The Multiple Actions of GLP-1 on the Process of Glucose-Stimulated Insulin Secretion

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The physiological effects of glucagon-like peptide-1 (GLP-1) are of immense interest because of the potential clinical relevance of this peptide. Produced in intestinal L-cells through posttranslational processing of the proglucagon gene, GLP-1 is released from the gut in response to nutrient ingestion. Peripherally, GLP-1 is known to affect gut motility, inhibit gastric acid secretion, and inhibit glucagon secretion. In the central nervous system, GLP-1 induces satiety, leading to reduced weight gain. In the pancreas, GLP-1 is now known to induce expansion of insulin-secreting β-cell mass, in addition to its most well-characterized effect: the augmentation of glucose-stimulated insulin secretion. GLP-1 is believed to enhance insulin secretion through mechanisms involving the regulation of ion channels (including ATP-sensitive K^+ channels, voltage-dependent Ca^{2+} channels, voltage-dependent K^+ channels, and nonselective cation channels) and by the regulation of intracellular energy homeostasis and exocytosis. The present article will focus principally on the mechanisms proposed to underlie the glucose dependence of GLP-1's insulinotropic effect. *Diabetes* 51 (Suppl. 3): S434–S442, 2002

Glucagon-like peptide 1 (GLP-1) is a potent incretin hormone produced in the L-cells of the distal ileum and colon. In the L-cells, GLP-1 is generated by tissue-specific posttranslational processing of the proglucagon gene (1). Nutrients, including glucose, fatty acids, and dietary fiber, are all known to upregulate the transcription of the gene encoding GLP-1, and they can stimulate the release of this hormone (2). Although the majority of L-cells are located in the distal ileum and colon, the levels of GLP-1 rise rapidly upon food ingestion. It is now well accepted that nutrients, principally sugars and fats, liberate GLP-1 and GLP-1–releasing factors, including glucose-dependent insulinotropic peptide (GIP), gastrin-releasing peptide, and selective neural regulators that also stimulate GLP-1 secretion (rev. in 1–3). Upon its release, GLP-1 affects multiple target tissues throughout the body, actions thought to be mediated by a single G-protein–coupled receptor isoform. GLP-1 receptor transcripts and/or protein have been identified in several tissues, including pancreatic islets, lung, gastrointestinal (GI) tract, and the central nervous system (CNS) (2,3). More questionable is the expression of functional GLP-1 receptors in liver and skeletal muscle tissues, where gene expression has been detected (4). GLP-1's ability to augment insulin release in a glucose-dependent manner is its most well-characterized physiological effect and one of its most promising characteristics from a clinical perspective.

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AKAP, A-kinase anchoring protein; $[Ca^{2+}]_i$, intracellular concentration of Ca^{2+}; CICR, Ca^{2+}-induced Ca^{2+} release; CNS, central nervous system; cPKA, catalytic subunit of protein kinase A; DP-IV, dipeptidyl-peptidase IV; ERK, extracellular signal-related kinase; GEF-II, guanine nucleotide exchange factor II; GI, gastrointestinal; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; HSL, hormone-sensitive lipase; IP_3, inositol triphosphate; K_{ATP}, ATP-sensitive K^+ channel; K_{Ca}, Ca^{2+}-sensitive voltage-dependent K^+ channel; K_v, voltage-dependent K^+ channel; MAPK, mitogen-activated protein kinase; NSCC, nonspecific cation channel; PDX-1, pancreatic duodenal homeobox-1; PI3-K, phophatidylinositol 3-kinase; PKA, protein kinase A; PKCζ, protein kinase Cζ; P_o, open probability; SU, sulfonylurea; VDCC, voltage-dependent Ca^{2+} channel.

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THE ACTIONS OF GLP-1

GLP-1 in the pancreas: insulin secretion and β-cell mass. There are several known and speculated pancreatic functions for GLP-1. The GLP-1 receptor is expressed in β-cells, where its activation is proposed to have multiple acute and long-term actions (3). With respect to β-cell function, GLP-1 rapidly and potently stimulates insulin secretion, a well-known action that will be discussed below. However, GLP-1 also stimulates insulin gene transcription, islet cell growth, and neogenesis, additional potentially important functions that may be clinically relevant for the treatment of diabetes. In mice, GLP-1 stimulates β-cell proliferation (5), and in rat β-cell lines, it stimulates DNA synthesis through a phosphatidylinositol 3-kinase (PI3-K)-dependent pathway (6). Effects on β-cell insulin gene transcription and proliferation are proposed to occur via the upregulation of the transcription factor pancreatic duodenal homeobox-1 (PDX-1) (6–8). PDX-1 translocation to the nucleus of RIN 1046-38 cells was also shown to be dependent on cAMP/protein kinase A (PKA) (9). The actions of GLP-1 on proliferation may involve the PI3-K downstream targets extracellular signal-related kinase (ERK) 1/2 and p38 mitogen-activated protein kinase (MAPK) (10). These studies have also demonstrated that a GLP-1–induced activation of protein kinase Cζ (PKCζ) is implicated in β-cell proliferation (10). Thus, at least two signaling pathways (PI3-K activation of ERK, MAPK, or

PKC and cAMP/PKA-mediated translocation of PDX-1) can be implicated in GLP-1's effects on β-cell proliferation. GLP-1 can also increase islet and β-cell mass by promoting cellular differentiation. Studies have shown that GLP-1 can induce the differentiation of rat (ARIP), pancreatic exocrine (AR4J), and human islet progenitor cells and implicate PDX-1 activation as a potential common mediator (3,11). A hint of the clinical relevance of GLP-1 treatment to circumvent the loss of β-cell capacity can be seen in vivo. Prolonged administration of GLP-1 to obese diabetic (*db/db*) mice stimulated insulin secretion, improved blood glucose/glycemic excursions, and also enhanced β-cell neogenesis and islet mass (7). Similar improvements in glycemic control and β-cell mass could be seen in mice with a partial pancreatectomy (12) and in neonatal mice treated with streptozotocin (13). Studies examining GLP-1 loss of function in GLP-1 receptor$^{-/-}$ mice demonstrated alterations in pancreatic insulin content and insulin gene transcription in one study (14) and islet size and composition in another (15). It is now thought that the phenotype of the GLP-1 receptor$^{-/-}$ mouse is likely diminished by compensatory upregulation of other signaling peptides, including GIP. Together, this work suggests that future therapies targeting the GLP-1 signal transduction pathways may be used to improve β-cell capacity by improving β-cell mass and secretion.

GLP-1 in the periphery: gut motility and insulin sensitivity. GLP-1 appears to exert a wide range of extrapancreatic effects. It participates in an ileal break phenomenon by playing an inhibitory role in gastric emptying and small intestinal transit (16,17). GLP-1 decreases gastric motility via direct effects on gastric smooth muscle and also inhibits postprandial acid secretion (4). It also decreases small intestine movement through inhibition of smooth muscle activity, resulting in an overall reduction in the absorption of nutrients from the GI tract (4). Reduced motility likely causes less severe postprandial glucose fluctuations and reduces the need for a large and rapid postprandial insulin response. GLP-1 also appears to improve insulin sensitivity and glucose uptake of both human and rat adipose tissue and skeletal muscle (4). Several studies suggest that GLP-1 may directly enhance glucose disposal in an insulin-independent fashion, although this may also result from the overall inhibition of glucagon secretion (3). GLP-1 also appears to have some cardiovascular effects, in part through actions on the CNS, as the administration of GLP-1 by intravenous or intracerebroventricular injection increases heart rate and blood pressure in rats, an effect that can be reversed by application of the antagonist exendin 9-39 or bilateral vagotomy, but not when GLP-1 is given by peripheral injection (3). The physiological relevance of this latter effect is unknown.

GLP-1 in the CNS: control of appetite and weight. GLP-1 has profound effects on feeding behavior. Although these actions of GLP-1 could be in part related to its effects on intestinal motility, they also appear to involve direct effects on hypothalamic feeding centers as GLP-1 receptors are found in specific nuclei within the hypothalamus (18). Acute administration of GLP-1 in humans and rodents induces satiety and decreases caloric intake (19–21). Administration of the GLP-1 antagonist exendin 9-39 abrogates the effect of GLP-1 and can itself promote weight gain (22). Long-term exendin 4 treatment of Zucker rats reduces food intake and decelerates weight gain (23). Interestingly, GLP-1 receptor$^{-/-}$ mice do not become obese, possibly because this peptide is not essential for weight regulation or because compensatory mechanisms are upregulated (24). In humans with type 2 diabetes, short-term GLP-1 or exendin 4 administration curbs appetite and food intake in addition to its insulinotropic actions (19,25), suggesting long-term delivery would promote weight loss in these patients. The ability of GLP-1 analogs to promote weight loss and improve β-cell function could prove to be ideal for the treatment of type 2 diabetes.

Type 2 diabetes and GLP-1 receptor agonists. It is well known that type 2 diabetes is characterized by defects in both insulin secretion and in peripheral insulin sensitivity (26). Current treatments for the β-cell defect include the sulfonylurea (SU) drugs, which were the first therapy targeted against insufficient insulin secretion. However, these compounds promote insulin secretion independent of blood glucose and can therefore cause hypoglycemia (27). In addition, as clearly demonstrated in the U.K. Prospective Diabetes Study, the ability of SUs to stimulate insulin secretion decreases over time, likely reflecting a deterioration in β-cell function (28). For these reasons, there is a need for the development of new drugs to treat the β-cell defect component of type 2 diabetes. Meglitinide analogs belong to a newer family of insulin secretagogues with actions similar to the SUs, e.g. ATP-sensitive K^+ (K_{ATP}) channel inhibition, but have distinct pharmacokinetic and pharmacodynamic properties (rapid onset/short duration) (27). The properties of GLP-1 described above, including glucose-dependent stimulation of insulin secretion and the expansion of β-cell mass, coupled with the inhibition of glucagon secretion and food intake, suggest that it would greatly complement current β-cell therapies. Trials with GLP-1 in diabetic patients have shown it to stimulate insulin secretion, inhibit gastric emptying, lower circulating glucagon, and improve overall glycemic control through both intravenous and subcutaneous injection (1).

Two major drawbacks in the use of GLP-1 therapy are related to its rapid inactivation in circulation and its delivery via injection. The issue regarding rapid inactivation has been addressed using two promising strategies. The first involves the development and use of GLP-1 analogs resistant to proteolytic cleavage by the enzyme dipeptidyl-peptidase IV (DP-IV) (1). Currently, several pharmaceutical companies are testing their GLP-1 analogs in a variety of models, including humans. The second strategy involves the neutralization of the DP-IV enzyme itself. Thus far, DP-IV inhibition has been shown to prolong the action of GLP-1, improve glycemia in diabetic models, and more recently to delay the onset of diabetes in Zucker diabetic fatty rats (29–32). Although both strategies are promising, several obstacles still remain. With respect to DP-IV inhibition, it is not clear what long-term effects such a therapy would have, given that this enzyme cleaves a number of biologically active peptides with various biological activities, including glucagon, vasoactive intestinal peptide, glucose-dependent insulinotropic polypeptide, neuropeptide Y, and substance P, for example (33). With respect to GLP-1 therapy, it is unlikely that it will gain widespread acceptance in the diabetes commu-

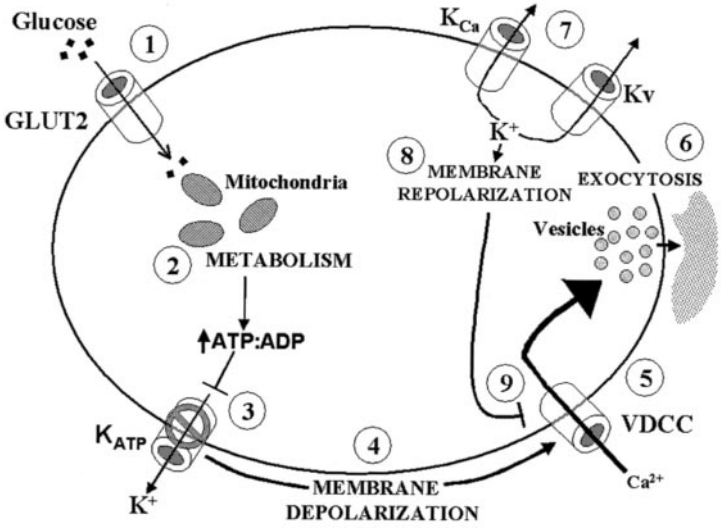

FIG. 1. The ionic mechanism (K_{ATP} channel–dependent) of stimulus-secretion coupling in the pancreatic β-cell. *1*: Glucose enters the β-cell via GLUT2 transporters. *2*: It is metabolized to produce an increase in the intracellular ATP-to-ADP ratio. *3*: This results in closure of K_{ATP} channels. *4*: This also results in membrane depolarization. *5*: Finally, it results in the opening of VDCCs. *6*: Entry of Ca^{2+} is the main trigger for exocytosis of insulin-containing granules. *7*: Also triggered by membrane depolarization, K_v and K_{Ca} channels open. *8*: This repolarizes the membrane. *9*: Repolarization leads to closure of VDCCs, limiting Ca^{2+} entry and insulin secretion.

nity when effective oral agents are available. However, one can anticipate that a small molecule that holds some or all of the properties of native GLP-1 will eventually be developed. To facilitate this process, it is important to understand how GLP-1 controls insulin secretion.

GLP-1 AND INSULIN SECRETION

Overview of the ATP-sensitive pathway. Glucose-stimulated insulin secretion (GSIS) is regulated by a number of ionic and nonionic signaling pathways, also known as the K_{ATP}-dependent and -independent pathways (34,35). The K_{ATP}-dependent mechanism of stimulus-secretion coupling is reviewed in Fig. 1. In general, the β-cell adapts insulin secretion to prevailing blood glucose levels through glucose metabolism. When glucose levels rise, the rate of glycolysis increases, which generates substrates (mainly pyruvate) for mitochondrial oxidative metabolism, the result of which is the generation of ATP, or more correctly an increase in the ATP-to-ADP ratio (36). This event provides the functional link between a glucose stimulus and insulin secretion. The increase in this ratio causes the closure of β-cell K_{ATP} channels, leading to plasma membrane depolarization, activation of voltage-dependent Ca^{2+} channels (VDCCs), and an increase in the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$), the main trigger for insulin secretion. Repolarization of β-cells is likely mediated by voltage-dependent K^+ (K_v) channels and Ca^{2+}-sensitive voltage-dependent K^+ (K_{Ca}) channels (37), which open in response to glucose-induced membrane depolarization to restore the outward flux of K^+. GLP-1 is proposed to modulate GSIS by regulating the activity of several ion channels involved in K_{ATP}-dependent insulin secretion as well as steps distal to channel modulation.

GLP-1 and β-cell K_{ATP} channels. One of the many observed cellular effects of GLP-1 is the inhibition of β-cell K_{ATP} channels (38–40). The resulting membrane depolarization induced by K_{ATP} channel closure initiates Ca^{2+} influx through VDCCs and triggers the exocytotic release of insulin. Figure 2 shows the excitatory effect of GLP-1 on membrane potential and its inhibitory effect on both native K_{ATP} channel currents from INS-1 cells and currents mediated by recombinant K_{ATP} channels (SUR1/Kir6.2) coexpressed with the GLP-1 receptor in a mammalian cell line. The physiological consequences of GLP-1–facilitated K_{ATP} channel closure would be to *1*) augment the excitability of cells already above the threshold for insulin

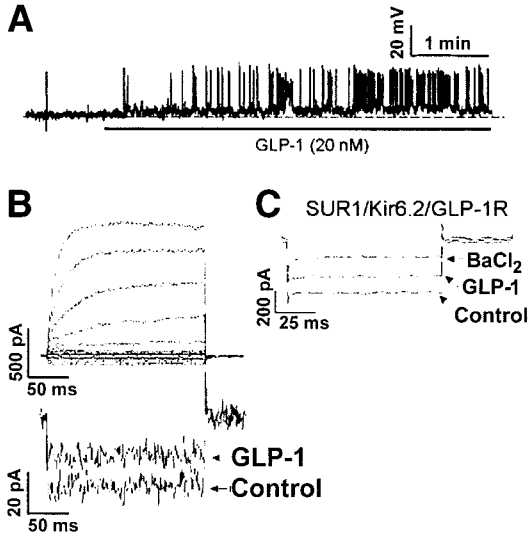

FIG. 2. Effects of GLP-1 on β-cell excitability and native and recombinant K_{ATP} channels. *A*: Membrane potential was recorded from an INS-1 cell using the patch-clamp technique in current-clamp mode. Application of GLP-1 (20 nmol/l) causes membrane depolarization and an increase in the action potential firing rate. A family of ionic currents elicited from an individual INS-1 cell using the patch-clamp technique in voltage-clamp mode is shown in *B*, upper panel. Cells were held at −80 mV and stepped at 10-mV intervals from −120 to 40 mV for 200 ms. Recordings were made with 5 mmol/l K^+ in the bath solution. In the lower panel of *B*, GLP-1 is shown to inhibit K_{ATP} channels in INS-1 cells. To measure inward K_{ATP} current, cells were held at 0 mV and stepped to −100 mV every 10 s. GLP-1 (20 nmol/l) was superfused over cells for 3–5 min until a steady-state current was obtained. Tolbutamide sensitivity was confirmed as a marker of K_{ATP} current (traces not shown). *C*: GLP-1 inhibits K_{ATP} channels in a recombinant system. tsA201 cells were transiently transfected with GLP-1R, Kir6.2, and SUR1 clones 48–72 h before recording. Current recordings were performed under symmetrical K^+ conditions (140 mmol/l K^+ in the bath solution). GLP-1 (20 nmol/l) was applied to the cells in a similar manner to *B*, upper panel. Barium chloride ($BaCl_2$, 2 mmol/l) was used in the recombinant system as a fully washable potassium channel blocker to assess the amount of recombinant K_{ATP} current present. The amphotericin-perforated patch-clamp technique mode was used to measure whole-cell K_{ATP} currents and membrane potentials while maintaining the integrity of the intracellular environment.

release and *2*) increase the percentage of β-cells actively secreting insulin at glucose concentrations normally subthreshold for the release of insulin.

The consensus view is that the inhibitory effect of GLP-1 on K_{ATP} channels is cAMP/PKA-dependent (38–41), although one study using rat β-cells disagrees (42). This assertion by Suga et al. (42) is based on their finding that the specific PKA inhibitor Rp-cAMPS (100 μmol/l) was unable to prevent the cellular depolarization and reduction in the whole-cell K_{ATP} current elicited by GLP-1. The completeness of PKA inhibition by Rp-cAMPS should be questioned because in the same study, forskolin induced significant insulin secretion even in the presence of Rp-cAMPS. Secondly, Suga et al. suggest that GLP-1 causes a slight increase in the ATP sensitivity of the K_{ATP} channel such that at low micromolar ATP concentrations, the K_{ATP} channel will be more susceptible to closure. However, in the normal rat pancreatic β-cell, millimolar levels of ATP are present (43), and at these physiological ATP levels, very similar K_{ATP} channel open probability (P_o) in the absence and presence of GLP-1 are predicted as follows. Our calculations indicate that with an intracellular [ATP] of 2 mmol/l, the K_{ATP} channel P_o is reduced from 0.005 to 0.003 in the presence of GLP-1. It is plausible that a leftward shift of ATP sensitivity does occur in the presence of GLP-1. However, the observed magnitude of GLP-1–induced K_{ATP} current reduction seen in whole-cell patch-clamp recordings (Fig. 2) is likely too large to be accounted for solely by the small decreases in P_o calculated from the data of Suga et al. (42). Recent work from our laboratory has shown that the membrane-permeant specific PKA inhibitor H-89 (44) is capable of completely inhibiting K_{ATP} current reduction by GLP-1 (45). Moreover, others have shown similar results using Rp-8-Br-cAMPS, a more membrane-permeable analog of Rp-cAMPS (38,41). The actions of GLP-1 on K_{ATP} channels may also involve other signaling pathways because K_{ATP} channel inhibition by GLP-1 in mouse β-cells was demonstrated to be calmodulin dependent, using the calmodulin inhibitors W-7 and calmidazolium (46).

The glucose dependency of GLP-1 actions has been well established, although the precise mechanisms for this dependence are unclear (41,47). However, the cellular actions of PKA on the K_{ATP} channel may provide a link between this kinase and the glucose sensitivity of GLP-1. Other groups have shown that addition of the catalytic subunit of PKA (cPKA) to excised patches containing K_{ATP} channels results in an augmentation of K_{ATP} current (39,48). Our laboratory has recently shown that the effect of cPKA on K_{ATP} current is dependent on ADP (45). When ADP levels are elevated, cPKA increases K_{ATP} channel current in a recombinant system, consistent with the results of Lin et al. (39). Conversely, as ADP levels are decreased, cPKA reduces K_{ATP} current (45). Physiologically, this may result in a negligible enhancement of β-cell excitability when glucose levels are low (high [ADP]), whereas when glucose levels rise (low [ADP]), GLP-1–mediated closure of K_{ATP} channels, via a PKA-dependent pathway, leads to membrane depolarization and subsequent increases in β-cell excitability. Special attention must be given to the cellular ATP-to-ADP ratio when considering K_{ATP} channel activity because it is changes in this ratio, more than simply changes in intracellular [ATP] *per se*, that govern the activity of K_{ATP} channels in the intact β-cell. Free ATP is highly buffered within the cell by membrane and cytosolic ATPases (43) and is predicted not to change significantly with increased glucose metabolism (36). In contrast, the reciprocal change in ADP, which is not buffered to the same extent, is more significant and results in a change in the ATP-to-ADP ratio (36). Indeed, the importance of ADP in controlling β-cell K_{ATP} channel activity has been demonstrated because mutations in the ADP-sensing region of the human K_{ATP} channel lead to uncontrolled insulin secretion and hypoglycemia (49). The molecular identity of the PKA phosphorylation site(s) is of significant importance and is still under investigation. Both the Kir6.2 and SUR1 subunits of the K_{ATP} channel contain putative target sequences for PKA-mediated phosphorylation (39,48), and systematic mutation of these residues should clarify the relative contributions of these sites to the action of PKA on the K_{ATP} channel.

GLP-1, VDCCs, and intracellular Ca^{2+} stores. GLP-1 has been shown to enhance currents through VDCCs in mouse, rat, and human β-cells (38,50–52), although the magnitude of this effect varies and often does not reach statistical significance. In single human β-cells, GLP-1 was shown to increase L-type VDCC activity and the amplitude of depolarization-evoked intracellular calcium transients, an effect which accounted for 40% of the increase in GLP-1–potentiated exocytosis (52). Although L-type VDCCs are classically regarded as the major regulators of Ca^{2+} influx leading to insulin secretion, β-cells are known to express multiple Ca^{2+} channel isoforms (53). Pereverzev et al. (54) reported recently that mice lacking the α1E-isoform of $Ca_v2.3$ have reduced glucose tolerance and diminished insulin responses to glucose. They speculate that G-protein regulation of this channel may modulate insulin secretion based on muscarinic acetylcholine receptor regulation of VDCCs in vitro (55).

We have found in HIT-T15 insulinoma cells transfected with the GLP-1 receptor that GLP-1 causes an increase in voltage-dependent Ca^{2+} currents (see 56 and Fig. 3*A*). This is due, at least partly, to a leftward shift in the voltage dependence of activation reminiscent of the effect of VDCC phosphorylation by PKA (57,58). We also observed a rightward shift in the voltage dependence of steady-state inactivation such that in the presence of GLP-1, less channels were effectively inactivated at a given holding potential (50). This is supported by Britsch et al. (50), who suggest that GLP-1 treatment of mouse β-cells slows the inactivation of voltage-dependent Ca^{2+} currents. Additionally, GLP-1 led to an increase in intracellular calcium only after glucose addition, an effect that was blocked in part by VDCC antagonists (Fig. 3*C*). The ability of GLP-1 to enhance Ca^{2+} currents is, like the effect on K_{ATP} channels, cAMP dependent (51,52), based on the ability of Rp-cAMPS to prevent an increase in currents. Indeed, treatment of rat β-cells with dibutyryl cyclic-AMP, a membrane-permeable cAMP analog, replicated the effect of GLP-1 on Ca^{2+} currents (51). Additionally, in our studies (56), the VDCC response to GLP-1 was lost in HIT-T15 cells expressing a mutant GLP-1 receptor lacking critical residues required for coupling to adenylyl cyclase, whereas VDCC activity could still be enhanced by the

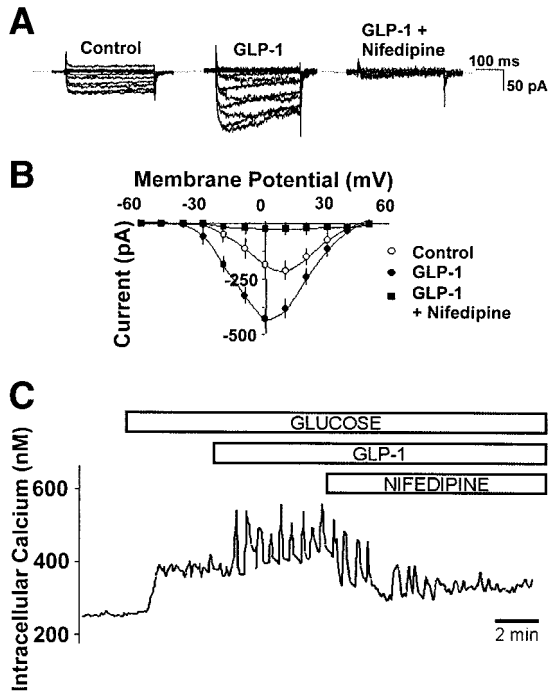

FIG. 3. GLP-1 enhances VDCC activity and $[Ca^{2+}]_i$ responses to glucose in HIT-T15 cells overexpressing the wild-type GLP-1 receptor. HIT-T15 cells overexpressing the wild-type GLP-1 receptor were voltage-clamped in the whole-cell configuration under conditions to measure voltage-dependent Ca^{2+} currents, held at −70 mV and stepped from −60 to 60 mV in 10-mV steps for 250 ms. *A*: Representative traces for basal control and after sequential cumulative addition of GLP-1 (10^{-8} mol/l) and nifedipine (10 μmol/l). Current-voltage relationships are shown in *B*. *C*: A representative trace of $[Ca^{2+}]_i$ in wild-type GLP-1 receptor–transfected HIT-T15 cell measured with Fura 2-AM after sequential cumulative addition of glucose (10 mmol/l), GLP-1, and nifedipine as in *A* and *B*.

cAMP independent agonist BAYK8644. Recent evidence suggests that an A-kinase anchoring protein (AKAP), AKAP18, targets PKA to VDCCs and that this kinase may be involved in GLP-1 modulation of these channels (59).

In addition to effects on VDCCs, GLP-1 can mobilize intracellular calcium stores in a cAMP-dependent manner (60,61), possibly contributing to the oscillatory $[Ca^{2+}]_i$ response to GLP-1 seen in HIT-T15 cells (Fig. 3*C*) (62). Our studies suggest that GLP-1 application results in oscillations in $[Ca^{2+}]_i$ in HIT-T15 cells and that these oscillations are not abolished (although they are diminished in amplitude) by removal of extracellular Ca^{2+} or by VDCC blockade (Fig. 3*C*). Indeed, in a number of cell types, Ca^{2+} oscillations induced by an agonist are primarily caused by Ca^{2+} release through inositol trisphosphate (IP_3) and/or ryanodine-sensitive stores (63–65). In β-cells, GLP-1 mobilizes Ca^{2+} stores in large part by sensitizing the ryanodine receptor (likely the type 2 isoform, RYR-2) to the process of Ca^{2+}-induced Ca^{2+} release (CICR) (61,66). Several studies have demonstrated that GLP-1 can increase $[Ca^{2+}]_i$ in a PKA-independent manner (67–69). This mechanism has recently been ascribed to CICR from ryanodine-sensitive stores via cAMP-regulated guanine nucleotide exchange factor II (GEF-II or Epac2) and its interaction with either the Ras-related small G-protein Rap1 or with Rab3 small G-protein effector Rim2 (68). The importance of cAMP-GEF-II–Rim2 has been demonstrated, because inactivation of this complex (by antisense oligonucleotides or mutant constructs) attenuated the secretory response of mouse islets or MIN6 insulinoma cells to GLP-1 (67). Because the affinity of cAMP for PKA is much higher (~100 nmol/l) compared with cAMP–GEF-II (~10 mmol/l), it is interesting to speculate that the GLP-1–stimulated cAMP–GEF-II pathway might operate on a rise in local cAMP rather than global changes. Although GLP-1 receptor signaling stimulates IP_3 production in GLP-1 receptor–expressing COS cells (70), a role for IP_3-sensitive Ca^{2+} stores in global $[Ca^{2+}]_i$ is in doubt because the GLP-1–stimulated IP_3 production in primary β-cells is reportedly minimal (71,72) and the IP_3 receptor antagonist xestospongin C failed to block release of intracellular Ca^{2+} stores by forskolin treatment (68). However, IP_3-regulated Ca^{2+} release from insulin granules has been suggested by the studies of Nakagaki et al. (62), who suggest that GLP-1 may uniquely regulate temporal and spatial release of intracellular calcium through local IP_3 signaling. Thus, GLP-1–mediated release of intracellular stores, along with potentiation of Ca^{2+} entry through VDCCs, likely contribute to the insulinotropic effect of GLP-1.

GLP-1 and β-cell K_v channels. Voltage-dependent K^+ currents, such as those mediated by K_v or K_{Ca} channels, mediate repolarization of β-cells after a depolarizing stimulus, such as glucose (37). Recently, we reported that K_v1 and K_v2 family channels regulate insulin secretion, because dominant-negative functional knockout of either of these channel families enhanced GSIS (73). $K_v2.1$ channels mediate the majority of this effect (>60%), the mechanism of which involves enhanced glucose-stimulated membrane depolarization and Ca^{2+} entry (unpublished observations). Because β-cell K_v currents are potent glucose-dependent regulators of insulin secretion, we hypothesized that physiological secretagogues, such as GLP-1, may regulate K_v channel function. Indeed, we report elsewhere in this supplement that the GLP-1 receptor agonist exendin 4 inhibits voltage-dependent outward K^+ currents in rat β-cells voltage-clamped in the whole-cell configuration by 40% and significantly prolongs the time course of β-cell repolarization after transient depolarization by current injection. This compares to an 86% reduction in outward K^+ currents achieved with the general K_v channel antagonist tetraethylammonium. GLP-1 antagonized voltage-dependent outward K^+ currents in rat β-cells in the absence of glucose. However, this effect may still contribute to the glucose dependence of GLP-1's insulinotropic effect, because K_v channels are not normally expected to be active until after a glucose-induced depolarization of the cell membrane (37). Additionally, and similar to the effect of GLP-1 on the other ion channels mentioned above, exendin 4–mediated inhibition of β-cell K_v channels is dependent on cAMP signaling. One recent study, however, suggested that cAMP signaling was not sufficient in itself to antagonize voltage-dependent K^+ currents in an insulin-secreting cell line (INS-1) (74).

Numerous studies have described effects of hormone-mediated alterations in voltage-dependent K^+ currents, both excitatory and inhibitory. The best-characterized of these effects is the voltage-dependent K^+ current down-regulation in lymphocytes and upregulation in cardiac myocytes (75,76). In both of these tissues, the cAMP/PKA

signaling pathway has been implicated in the regulation of these channels (76,77). Reports suggest that cAMP can reduce voltage-dependent K^+ currents in murine lymphocytes (76) and a pituitary cell line (78) but enhance voltage-dependent K^+ currents in cardiac myocytes (77), a finding that has been confirmed at the single-channel level in frog atrial myocytes (79) and the giant squid axon (80). Phosphorylation may occur directly on the channel, because PKA phosphorylation of an atrial K_v channel near the NH_2-terminus enhanced channel activity (81), and phosphorylation of K_v1 channel α-subunits regulates the extent of inhibition of these channels conferred by a regulatory β-subunit (82). Phosphorylation of β-subunits themselves may also modulate the regulatory interaction with pore-forming α-subunits (83). It has recently been demonstrated that regulation of a cardiac K_v channel (K_vLQT) by cAMP requires the expression of AKAP15/18 or AKAP79 (84). Additionally, an increase in voltage-dependent K^+ current is implicated in epinephrine-induced inhibition of the glucose-dependent increase in $[Ca^{2+}]_i$ in *ob/ob* and +/+ mouse β-cells (85) since the effect was reversed by tetraethylammonium. Interestingly, the inhibitory effect of epinephrine on $[Ca^{2+}]_i$ was also reversed by the adenylyl cyclase activator forskolin (85). Therefore, we believe there is mounting evidence to suggest that hormonal modulation of K_v currents is physiologically important. Specifically, GLP-1 inhibition of these currents is expected to lead to enhanced β-cell excitability.

GLP-1 and other β-cell ion channels. Increases in intracellular cAMP have long been known to enhance Na^+ currents (86), an effect that may be mediated through direct channel phosphorylation by PKA (87). A transient Na^+ current response to cAMP was first described in gastropod neurons and termed $I_{Na(cAMP)}$ (88). In insulin-secreting cells, RNA expression of the nonselective cation genes mSTRPC4 and LTRPC2 has recently been detected in insulinoma cells and human islets, respectively (89), and cAMP has been reported to induce the gene expression of mNSC1, which encodes a mouse nonspecific cation channel (NSCC) (90). GLP-1 is thought to enhance an NSCC carrying predominantly Na^+ currents (91,92). This effect occurs through GLP-1's activation of cAMP signaling and release of intracellular Ca^{2+} stores and may serve as yet another important modulatory pathway for GLP-1 in the β-cell (40). It is not clear whether the NSCCs activated by GLP-1 correspond to the nonselective cation current produced by activation of the Ca^{2+}-sensing receptor (93), but the latter effect is reported not to involve activation of the G_s subunit and may therefore not involve the cAMP/PKA pathway.

There is little known regarding the effects of GLP-1 on other ion channels. Cell swelling–activated Cl^- currents have been detected in insulin-secreting cells (94), but a role for these channels in insulin secretion is unclear. Cl^- channels such as cystic fibrosis transmembrane conductance regulator and outwardly rectifying Cl^- channels are activated by cAMP/PKA signaling (95). If occurring in the β-cell, this effect would tend to promote depolarization. One report suggests that GLP-1 activates a Ca^{2+}-sensitive Cl^- current in *Xenopus* oocytes expressing the GLP-1 receptor (96), an effect that was dependent on $Ins(1,4,5)P_3$-dependent intracellular Ca^{2+} mobilization (96). It remains to be determined, however, whether GLP-1 can stimulate Cl^- currents in insulin-secreting cells.

GLP-1 and exocytosis. Glucose can exert a stimulatory effect on insulin exocytosis, independent of its well-characterized actions initiated by the inhibition of K_{ATP} channels. The importance of this pathway can in part be realized by the fact that mice with a targeted disruption in K_{ATP} (Kir 6.2 or SUR1) do not display overt abnormalities in glucose tolerance (97,98). K_{ATP}-independent insulin secretion is not well understood and is thought to involve several signals that act on nonionic targets, in particular the distal steps of exocytosis. It has been proposed that glucose metabolism is required for this stimulatory effect and that likely signals include ATP, cAMP, glutamate, and malonyl-CoA (rev. in 99 and 100). Given that ATP, cAMP, and PKA are all implicated in the exocytotic process, it is plausible to think that GLP-1 may have effects distal to actions on ion channels, further augmenting insulin secretion. It is well known that actions that suppress GLP-1–induced cAMP accumulation and PKA activation inhibit insulin secretion, suggesting that cAMP and/or PKA are the plausible effectors (101). In mouse β-cells, only a fraction of exocytosis can be accounted for by actions on stimulus-secretion coupling (102). From studies showing that cAMP induces secretion in the presence of low and high $[Ca^{2+}]_i$, it is suggested that cAMP sensitizes the exocytotic machinery. Studies using photoreleasable cAMP and PKA inhibitors demonstrate that cAMP evokes PKA-dependent and -independent effects on exocytosis. Increases in cAMP, independent of PKA activation, appear to accelerate exocytosis of the readily releasable pool in β-cells (103). PKA-dependent mobilization of secretory granules, unlike the generation of cAMP itself, appears to require glucose metabolism (increased ATP/ADP) and involves the translocation of granules (103,104). Such an effect would increase the size of the readily releasable pool, increase the rate of replenishment of the pool, and enhance exocytosis. Because GLP-1 can increase both cAMP and PKA, effects on exocytosis can be implied from this data. There are several potential target proteins for the actions of GLP-1, including β-cell soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) proteins (105).

GLP-1 and intracellular energy homeostasis. Recent studies in clonal β-cells suggest that the insulinotropic actions of GLP-1 are in part mediated by a PKA-dependent stimulation of hormone-sensitive lipase (HSL) (106). It is proposed that lipolytic actions of GLP-1 cause the breakdown of trigycerides to free fatty acids in β-cells, which are then converted to long-chain CoA. An increase in free fatty acids could then provide substrate for mitochondrial oxidation and ATP production, leading to a larger increase in the intracellular ATP-to-ADP ratio and further inhibition of K_{ATP} channels. Additionally, because ATP can itself influence exocytosis, some of the actions of GLP-1 may be to target the distal steps of exocytosis, as mentioned above. It has been shown in several studies that ATP markedly facilitates exocytosis independent of cellular depolarization but is dependent on Ca^{2+} (99). Thus, yet another potential mechanism could explain GLP-1–induced insulin secretion.

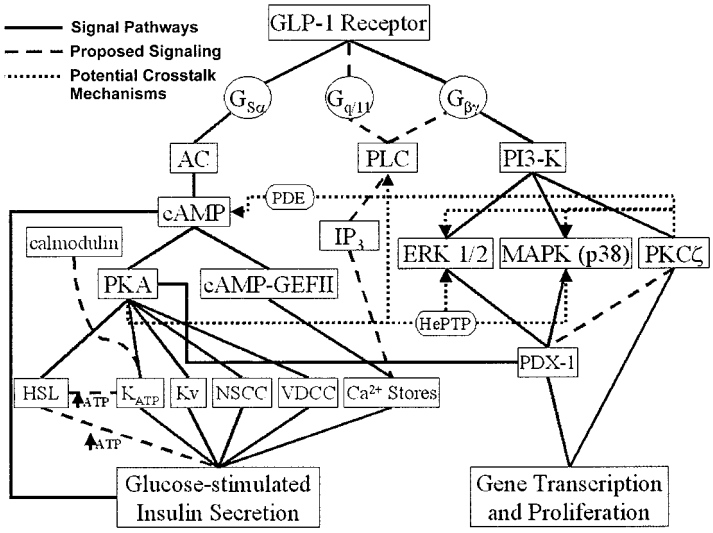

FIG. 4. Signaling pathways triggered by GLP-1 receptor activation. The signaling pathways shown include the more well-established pathways (solid lines), less well-established and putative pathways (dashed lines), and potential mechanisms of pathway cross talk not reviewed here (dotted lines). AC, adenylyl cyclase; HePTP, hematopoietic protein tyrosine phosphatase; PDE, phosphodiesterase; PLC, phospholipase C.

SUMMARY AND CONCLUSIONS

The ability of GLP-1 to enhance insulin secretion in a glucose-dependent manner remains perhaps its most promising characteristic from a clinical perspective. The recent discovery that GLP-1 is able to promote β-cell mass expansion has added to the clinical promise of this peptide, or more likely a molecule that activates one or all of GLP-1's signaling pathways. The signaling molecules thus far implicated in GLP-1 receptor signaling include adenylyl cyclase/cAMP/PKA, PI3-K/ERK/MAPK, phospholipase C, PKC and cAMP–GEF-II (Fig. 4). Of these, only cAMP/PKA signaling has been firmly established as a mediator of GLP-1's insulinotropic effect. PKA phosphorylation has been implicated in the regulation of K_{ATP} channels, VDCCs, K_v channels, NSCCs, intracellular Ca^{2+} stores, docking and fusion of vesicles, and intracellular energy homeostasis through HSL. However, the view that cAMP/PKA signaling is the sole mediator of GLP-1's insulinotropic effect, and is not affected by other signaling pathways, seems unlikely in the face of potential cross talk between pathways (Fig. 4) and recent evidence suggesting involvement of the cAMP-GEF-II–Rim2 complex (67). Elucidation of the mechanism by which GLP-1 exerts its clinically relevant effects and the contribution of various signaling pathways and effector targets remains an important task toward developing effective therapies for type 2 diabetes.

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