channels, showed the reverse dependence, triggering GIP responses in the absence, but not presence, of the cAMP-raising agents, forskolin and 3-isobutyl-1-methylxanthine (IBMX)\textsuperscript{14}. $K_{\text{ATP}}$ channel closure has itself been postulated as a mechanism underlying K- and L cell glucose sensing, similar to its well-established role in mediating glucose-dependent insulin secretion from pancreatic $\beta$-cells. In support of this idea, $K_{\text{ATP}}$ channel inhibition enhanced GIP and GLP-1 secretion from intestinal cultures, triggered GIP-1 secretion from the enteroendocrine cell line, GLUTag\textsuperscript{20}, and increased GLP-1 release from perfused rat intestine\textsuperscript{21}. However, there is little data supporting the idea that $K_{\text{ATP}}$ channel closure triggers gut hormone secretion in an intact animal. Mice lacking the $K_{\text{ATP}}$ channel subunit Kir6.2 did not show impaired glucose-triggered gut hormone secretion\textsuperscript{22,23}; and in humans, the $K_{\text{ATP}}$ channel inhibitor, glibenclamide, had no effects on GIP and GLP-1 concentrations before or during an oral glucose tolerance test\textsuperscript{24}. $K_{\text{ATP}}$-channel closure has, however, been suggested to underlie fructose-stimulated GLP-1 secretion\textsuperscript{25}. Fructose is not a substrate for sodium-coupled monosaccharide transport, and instead enters cells through the facilitative transporter GLUT5. Interestingly, fructose does not stimulate GIP secretion in healthy rodents and humans\textsuperscript{25}, but GIP release in response to fructose has been reported in diabetic mouse models\textsuperscript{22,26}. However, opening of $K_{\text{ATP}}$-channels with diazoxide did not abolish fructose-stimulated GIP secretion in diabetic mice, and even the GLP-1 response to oral fructose remained intact in mice lacking the $K_{\text{ATP}}$ channel subunit, Kir6.2\textsuperscript{23}. In view of the widespread agreement that $K_{\text{ATP}}$ channels are expressed and functional in K- and L cells, the results unanimously support the idea that the resting $K_{\text{ATP}}$ conductance is already very small in native K- and L cells in vivo, and that any differences observed between in vivo and in vitro experiments might reflect differences in the metabolic status of the enteroendocrine cells under the different conditions.

Another physiological glucose-sensing mechanism involves the G-protein-coupled receptor heterodimer of TAS1R2 and TAS1R3, that underlies sweet taste sensation in the tongue. Impaired postprandial GLP-1 levels have been observed in mice lacking $\alpha$-gustducin, a component of the downstream taste receptor signaling pathway\textsuperscript{27}, and both GIP and GLP-1 secretion have been reported from the GLUTag cell line in response to high concentrations of artificial sweeteners\textsuperscript{28}. We, however, were unable to show a role for TAS1R2/3 in hormone secretion from murine K- or L cells in primary culture\textsuperscript{14,16}, and artificial sweeteners failed to elevate plasma incretin hormone levels in human volunteers\textsuperscript{29}. Other studies also failed to show artificial sweetener-stimulated GIP secretion in mice in vivo\textsuperscript{22} or GLP-1 secretion in a perfused rat intestinal preparation\textsuperscript{31}, questioning the importance of TAS1R2/3 in incretin secreting cells.

**G-PROTEIN-COUPLED RECEPTORS: CANDIDATES FOR SELECTIVELY TARGETING K- AND L CELLS**

The development of transgenic mice with fluorescently tagged K- or L cells has enabled the transcriptomic analysis of these different cell types. Similarities and differences between K- and L cell populations were observed for the expression of a number of G-protein-coupled receptors. Both K- and L cell populations, for example, were found to express messenger ribonucleic acids (mRNAs) encoding the free-fatty acid receptors, FFAR1 (GPR40) and FFAR4 (GPR120)\textsuperscript{14,16}. GPR40 activation has, for
example, been linked to the stimulation of GLP-1 secretion in experiments using Gpr40 knockout mice\textsuperscript{30}, as well as by the application of GPR40 agonists to the perfused rat intestine\textsuperscript{31}. Recent data, by contrast, convincingly showed a reduction in GPR119 mRNA for which is expressed in both K- and L cells\textsuperscript{14,16}. Whereas a small molecule GPR119 agonist elevated GLP-1 secretion and GPR40 activation to GLP-1 release are robust, it is currently unclear whether either of these receptors plays a relatively greater role in any particular enteroendocrine cell type.

Other lipid-derived stimulants, such as mono-acyl-glycerol, activate GPR119, mRNA for which is expressed in both K- and L cells\textsuperscript{34,16}. Whereas a small molecule GPR119 agonist elevated GLP-1 and GIP concentrations in mice\textsuperscript{34}, however, the natural GPR119 ligand oleoylethanolamide (OEA) was not a good stimulus of GIP-secretion from primary epithelial cultures\textsuperscript{14}. GLP-1 secretion, by contrast, was stimulated by OEA from both small and large intestinal-derived murine cultures\textsuperscript{34}. In the colon, this was mediated by GPR119, as shown by the loss of mRNA for GLP-1 release in colonic cultures from mice lacking GPR119 specifically in proglucagon expressing cells. Similar experiments carried out in small intestinal cultures from these mice, however, showed that the OEA-activated GPR119 GLP-1 secretory response in the upper gastrointestinal tract was not GPR119-dependent\textsuperscript{34}, suggesting that OEA recruits alternative mechanisms in duodenaljejunal L cells, and that this pathway might not be sufficiently active in K cells to trigger GIP secretion.

The related compounds, mono-arachidonoyl-glycerol and arachidonylethanolamine (anandamide), are agonists for the endocannabinoid receptor, CB1 (encoded by the gene Cnr1), which is predominantly G\textsubscript{i}-coupled. Cnr1 is highly expressed in small intestinal K- and L cells, with a tendency for higher expression in K- than L cells, but was not detected in L cells from the colon\textsuperscript{35}. Consistent with the known G\textsubscript{i} coupling of CB1, anandamide was shown to inhibit IBMX-triggered GIP secretion in vivo, an effect that was blocked by the CB1-antagonist AM251. Interestingly, GLP-1 secretion from the same cultures was not affected by anandamide, despite the relatively high expression of Cnr1 in small intestinal L cells. Pretreatment of mice with anandamide delayed GIP, but not GLP-1 responses to an oral glucose tolerance test\textsuperscript{35}. These findings raise questions about why the same receptor is apparently more effectively coupled to inhibition of secretion in K- than L cells, despite relatively high expression levels in both.

Ligands for other predominantly G\textsubscript{i}-coupled receptors expressed in both K- and L cells, by contrast, tend to have similar effects on both GIP and GLP-1 secretion. Somatostatin is nearby intestinal D cells, this being an example of how paracrine signals can integrate responses to luminal signals within the epithelium. Galanin, most likely secreted from enteric neurons, similarly recruits GALR1 in both K- and L cells, resulting in a G\textsubscript{q}-dependent suppression of CAMP levels\textsuperscript{36}, and exemplifying that enteroendocrine cells likely integrate responses to luminal nutrients with signals arriving through the enteric nervous system. G\textsubscript{q}-coupled receptors, such as GALR1, have been reported to inhibit electrically excitable cells through activation of G-protein activated inwardly rectifying potassium (GIRK) channels, mediated by the G-protein \(\gamma\)-subunit. Interestingly, whereas both K- and L cells showed enriched expression of mRNAs encoding GIRK channels, and although GIRK-inhibition had no effect on the ability of galanin to inhibit GIP-1 secretion, only GLP-1, but not GIP, secretion could be inhibited by co-application of the GIRK-activator, ML297, with IBMX or glucose\textsuperscript{34}. This might point to a difference in the role of potassium conductances in the stimulus secretion coupling of K- and L cells, but further work assessing possible differences in the electrical activity of these enteroendocrine cell types and its relationship to hormone secretion is required.

Given the calcium dependence of a number of proteins involved in the exocytotic pathway, predominantly G\textsubscript{q}-coupled receptors should be good targets to stimulate incretin secretion. The similar expression levels in K- and L cells of mRNAs encoding the G\textsubscript{q}-coupled receptor, FFAR1, have been mentioned above. The related short-chain fatty acid receptor FFAR2 (GPR43) by contrast seems more abundant in L- than K cells (Affymetrix chip array probe 1425216 RMA-values are 2,143 for fluorescently tagged L- and 154 for fluorescently tagged K cells isolated from the small intestine, with the latter value being similar to values observed for the non-fluorescent control cells; Reimann and Griibble unpubl.), supporting the reported importance of GPR43 in short-chain fatty acid stimulated GLP-1 secretion\textsuperscript{35}. However, whereas fluorescent reporter mice for FFAR2 had only a few labeled enteroendocrine cells, reporter mice for the other short chain fatty acid receptor FFAR3 (GPR41) showed strong labeling of a number of enteroendocrine cells in the small intestine, including K- and L cells\textsuperscript{37}. A recent publication reported a blunting of GLP-1, but not GIP, secretion in response to orally-administered butyrate in Ffar3 knockout mice\textsuperscript{36}, and further work will be required to clarify the relative roles of these receptors in incretin-secreting cells. Another G\textsubscript{q}-coupled receptor, presumably underlying modulation of enteroendocrine secretion by the enteric nervous system, as it is activated by neuromedin C and gastrin-releasing peptide, is the bombesin receptor 2. Bombesin receptor 2 mRNA was found to be selectively enriched in L cells, but not K cells, and, consistent with this finding, bombesin increased calcium concentrations in L cells, but not K cells, and triggered GLP-1, but not GIP, secretion from primary small epithelial cultures as well as in a perfused intestinal preparation\textsuperscript{39}.

Whether any of the predominantly G\textsubscript{q}-coupled receptors will be good targets for selective stimulation of GLP-1 secretion
in vivo will have to await further research. It should be noted, however, that a recent report of a so-called FFAR1 ‘superagonist,’ capable of triggering robust GLP-1 secretory responses, stresses the importance of the dual action of such compounds in recruiting both G$_{q}$- and G$_{s}$-coupled pathways. This latter notion is supported by our observation that raising intracellular cAMP levels with forskolin and IBMX boosts GIP and GLP-1 secretory responses to a number of agents that elevate enteroenodocrine cell cytosolic Ca$^{2+}$ concentrations, in primary epithelial cultures. A predominantly G$_{s}$-coupled receptor expressed in L cells, but not K cells, is the melanocortin receptor 4, and increased GLP-1 and PYY secretion in response to agonists has been shown, although currently the nature or source of the physiological ligand for this receptor on L cells is uncertain. Other predominantly G$_{q}$-coupled receptors, expression of which is enriched in L cells, include GPR119 (see above) and the bile acid-sensitive receptor, GPBAR1 (TGR5). Although there seems to be some expression of Gpbar1 in K cells, it is not enriched compared with the surrounding cells (Reimann and Gribble unpublished observation), making GPBAR1 one of the more promising receptors to ‘selectively’ stimulate GLP-1 secretion. However, we recently showed that bile acids need to access the basolateral rather than the apical/luminal surface of L cells to stimulate GLP-1 secretion through GPBAR1, and a similar observation was reported for agonists of GPR40. As melanocortin receptor 4 was also located to the basolateral side, the interesting question arises if any GPCRs directly sample the luminal contents. If all L cell GPCRs are instead located on the basolateral membrane, the potential hope to develop agents with limited systemic bioavailability to avoid off-target side-effects as a result of action on other cells expressing the receptors in question would be unfounded.

CONCLUSION
Although the success of GLP-1 analogs/mimetics in the treatment of type 2 diabetes and the correlation of strongly elevated postprandial GLP-1 levels after Roux-Y gastric bypass surgery strongly suggests benefits of recruiting endogenous GLP-1 reserves as a not yet exploited treatment alternative, the situation for GIP is less clear. Arguments have been put forward for developing both GIP-receptor agonists and antagonists. The differences observed in murine K- and L cells suggest that it is possible to elevate one incretin preferentially by external stimuli, but further work will be required before translation into a clinical therapy.

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DISCLOSURE
The authors declare no conflict of interest.

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