

Glucagon-Like Peptide 1 Secretion by the L-Cell

The View From Within

Gareth E. Lim¹ and Patricia L. Brubaker^{1,2}

Glucagon-like peptide 1 (GLP-1) is a gut-derived peptide secreted from intestinal L-cells after a meal. GLP-1 has numerous physiological actions, including potentiation of glucose-stimulated insulin secretion, enhancement of β -cell growth and survival, and inhibition of glucagon release, gastric emptying, and food intake. These antidiabetic effects of GLP-1 have led to intense interest in the use of this peptide for the treatment of patients with type 2 diabetes. Oral nutrients such as glucose and fat are potent physiological regulators of GLP-1 secretion, but non-nutrient stimulators of GLP-1 release have also been identified, including the neuromodulators acetylcholine and gastrin-releasing peptide. Peripheral hormones that participate in energy homeostasis, such as leptin, have also been implicated in the regulation of GLP-1 release. Recent studies have begun to elucidate the intracellular signaling pathways that mediate the effects of GLP-1 secretagogues on the intestinal L-cell. The purpose of this review is to summarize the known signaling mechanisms of GLP-1 secretagogues based on the available literature. A better understanding of the pathways underlying GLP-1 secretion may lead to novel approaches by which the levels of this important insulinotropic hormone can be enhanced in patients with type 2 diabetes. *Diabetes* 55 (Suppl. 2):S70–S77, 2006

Glucagon-like peptide 1 (GLP-1) is an intestinal hormone that exerts profound effects in the regulation of glycemia, stimulating glucose-dependent insulin secretion, proinsulin gene expression, and β -cell proliferative and anti-apoptotic pathways, as well as inhibiting glucagon release, gastric emptying, and food intake (1). The demonstrated success of GLP-1 to lower glycemia has led to approval of the GLP-1 receptor agonist exendin-4 (Byetta) for the treatment of patients with type 2 diabetes (2). Studies using

GLP-1 receptor antagonists as well as GLP-1 receptor null mice have demonstrated that GLP-1 makes an essential contribution to the “incretin” effect after a meal (3,4). However, GLP-1 secretion is reduced in patients with type 2 diabetes (5–7), and this may contribute in part to the reduced incretin effect and the hyperglycemia that is observed in these individuals (8). Thus, interest has now focused on the factors that regulate the release of this peptide after nutrient ingestion. Many different GLP-1 secretagogues have been described in the literature over the past few decades, including nutrients, neurotransmitters, neuropeptides, and peripheral hormones (rev. in 9,10). However, the specific receptors, ion channels, and intracellular signaling proteins expressed by the GLP-1-producing intestinal L-cell have only recently begun to be characterized. The purpose of this review is to integrate the literature regarding the signal transduction pathways used by the major GLP-1 secretagogues. An improved understanding of the mechanisms regulating GLP-1 secretion may lead to novel approaches to enhance GLP-1 levels in vivo, thereby providing an alternative approach to the use of this peptide in the treatment of patients with type 2 diabetes.

SYNTHESIS AND DEGRADATION OF GLP-1

Although the proglucagon gene is expressed in enteroendocrine L-cells and pancreatic α -cells (11), GLP-1 is synthesized by posttranslational processing of proglucagon only in the intestine. The L-cells are predominantly located in the ileum and colon and have been identified as open-type epithelial cells that are in direct contact with nutrients in the intestinal lumen (12). Furthermore, L-cells are located in close proximity to both neurons and the microvasculature of the intestine (13,14), which allows the L-cell to be affected by both neural and hormonal signals. In fetal rat intestinal L-cell cultures and immortalized murine L-cells, proglucagon gene expression is enhanced by activation of the protein kinase A (PKA) pathway (15,16). Although originally thought to be mediated through CREB binding to the cAMP response element in the proglucagon gene, recent studies have demonstrated that cAMP-dependent proglucagon gene expression in the L-cell occurs via β -catenin-mediated activation of the bipartite transcription factor, TCF4 (17). Interestingly, a very recent report has linked variants of TCF4 to the risk for development of type 2 diabetes (18), implicating GLP-1 in the etiology of this disease.

Tissue-specific expression of prohormone convertase isoforms directs the synthesis of specific proglucagon-derived peptides in the L-cell and α -cell (Fig. 1). Hence, cleavage of proglucagon by prohormone convertase 1/3, which is expressed in the L-cell, liberates GLP-1 and GLP-2, as well as the glucagon-containing peptides, glicen-

From the ¹Department of Physiology, University of Toronto, Toronto, Ontario, Canada; and the ²Department of Medicine, University of Toronto, Toronto, Ontario, Canada.

Address correspondence and reprint requests to Dr. P.L. Brubaker, Room 3366, Medical Sciences Building, University of Toronto, 1 King's College Circle, Toronto, ON M5S 1A8 Canada. E-mail: p.brubaker@utoronto.ca.

Received for publication 23 March 2006 and accepted in revised form 2 May 2006.

P.L.B. has received honoraria from and is a consultant for Amgen.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

GABA, γ -aminobutyric acid; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1; GRP, gastrin-releasing peptide; K_{ATP} channel, ATP-sensitive K^+ channel; MAPK, mitogen-activated protein kinase; MUFA, monounsaturated fatty acid; PKA, protein kinase A; PKC, protein kinase C; STAT, signal transducer and activator of transcription.

DOI: 10.2337/db06-S020

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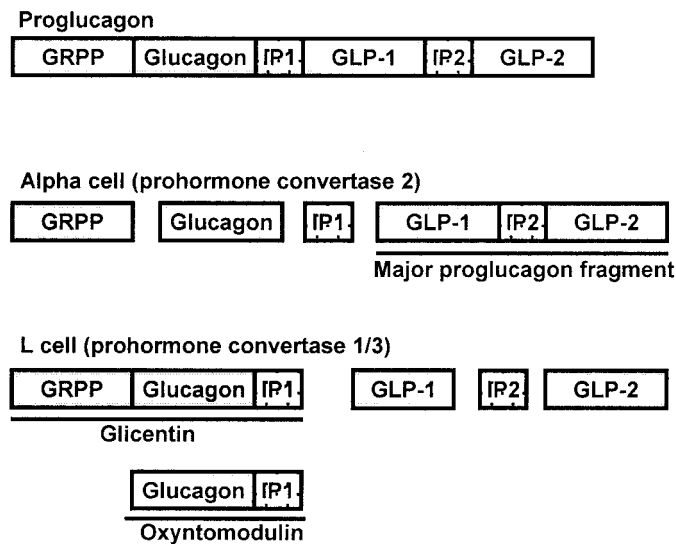


FIG. 1. Tissue-specific posttranslational processing of proglucagon liberates different proglucagon-derived peptides. Prohormone convertase 2 in the α -cell releases glicentin-related pancreatic peptide (GRPP), glucagon, intervening peptide-1 (IP1), and the major proglucagon fragment, which contains within its sequence both GLP-1 and GLP-2. Early studies on L-cell secretagogues used antibodies that targeted a mid-sequence epitope of glucagon, resulting in detection of glicentin/oxyntomodulin (i.e., enteroglucagon or gut glucagon-like immunoreactivity) as well as of glucagon. Because these peptides, as well as GLP-2, are synthesized and secreted from the L-cell in a 1:1 stoichiometric ratio with GLP-1 (21,22), such studies therefore indirectly also examined GLP-1 synthesis and secretion. Hence, the results of studies using antisera against any of the intestinal proglucagon-derived peptides are discussed in this review synonymously. Finally, bioactive GLP-1 exists in two equipotent forms, GLP-1^{7-36NH₂} and GLP-1⁷⁻³⁷, in the circulation, of which the former is predominant (23). Secreted GLP-1 is rapidly degraded by the ubiquitous enzyme dipeptidyl peptidase IV (24), resulting in an extremely short half-life for GLP-1 of ~2 min (23).

tin and oxyntomodulin (19,20). In contrast, α -cell expression of prohormone convertase 2 leads to synthesis of glucagon, glicentin-related pancreatic peptide, and the major proglucagon fragment, which contains within its sequence both GLP-1 and GLP-2. Early studies on L-cell secretagogues used antibodies that targeted a mid-sequence epitope of glucagon, resulting in detection of glicentin/oxyntomodulin (i.e., enteroglucagon or gut glucagon-like immunoreactivity) as well as of glucagon. Because these peptides, as well as GLP-2, are synthesized and secreted from the L-cell in a 1:1 stoichiometric ratio with GLP-1 (21,22), such studies therefore indirectly also examined GLP-1 synthesis and secretion. Hence, the results of studies using antisera against any of the intestinal proglucagon-derived peptides are discussed in this review synonymously. Finally, bioactive GLP-1 exists in two equipotent forms, GLP-1^{7-36NH₂} and GLP-1⁷⁻³⁷, in the circulation, of which the former is predominant (23). Secreted GLP-1 is rapidly degraded by the ubiquitous enzyme dipeptidyl peptidase IV (24), resulting in an extremely short half-life for GLP-1 of ~2 min (23).

SYSTEMIC REGULATION OF GLP-1 SECRETION

Nutrient ingestion is the primary physiological stimulus to the L-cell and results in a biphasic pattern of GLP-1 secretion. An initial rapid rise in circulating GLP-1 levels occurs 15–30 min after a meal, followed by a second minor peak at 90–120 min (25). Glucose and fat have been found to be potent stimulators of GLP-1 secretion when ingested (26), but also after direct administration into the intestinal lumen (22,27) or into perfused ileal segments (28). Unlike glucose and fat, protein does not appear to stimulate proglucagon-derived peptide secretion from L-cells (26), although protein hydrolysates have been found to stimulate GLP-1 release in a perfused rat ileum model and in immortalized human L-cells (28,29).

Previous studies have indicated that L-cells are not present in the proximal small intestine of humans and rodents (12). Hence, we proposed that the initial rapid rise

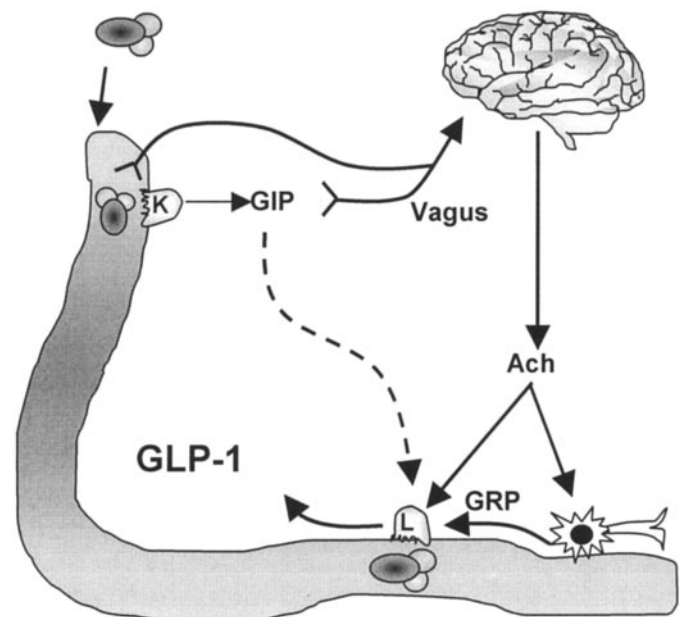


FIG. 2. Regulation of GLP-1 secretion by ingested nutrients. After a meal, nutrients in the duodenum activate a proximal-distal neuroendocrine loop, which stimulates GLP-1 secretion from L-cells in the ileum and colon. In rodents, GIP, released from K-cells, activates vagal afferents, which subsequently causes GLP-1 secretion through vagal efferents and enteric neurons that release acetylcholine (ACh) and GRP. Movement of nutrients toward more distal sections of the intestine leads to the direct interaction of nutrients with L-cells, which also stimulates GLP-1 secretion.

in GLP-1 secretion must be mediated indirectly, through a neuro/endocrine pathway, rather than through direct interactions of the luminal contents with L-cells (27). Although a very recent study has detected GLP-1-immunoreactive cells in the duodenum of humans (30), this remains controversial. Therefore, to elucidate the mechanism(s) underlying proximal nutrient-stimulated GLP-1 secretion, we developed a rodent model of GLP-1 secretion in which nutrient flow to the distal intestine was prevented, thereby excluding the possibility of direct interactions of the luminal nutrients with the L-cell (Fig. 2). In this model, placement of glucose or fat into the duodenum induced an immediate and prolonged stimulation of the L-cell that was comparable in magnitude to increments in proglucagon-derived peptides observed when nutrients were placed directly into the ileum (27). Furthermore, when nutrients were placed in the duodenum of the rat, a prompt rise in glucose-dependent insulinotropic peptide (GIP) levels was also observed, and infusion of GIP or treatment of primary rat L-cells in culture with GIP also stimulated proglucagon-derived peptide secretion (22,27,31), thus implicating GIP in the proximal regulation of GLP-1 secretion. The more important role of the vagus nerve in mediating the proximal-distal loop was elucidated when L-cell stimulation by placement of fat into the duodenum or by infusion of physiological concentrations of GIP was completely abrogated by subdiaphragmatic vagotomy (22). Furthermore, activation of the efferent celiac branch was demonstrated to increase GLP-1 secretion. Finally, acetylcholine has now been identified as a key neurotransmitter mediating the proximal-distal loop. In vitro studies have determined that human and rodent L-cells are sensitive to the stimulatory effects of acetylcholine (13,32). Furthermore, when

rodents were infused with atropine or pirenzepine (an M1 muscarinic receptor antagonist), duodenal nutrients were unable to increase GLP-1 secretion (13). When taken together, therefore, these studies demonstrated that, in rodents, the regulation of GLP-1 release by proximal nutrients is mediated via GIP actions on cholinergic fibers of the vagus nerve.

A second neural regulator of the proximal-distal loop has been identified as gastrin-releasing peptide (GRP), a neuropeptide that is locally released from GRPergic neurons in the enteric nervous system. GRP has been demonstrated to be a potent GLP-1 secretagogue in both in vitro and in vivo studies of the L-cell (31,33). The role of GRP in the proximal-distal loop regulating the L-cell has also been demonstrated using a GRP receptor antagonist, as well as GRP null mice (33,34).

Although the early rise of GLP-1 does occur in humans (25,26), the specific mediators of the proximal-distal loop have yet to be defined. The human L-cell is responsive to acetylcholine in vitro (32), and administration of atropine prevents GLP-1 secretion after nutrient ingestion (35). Reimer et al. (29) have also demonstrated that human L-cells are responsive to GRP in vitro. However, GIP does not stimulate GLP-1 secretion in humans in vivo (36). Further work is clearly required to identify the exact mechanisms mediating the rapid release of GLP-1 secretion after nutrient ingestion in humans.

The second later peak of GLP-1 secretion is believed to occur consequent to transit of ingested nutrients down the lumen to directly interact with the distal L-cell. Placement of nutrients directly into the lumen of the ileum stimulates GLP-1 release (27,37), whereas treatment of rodent and human L-cells in culture with either glucose or fatty acids induces dose-dependent increases in GLP-1 secretion (29,38–41). As glucose does not reach the distal gut in high concentrations, it has been proposed that fat, which does transit to the ileum, is the more physiological direct regulator of GLP-1 release (9). Nonetheless, under conditions of nutrient dumping/rapid transit or after administration of digestive enzyme inhibitors, both fat- and glucose-stimulated GLP-1 secretion may be of importance (42–44).

Finally, in addition to luminal nutrients, a number of additional intestinal peptides and neurotransmitters, as well as systemic hormones, have been demonstrated to modulate GLP-1 secretion. These include intestinal somatostatin, for which a negative feedback loop with GLP-1 has been proposed (31,45); the neurotransmitter γ -aminobutyric acid (GABA), which enhances GLP-1 release (46); and α - and β -adrenergic agonists, which respectively inhibit and/or stimulate GLP-1 secretion from L-cells in the perfused rat intestine (47). More recently, the adipocyte hormone leptin has also been found to stimulate GLP-1 release from the human and rodent L-cell, and this effect is abolished in leptin-resistant diet-induced obese mice (48). Furthermore, preliminary findings have demonstrated insulin to be a GLP-1 secretagogue, while insulin resistance in the L-cell reduces basal and stimulated GLP-1 release (49). Although the physiological significance of many of these findings remains to be firmly established, these studies suggest that impairments at the level of the L-cell may account, at least in part, for the reduced GLP-1 secretion that is observed in patients with type 2 diabetes (5–7), as well as in obesity (50).

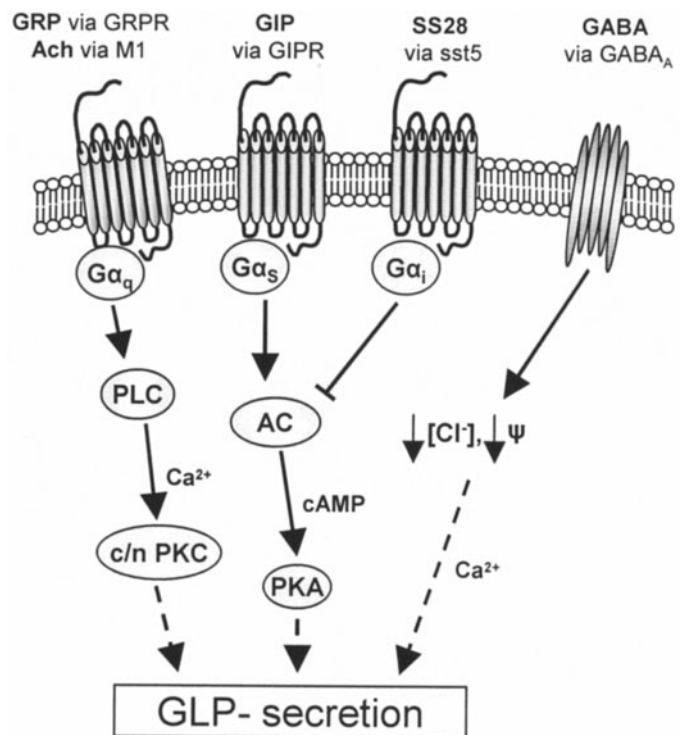


FIG. 3. Intracellular pathways of GLP-1 secretagogues activated by proximal nutrients. Binding of GRP or acetylcholine (Ach) to $G\alpha_q$ -linked GRP or M1 receptors, respectively, is associated with phospholipase C (PLC) activation and subsequent stimulation of conventional and novel isoforms of protein kinase C (c/n PKC). Activation of these receptors is also associated with increases in intracellular calcium and the phosphorylation of p44/42 mitogen-activated kinase. GIP receptor activation causes the stimulation of adenylyl cyclase (AC) via $G\alpha_s$. This leads to an increase in cAMP and activation of PKA. The somatostatin receptor is coupled with $G\alpha_i$, which inhibits AC, therefore inhibiting GLP-1 secretion. Binding of GABA to $GABA_A$ receptors depolarizes L-cells by channel opening and the efflux of chloride. Solid arrows = known pathways; dashed arrows = unknown pathways.

INTRACELLULAR SIGNALING PATHWAYS REGULATING GLP-1 SECRETION

Until recently, there has been a relative paucity of published literature on the intracellular pathways mediating the effects of GLP-1 secretagogues in the L-cell. However, the development of in vitro models of the murine, rat, and human intestinal L-cell has now permitted more detailed examination of signaling pathways expressed in the L-cell, although in some instances, information has been assumed by extrapolation from what is known regarding other neuroendocrine cells (Figs. 3–5). An improved understanding of the intracellular mechanisms underlying GLP-1 secretion may lead to novel approaches to augment GLP-1 secretion in vivo, particularly if used in conjunction with agents designed to prevent the rapid degradation of GLP-1 in the circulation (1).

Acetylcholine. As discussed above, acetylcholine stimulates GLP-1 secretion in vitro and in vivo. M1 muscarinic receptor agonists increase GLP-1 secretion from rat L-cells, and inhibition of M1 muscarinic receptors prevents proximal nutrient-induced release of GLP-1 by bethanechol, a nonselective muscarinic agonist (13). In human L-cells, inhibition of M1 and M2 muscarinic receptors also prevents bethanechol-induced GLP-1 release (32). M1 muscarinic receptors are G protein-coupled receptors that are linked to $G\alpha_{q/11}$, and ligand binding results in the activation of phospholipase C, which cleaves phosphati-

dylinositol (4,5)biphosphate into inositol-1,4,5-trisphosphate and diacylglycerol, leading to increases in intracellular calcium and activation of both conventional and novel protein kinase C (PKC) isoforms (51) (Fig. 3). M2 receptors are thought to be coupled to $G\alpha_i$, which inhibits adenylyl cyclase (51), but the enhanced GLP-1 secretory response to M2 receptor activation in human L-cells suggests the existence of an alternative intracellular pathway.

GRP. GRP is a potent stimulator of the intestinal L-cell *in vivo* and *in vitro* (31,33), but the signal transduction cascade that occurs in response to GRP treatment in the L-cell has yet to be defined. Based on studies using other neuroendocrine cells, GRP binds to a G protein-coupled receptor that is coupled to $G\alpha_q$ (52). For example, the plurihormonal murine secretin tumor cell line (STC-1) releases not only secretin, but also GLP-1 and cholecystokinin. Treatment with GRP stimulates hormone secretion by these cells in association with activation of mitogen-activated protein kinase (MAPKK) and subsequent phosphorylation of p44/42 mitogen-activated protein kinase (MAPK). GRP-stimulated cholecystokinin secretion was also found to be dependent on the activation of PKC (53). Consistent with these findings, downregulation of PKC activity by prolonged treatment with phorbol myristate acetate to inactivate classic and novel PKCs prevents GRP-mediated insulin secretion from pancreatic β -cells (54). GRP also enhanced insulin secretion in association with an increase in intracellular calcium. Although p44/42 MAPK is expressed in the mouse and human L-cell (49; R. Iakoubov, A. Izzo, A. Yeung, C.I. Whiteside, P.L.B., unpublished data) and changes in intracellular calcium levels have been linked to GLP-1 release in the rodent L-cell (55,56), further work is clearly required to determine the exact mechanism of action of GRP to stimulate GLP-1 secretion.

GABA. GABAergic neurons are components of the enteric nervous system located primarily in the myenteric plexus of the colon. Three isoforms of the GABA receptor exist ($GABA_A$, $GABA_B$, and $GABA_C$), and their expression and distribution is tissue specific. Of the three isoforms, $GABA_A$ and $GABA_C$ receptors are ion-channel linked receptors, whereas the $GABA_B$ receptor is a metabotropic G protein-coupled receptor (57). Gameiro et al. (46) confirmed the expression of $GABA_A$ receptors in the murine L-cell, and GABA treatment of these cells caused an efflux of chloride ions from the cell, leading to depolarization, opening of voltage-gated calcium channels, and GLP-1 secretion. These *in vitro* findings suggest that GABA from GABAergic neurons may act in a paracrine manner to modulate hormone secretion. Nonetheless, the physiological role of GABA modulation of GLP-1 secretion *in vivo* still remains to be demonstrated.

Glucose-dependent insulinotropic peptide. GIP mediates its biologic actions through a G protein-coupled receptor belonging to the glucagon receptor superfamily, which includes receptors for other structurally related gut-derived peptides, including GLP-1, GLP-2, glucagon, secretin, and growth hormone-releasing hormone (58). GIP receptor activation in the β -cell leads to the activation of adenylyl cyclase through $G\alpha_s$, resulting in increases in cAMP as well as in cytosolic calcium (59). This pathway leads to downstream activation of PKA and enhances hormone release, most notably that of insulin from the β -cell (60). However, GIP has also been reported to stimulate insulin secretion through cAMP-dependent PKA-

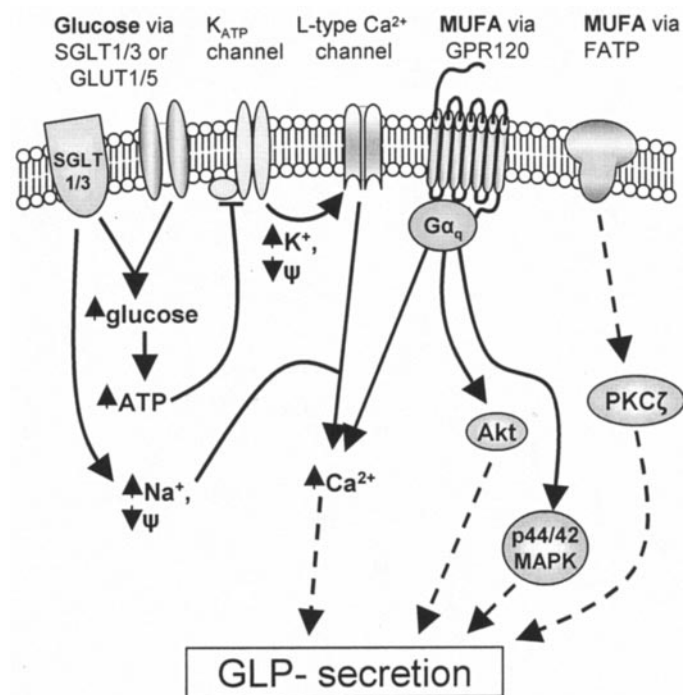


FIG. 4. Signaling components involved in direct nutrient-induced GLP-1 secretion. Glucose entry into L-cells via sodium glucose transporters (SGLT) causes an increase in ATP, which leads to K_{ATP} channel closure. Together with Na^+ entry via sodium glucose transporters, this results in a change in membrane potential (ψ), which opens L-type voltage-gated calcium channels and leads to GLP-1 release. MUFA-induced GLP-1 secretion is mediated by GPR120 and is associated with increases in intracellular calcium and phosphorylation of Akt and p44/42 MAPK via $G\alpha_q$. Alternatively, MUFAs may be transported via fatty acid transport proteins (FATP), leading to activation of PKC- ζ through an unknown mechanism to stimulate GLP-1 secretion. Solid arrows = known pathways; dashed arrows = unknown pathways.

independent activation of the cAMP guanine nucleotide exchange factor-II (Epac2) pathway (61). Although a direct stimulatory effect of GIP on the canine and rodent L-cell has been demonstrated (31,39,62), the expression of the GIP receptor and its downstream signaling pathways has yet to be fully examined. Furthermore, although cAMP-dependent GLP-1 secretion has been demonstrated in the human, murine, and rat L-cell (29,39,56), whether this is modulated through a PKA-dependent pathway and/or Epac2 has yet to be determined.

Somatostatin. Two distinct forms of somatostatin are produced in the intestine, SS14 by enteric neurons, and SS28 by enteroendocrine D-cells. However, in both rats and pigs, SS-28 is a more potent inhibitor of GLP-1 secretion (31,63,64). Somatostatin receptors exist as five isoforms (sst1–sst5), of which sst5 is expressed by the rat L-cell (63). These receptors are coupled with a pertussis-sensitive $G\alpha_i$ protein, and activation both inhibits adenylyl cyclase and decreases intracellular calcium levels (65). Because GLP-1 stimulates the secretion of both forms of somatostatin from the intestine, these findings suggest the existence of a feedback loop through which locally produced intestinal somatostatin can modulate GLP-1 release after the ingestion of nutrients (31,45,63,64).

Fatty acids. Long-chain monounsaturated fatty acids (MUFAs) directly stimulate GLP-1 secretion from the murine, rat, and human L-cell (29,38,39). Several recent studies have now begun to elucidate the mechanism of action of fatty acids on the L-cell (Fig. 4). Recently,

long-chain fatty acids have been found to interact with two distinct orphan G protein-coupled receptors: GPR40 and GPR120. In the β -cell, GPR40 is coupled to both $G\alpha_q$ and $G\alpha_i$, as demonstrated by increases in cytosolic calcium and inhibition of forskolin-induced cAMP production, respectively (66). GPR40 activation also increases p44/42 MAPK phosphorylation, and siRNA-mediated knock-down of GPR40 prevents fatty acid-induced insulin secretion. mRNAs for both GPR40 and GPR120 have also been detected in the murine L-cell, as well as in STC-1 cells (R. Iakoubov, A. Izzo, A. Yeung, C.I. Whiteside, P.L.B., unpublished data; 67). Although GPR120, but not GPR40, is required for fatty acid-induced GLP-1 release from the STC-1 cells, and activation of GPR120 is linked to increases in cytosolic calcium as well as p44/42 MAPK phosphorylation in these cells, neither of these pathways appear to be required for fatty acid-induced GLP-1 release (67). Furthermore, despite expression of both GPRs in the murine L-cell, MUFA treatment of these cells does not increase either intracellular calcium levels or p44/42 MAPK phosphorylation (R. Iakoubov, A. Izzo, A. Yeung, C.I. Whiteside, P.L.B., unpublished data). Hence, the exact role of these novel fatty acid receptors in GLP-1 secretion remains to be clearly elucidated.

The murine L-cell also expresses all three classes of PKCs (conventional, novel, and atypical) (R. Iakoubov, A. Izzo, A. Yeung, C.I. Whiteside, P.L.B., unpublished data). However, MUFA-induced GLP-1 secretion is mediated via activation of the atypical isoform PKC- ζ only (R. Iakoubov, A. Izzo, A. Yeung, C.I. Whiteside, P.L.B., unpublished data). The mechanism by which oleic acid activates PKC- ζ has yet to be determined, nor is it clear how oleic acid enters the cell. RT-PCR has confirmed the expression of fatty acid transport proteins, but their functional significance in MUFA-induced PKC- ζ activation and GLP-1 secretion is unclear. Further studies are clearly required to elucidate the exact mechanism of action of this important physiological regulator of GLP-1 release.

Glucose. As previously mentioned, it is not likely that glucose reaches the distal intestine to stimulate GLP-1 secretion, and in the physiological setting, GLP-1 secretion in response to ingested glucose is mediated indirectly by the vagus. Consistent with this hypothesis, GLP-1 release by isolated canine and rat L-cells is not directly affected by glucose (V.S.C. Wong, P.L.B., unpublished data; 62). Nonetheless, placement of glucose directly into the rat ileum has been found to stimulate the L-cell (27). Furthermore, murine L-cells do demonstrate dose-dependent GLP-1 secretion in response to glucose in vitro (40,41). Electrophysiological studies have shown that glucose effects a change in the membrane potential of the murine L-cell through closure of ATP-sensitive K^+ (K_{ATP}) channels (40), as well as via depolarization due to co-transport of sodium and glucose through the sodium glucose transporter (41). Expression of the glucose sensor glucokinase has also been detected in the mouse intestinal L-cell in vivo (68). The opening of L-type Ca^{2+} channels is also associated with glucose treatment, which leads to GLP-1 secretion (40,55). Taken together, the electrogenic response of the murine L-cell to glucose is similar to the stimulus secretion coupling events that occur during glucose-stimulated insulin secretion, but unlike the β -cell, the absence of GLUT2 mRNA expression in the murine L-cell suggests that glucose transport is primarily facilitated by sodium glucose transporters. Nonetheless, a recent report has indicated that the K_{ATP} channel cannot be detected in the

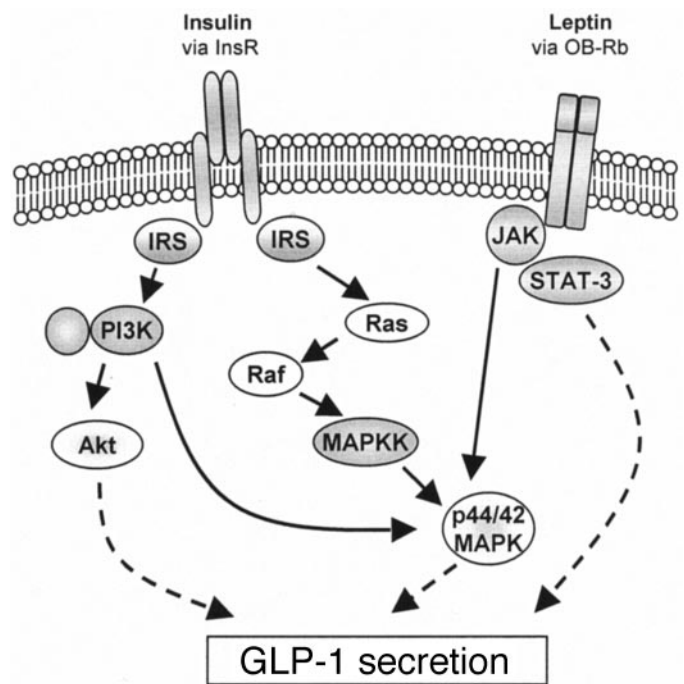


FIG. 5. Signal transduction pathways of metabolic hormones that stimulate GLP-1 secretion. Insulin receptor activation results in phosphorylation of insulin receptor substrate (IRS) molecules and subsequent activation of Akt and p44/42 MAPK through phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase kinase (MAPKK), respectively. Binding of leptin to its receptors in the L-cell results in STAT-3 phosphorylation, likely through janus kinase (JAK). Leptin receptor activation may also result in p44/42 MAPK phosphorylation. Solid arrows = known pathways; dashed arrows = unknown pathways.

mouse L-cell in vivo (69) and K_{ATP} null mice do not show alterations in circulating GLP-1 levels (70). Thus, the physiological importance of the findings made with the murine L-cell in vitro remain to be confirmed.

Leptin. Leptin is a cytokine derived from adipocytes with potent effects on food intake (71). When administered to rats and mice in vivo, leptin demonstrates stimulatory effects on GLP-1 secretion, and these effects have also been observed in rodent and human L-cells in vitro (48). Furthermore, leptin resistance induced by a high-fat diet in mice is associated with reduced basal and nutrient-stimulated GLP-1 secretion. The leptin receptor is a product of the *Ob-R* gene, which is related to other class I cytokine receptors. *Ob-R* is spliced into five isoforms, but only the OB-Rb isoform, which is the long form of the receptor, has the necessary intracellular motifs for leptin signaling (71). After leptin binding to OB-Rb, janus kinase (JAK) phosphorylates residues on OB-Rb, which then serve as docking sites for signal transducer and activator of transcription (STAT) molecules (Fig. 5). Once phosphorylated, STAT molecules dimerize and enter the nucleus to mediate effects on gene transcription (71). Consistent with these findings, human and rodent L-cells express OB-Rb, and leptin treatment increases STAT-3 phosphorylation (48); however, it remains unknown as to how this results in enhanced GLP-1 release. Nonetheless, stimulatory effects of leptin have also been reported in STC-1 cells, such that cholecystokinin release occurs in response to leptin, and this appears to be dependent on p44/42 MAPK phosphorylation (72). Whether the effect of leptin on GLP-1 secretion from the L-cell is mediated by p44/42 MAPK

remains to be determined. Nonetheless, these findings suggest a possible mechanism by which circulating GLP-1 levels are reduced in obese individuals (50).

Insulin. The insulin receptor is a tetrameric protein that consists of two extracellular α -subunits and two intracellular β -subunits linked together by disulfide bonds (71). Insulin binding to the α -subunits results in the phosphorylation of tyrosine residues in cytoplasmic domains of the β -subunits, thereby generating both tyrosine kinase activity and docking sites for a number of different intracellular proteins (Fig. 5). We have recently found that murine and human L-cells express the insulin receptor, and treatment of these cells with insulin results in insulin receptor, Akt, and p44/42 MAPK phosphorylation (49). Furthermore, insulin treatment stimulates GLP-1 secretion from the murine L-cell in a dose-dependent fashion. As Akt activation has recently been found to regulate catecholamine release from the chromaffin cell (73), these findings suggest one possible mechanism by which insulin stimulates GLP-1 release. Excitingly, both the murine and human L-cell were also found to exhibit insulin resistance after 24-h pretreatment with high concentrations of insulin. Furthermore, this insulin resistance was associated with altered basal and attenuated insulin-stimulated GLP-1 secretion, as well as with heterologous desensitization of the L-cell response to GIP (G.E.L., N. Flora, P.L.B., unpublished data; 49). When taken together, these findings suggest a possible mechanism by which GLP-1 levels are reduced in patients with type 2 diabetes (5–7), as well as in normal men in the lowest tertile of insulin sensitivity (25).

Relevance to diabetes and insulin resistance. Numerous studies have now shown that GLP-1 levels after a meal are reduced in subjects with type 2 diabetes (5–7). These changes are not due to altered clearance of GLP-1 from the circulation (6) and appear to be independent of obesity (5). Although direct studies of the intestinal L-cell have implicated insulin resistance in this phenomenon (49), type 2 diabetes is associated with a broad range of metabolic disorders, and it therefore remains unclear as to the exact mechanism underlying the reductions in GLP-1 in this condition. Furthermore, because obesity is linked to leptin and insulin resistance, as well as to impaired secretion of GLP-1 (48), the coexistence of obesity and type 2 diabetes may result in further defects in the release of GLP-1. Although further studies are clearly required to elucidate the specific mechanisms leading to impaired GLP-1 secretion in both type 2 diabetes and obesity, several recent studies conducted in vivo have suggested that the insulin sensitizer metformin may increase circulating GLP-1 levels (74), possibly through effects on the L-cell. Detailed mechanistic studies of metformin action using in vitro models of the intestinal L-cell are clearly warranted.

CONCLUSIONS

To date, only a limited number of studies have directly examined the signaling pathway used by the L-cell. Nonetheless, demonstration of the utility of GLP-1 as an anti-diabetic agent has highlighted the importance of the intestinal L-cell as a potential target for pharmacological enhancement of GLP-1 levels. An improved understanding of the specific intracellular proteins that are crucial for GLP-1 secretion may lead to novel approaches to enhance GLP-1 levels in patients with type 2 diabetes.

ACKNOWLEDGMENTS

This work was supported by an operating grant from the Canadian Diabetes Association. G.E.L. was supported by graduate studentships from the Natural Sciences and Engineering Research Council of Canada and from the Banting and Best Diabetes Centre, University of Toronto. P.L.B. was supported by the Canada Research Chairs Program.

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