# Glucagon-Like Peptide 1 Stimulates Lipolysis in Clonal Pancreatic β-Cells (HIT)

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**Glucagon-like peptide 1 (GLP-1) is the most potent physiological incretin for insulin secretion from the pancreatic β-cell, but its mechanism of action has not been established. It interacts with specific cell-surface receptors, generates cAMP, and thereby activates protein kinase A (PKA). Many changes in pancreatic β-cell function have been attributed to PKA activation, but the contribution of each one to the secretory response is unknown. We show here for the first time that GLP-1 rapidly released free fatty acids (FFAs) from cellular stores, thereby lowering intracellular pH ($pH_i$) and stimulating FFA oxidation in clonal β-cells (HIT). Similar changes were observed with forskolin, suggesting that stimulation of lipolysis was a function of PKA activation in β-cells. Triacsin C, which inhibits the conversion of FFAs to long-chain acyl CoA (LC-CoA), enhanced basal FFA efflux as well as GLP-1–induced acidification and efflux of FFAs from the cell. Increasing the concentration of the lipase inhibitor orlistat progressively and largely diminished the increment in secretion caused by forskolin. However, glucose-stimulated secretion was less inhibited by orlistat and only at the highest concentration tested. Because the acute addition of FFAs also increases glucose-stimulated insulin secretion, these data suggest that the incretin function of GLP-1 may involve a major role for lipolysis in cAMP-mediated potentiation of secretion.**
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Glucagon-like peptide 1 (GLP-1) is the most potent potentiator of glucose-induced insulin secretion that has been described (1,2). This peptide causes the elevation of cAMP and the activation of protein kinase A (PKA) (3); however, it releases insulin only in the presence of stimulatory glucose (4) and thus serves as an incretin rather than a secretagogue (5). Activation of PKA leads to phosphorylation of multiple β-cell proteins, many of which have been hypothesized to play a role in insulin secretion (6–8). The nature of the endogenous substrates for PKA that may potentiate insulin secretion is unknown. Because the islet contains large stores of triglycerides (9), particularly in diabetes (10), another possible role of the normal rise in cAMP could be to stimulate lipolysis (via lipase activation), thereby providing the cell with free fatty acids (FFAs). Recent research on hormone-sensitive lipase (HSL) in β-cells yielded results consistent with that notion (11). The released FFAs may directly effect secretion, or they may do so indirectly via generation of other lipids, including the putative long-chain acyl CoA (LC-CoA) signal, diacylglycerol (DAG), and phosphatidic acid (PA) (12). The acute addition of exogenous FFAs is also known to enhance glucose-stimulated secretion (9,13–15).

We have shown in previous studies that added FFAs cause acidification in β-cells (16) and fat cells (17) as a consequence of the flip-flop mechanism of diffusion across the plasma membrane (16,17). Furthermore, we have shown in adipocytes that the elevation of cAMP stimulates lipolysis, with a resulting decrease in the intracellular pH ($pH_i$) caused by the release of FFAs, which become partially ionized (17). Therefore, in this study we assessed whether GLP-1 has a similar effect on lipolysis in β-cells. Our data show a decrease in $pH_i$ and a release of FFAs by agents that increase cAMP, presumably via activation of HSL. Furthermore, the incretin effect was largely diminished by a lipase inhibitor, whereas glucose-stimulated secretion was less affected. These findings indicate that cAMP-mediated lipolysis may play an important role in β-cell signal transduction and the incretin effect of GLP-1.

## RESEARCH DESIGN AND METHODS

**Growth and incubation of cells.** Clonal pancreatic β-cells (HIT-T15) were cultured in a RPMI-1640 medium supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, and 10% fetal calf serum (used between passages 64 and 80), harvested with phosphate-buffered saline containing 0.02% EDTA or with 0.25% trypsin and 0.06% EDTA diluted in phosphate-buffered saline, and washed in a buffer (pH 7.4) (18).

The effect of GLP-1 on FFA oxidation was measured as the $^{14}CO_2$ released from cells that were labeled overnight in RPMI-1640 with [U-$^{14}$C]oleic acid (1 μmol/l), washed free of remaining labeled FFAs, and incubated in the absence of added labeled or unlabeled FFAs (19).

For insulin secretion measurements, HIT-T15 cells were grown under standard conditions in 24-well plates and then used 2–4 days after passaging for secretion assays. Cells were washed twice in Krebs-Ringer bicarbonate (containing 2 mmol/l $CaCl_2$ and 0.25% bovine serum albumin) and buffered with 10 mmol/l HEPES at pH 7.4. The cells were preincubated with the above assay buffer for 30 min at 37°C. The buffer was replaced and the cells were then incubated for 30 min. Secretion was stopped by cooling the plates on ice. The sample solution was removed and centrifuged to remove any loose cells,

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ACS, acyl CoA synthetase; AM, acetoxymethyl ester; BCECF, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; DAG, diacylglycerol; FFA, free fatty acid; GLP-1, glucagon-like peptide 1; HSL, hormone-sensitive lipase; LC-CoA, long-chain acyl CoA; PA, phosphatidic acid; PCR, polymerase chain reaction; $pH_i$, intracellular pH; PKA, protein kinase A.

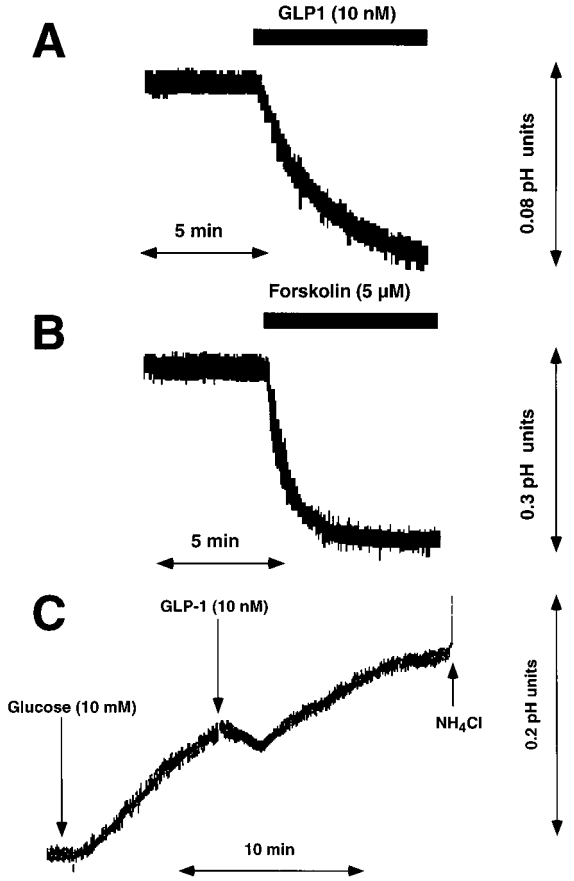


**FIG. 1. The effect of GLP-1 and forskolin on $pH_i$ in clonal pancreatic β-cells. HIT cells (0.2 mg/ml) loaded with the pH indicator BCECF were suspended in 1.3 ml Krebs buffer containing no albumin (pH 7.4) at 30°C. Traces A and B show the acidification caused by the addition of GLP-1 or forskolin. Trace C shows the alkalinization caused by glucose addition followed by a small and transient acidification caused by GLP-1. The illustrations shown are representative of experiments repeated at least three times.**

then the insulin level was measured by radioimmunoassay using the assay protocol for rat insulin distributed by Linco Research.

**Fluorescence measurements of $pH_i$, redox state, intracellular $Ca^{2+}$, and extracellular FFA.** Cells were loaded with 1 μmol/l 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF), acetoxymethyl ester (AM) for 30 min at 37°C with continuous shaking (20). For fluorescence recordings, cells (0.2 mg protein/ml) were maintained in suspension by continuous stirring in a modified Krebs buffer at 30°C in a computer-controlled Hitachi F-2000 spectrofluorometer. BCECF fluorescence was monitored at excitation wavelengths of 440 and 495 nm and an emission wavelength of 535 nm. The average basal $pH_i$ (mean ± SD) was 7.0 ± 0.1 ($n$ = 14). The $pH_i$ was calibrated by measuring the fluorescence of the dye at different pH values between 6 and 8 as determined with a pH electrode after the cells were permeabilized to equilibrate $pH_i$ with extracellular pH (16). Traces shown are those obtained at the excitation wavelength of 495 nm. $NH_4Cl$ (10 mmol/l final concentration) was added at the end of each experiment to confirm cell viability (16); the increase in $pH_i$ followed by the restoration of the basal $pH_i$ indicated viable cells and an intact plasma membrane. The engineered intestinal fatty acid–binding protein ADIFAB (0.2 μmol/l) (dissociation constant [$K_D$] for oleic acid = 390 nmol/l [21]), was monitored at an excitation wavelength of 390 nm and emission wavelengths of 432 and 505 nm (21,22) to measure the release of FFAs into the media. The redox state of the cells was determined by measuring reduced pyridine and oxidized flavin nucleotide fluorescence at the excitation and emission wavelengths of 340 and 460 nm and 460 and 540 nm, respectively, as described previously (20). Intracellular $Ca^{2+}$ changes were measured in cells loaded with the $Ca^{2+}$ indicator fura 2, as described previously (20). When $pH_i$ and extracellular FFAs were measured simultaneously, only single emission and excitation wavelengths were used. Baseline drift was subtracted from traces when single wavelength measurements were used.

**HSL.** The total RNA from pancreatic islets was isolated using the guanidinium isothiocyanate-phenol method and was reverse transcribed using random hexamers. Polymerase chain reaction (PCR) was performed using the rat HSL sense and antisense primers 5′-gaacactacaaacgcaacgag-3′ and 5′-caaggggtgagatggtaac-3′. The PCR cycles included a hot start at 97°C, followed by 30 cycles of 94°C for 30 s, 62°C for 20 s, and 72°C for 1 min. Experimental samples were also processed in the absence of reverse transcriptase to rule out amplification from genomic DNA. The PCR products were displayed on a 1.2% agarose gel, stained with ethidium bromide, and photographed with UV light. Negative control reactions lacking cDNA were amplified simultaneously with the experimental samples.

**Materials.** BCECF-AM, fura 2-AM, and ADIFAB were purchased from Molecular Probes (Eugene, OR). Radioactive tracers were from New England Nuclear (Boston, MA), and triacsin C was from Biomol Research Laboratories (Plymouth Meeting, PA). Other reagents were from Sigma (St. Louis, MO). Orlistat (Xenical, Roche) capsule material was extracted with ethanol to make a nominally 500 mmol/l stock solution. Forskolin (Research Biochemicals International, Natick, MA) was dissolved in DMSO. Cells used for secretion studies contained 0.025% ethanol and 0.1% DMSO in Krebs-Ringer bicarbonate solution containing 0.25% FFA-free bovine serum albumin.

## RESULTS

**GLP-1 decreases $pH_i$.** An acidification in clonal β-cells was observed when GLP-1 was added (Fig. 1*A*). The decrease in $pH_i$ began immediately after the addition and continued over a period of minutes. A similar but larger decrease in $pH_i$ was caused by the addition of forskolin (Fig. 1*B*), which indicated that this change was probably caused by an increase in cAMP. The extent of the decrease in $pH_i$ was similar to that observed in adipocytes and attributed to the generation of FFAs from triglycerides after the stimulation of lipolysis (17). The data in Fig. 1*A* and *B* were obtained in the absence of glucose. Glucose has been shown to alkalinize $pH_i$ (23), and this effect appears to dominate $pH_i$ in the presence of glucose, although a transient acidification occurred when GLP-1 was added (Fig. 1*C*).

**Elevation of $Ca^{2+}$ does not influence $pH_i$.** Agents that elevate cAMP may also elevate $Ca^{2+}$ (24). Thus, if acidification results from the release of intracellular FFAs, the FFAs could originate from either triglyceride stores (via lipolysis) or from phospholipids (via $Ca^{2+}$-activated phospholipase activity). Figure 2 shows that the elevation of cytosolic free $Ca^{2+}$

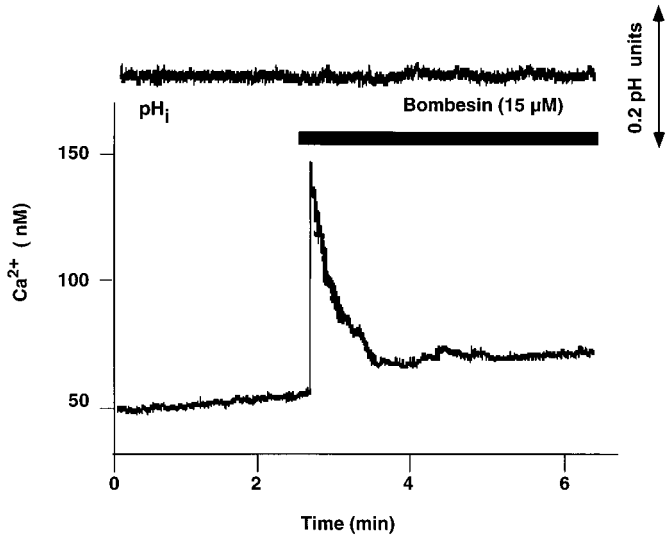


**FIG. 2. The effect of bombesin on $Ca^{2+}$ and $pH_i$. Experiments were performed and cells were incubated as described in Fig. 1. In addition, half of the cells were loaded with the $Ca^{2+}$ indicator fura 2 and monitored separately to assess changes in cytosolic free $Ca^{2+}$. The illustrations shown are representative of experiments repeated at least three times.**

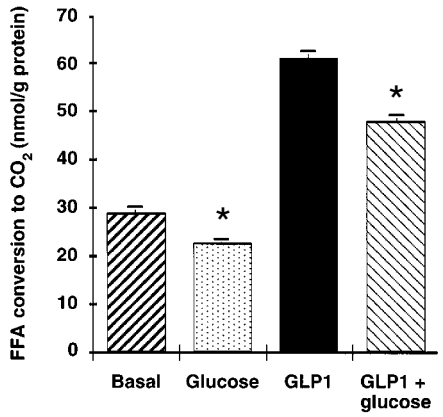


**FIG. 3. The effect of GLP-1 and glucose on FFA oxidation. Clonal β-cells (0.2 mg/ml) preloaded for 24 h with [U-$^{14}$C]oleate were washed three times and preincubated in 1.3 ml Krebs buffer containing 5 mmol/l glutamine without glucose or FFAs (pH 7.4) at 30°C. After changing to a test medium containing no glucose, 10 mmol/l glucose, 10 nmol/l GLP-1, or GLP-1 plus 10 mmol/l glucose, $^{14}CO_2$ evolution was determined during a period of 60 min. The data are means ± SE from six or eight flasks from two separate experiments. *$P$ < 0.01 vs. basal or GLP-1 alone.**

by bombesin is not accompanied by a change in $pH_i$ (top trace). As noted above, previous studies from our laboratories have shown that glucose stimulation of β-cells, which also elevates cytosolic free $Ca^{2+}$, causes alkalinization (23). Other agonists (e.g., carbachol and bradykinin) that increase $Ca^{2+}$ do not also decrease $pH_i$ (data not shown). These findings make it very unlikely that $Ca^{2+}$-stimulation of a phospholipase is responsible for the cAMP-induced decrease in $pH_i$.

**GLP-1 stimulates lipolysis.** If GLP-1 stimulates lipolysis, there should be evidence for an increase in FFA production. Evidence supporting an action of GLP-1 to stimulate lipolysis is presented in Fig. 3. Cells were incubated overnight with trace amounts of $^{14}$C-oleic acid to label the internal triglyceride pool, then the production of $^{14}CO_2$ was measured in cells that had been thoroughly washed to remove free oleate. We expected the stimulation of triglyceride breakdown to lead to increased oxidation of FFAs released from the labeled triglyceride pool. Indeed, cells acutely treated with GLP-1 exhibited more than a twofold increase in FFA oxidation, whereas the addition of glucose inhibited FFA oxidation in both control and GLP-1–treated cells. Inhibition of FFA oxidation by glucose (in the absence of GLP-1) was previously shown (19).

To determine whether FFAs were released from the β-cell into the medium, we used ADIFAB, a fluorescent fatty acid–binding protein that reports the binding of FFAs to the protein (21). Figure 4 directly demonstrates the basal FFA efflux from clonal β-cells by showing the increase in the ADIFAB fluorescence ratio. Glucose prevented FFA efflux (Fig. 4*A*), presumably by stimulating re-esterification. Lipolysis yields FFAs, which are converted to their active metabolite LC-CoA by acyl CoA synthetase (ACS). Triacsin C, an inhibitor of some isoforms of ACS (25), increased the release of FFAs when added under basal conditions (Fig. 4*B*) or after glucose addition (Fig. 4*A*). Control experiments showed that triacsin C itself neither altered the redox state nor qualitatively prevented the redox changes induced by glucose (Fig. 5). Also, it did not alter the $Ca^{2+}$ changes induced by a variety of agonists (data not shown).

To determine whether the $pH_i$ effects of GLP-1 (which we attributed to the stimulation of lipolysis) corresponded to FFA exit from the cells, we monitored the release of FFAs to the external buffer using ADIFAB while simultaneously measuring the $pH_i$. Figure 6 directly demonstrates that GLP-1 increased FFA release into the medium; as in Fig. 1, GLP-1 caused acidification of the cytosol (Fig. 6*A*, bottom trace) and a small release of FFAs into the medium (Fig. 6*A*, top trace). FFA release in the presence of glucose plus GLP-1 was quite low, presumably because of glucose-promoted re-esterification similar to that seen in Fig. 4; however, the FFA production was unmasked by the addition of triacsin C, which sharply increased FFA release and intracellular acidification (Fig. 6*B*). The increases in ADIFAB fluorescence (top traces) showed a time dependence for the release of FFAs into the medium that corresponded to the time course of the decreases in $pH_i$ (bottom traces). This implies that the FFAs can leave cells almost as quickly as they are generated.

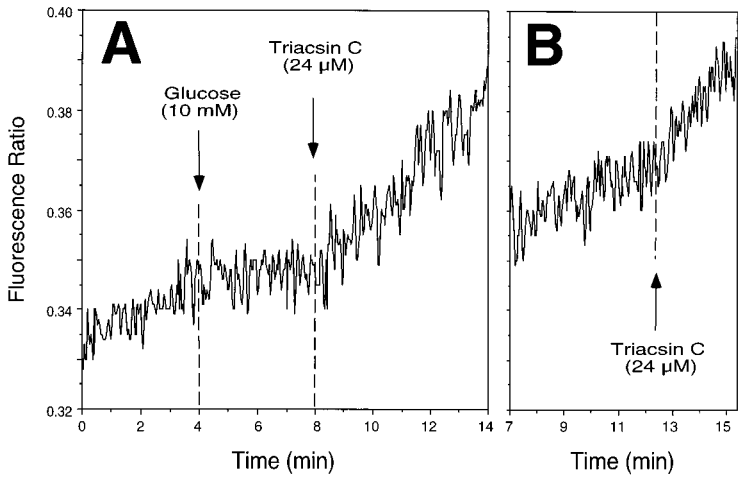


**FIG. 4. Illustration of the effect of glucose and triacsin C on FFA efflux from clonal β-cells. Experiments were performed and HIT cells were incubated as described in Fig. 1 but without BCECF. The relative FFA concentration in the medium was monitored with 0.2 μmol/l ADIFAB; an increase in the fluorescence ratio indicates an increase in medium FFAs. The illustrations shown are representative of experiments repeated at least three times.**

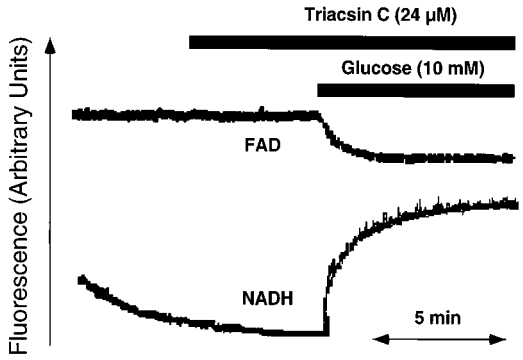


**FIG. 5. Triacsin C does not prevent glucose-mediated redox changes. HIT cells (0.2 mg/ml) were suspended in 1.3 ml Krebs buffer containing no albumin (pH 7.4) at 30°C. The top trace shows a decrease in oxidized flavin and the bottom trace shows an increase in reduced pyridine nucleotide fluorescence caused by the addition of glucose. Reduced pyridine (NADH) and oxidized flavin nucleotide (FAD) fluorescence were measured at excitation and emission wavelengths of 340 and 460 nm and 460 and 540 nm, respectively. The illustration shown is representative of experiments repeated at least three times.**

**HSL in the β-cell.** To explain the cAMP-mediated stimulation of lipolysis, the presence of an HSL-like activity must be postulated. To confirm that the RNA for adipocyte HSL was expressed in islets, rat islet cDNA was used in a PCR with HSL-specific sense and antisense primers. The PCR products were separated by agarose gel electrophoresis and the gel was stained with ethidium bromide. An amplification product of the expected size (280 bp) was present in the rat islet cell lane (Fig. 7). DNA sequencing of the isolated band confirmed its identity to HSL. There was no DNA amplification in the negative control lane, which was lacking a cDNA template. There was also no amplification product in RNA samples processed without reverse transcriptase enzyme (data not shown), ruling out amplification from genomic DNA.

**Inhibition of the incretin effect by a lipase inhibitor.** Forskolin increased glucose-stimulated insulin secretion, and this incretin effect was progressively and largely diminished by 30 min preincubation of the cells with increasing concentrations of the lipase inhibitor orlistat (tetrahydrolipstatin) (26–28) (Fig. 8). Similar results were seen in a second experiment with a different passage of cells. Glucose-stimulated secretion itself was less potently affected by orlistat in the experiment illustrated by Fig. 8, and it was not affected at all in the second experiment. There was no effect of orlistat on basal secretion (data not shown). It should be noted that though orlistat acts in the intestinal lumen in vivo and is not taken up systemically (29), orlistat can nevertheless cross biological model membranes (30). Furthermore, orlistat inhibits HSL (28) in addition to pancreatic lipase, but it does not inhibit phospholipase $A_2$ (31).

## DISCUSSION

The data presented here are consistent with a model (Fig. 9) in which GLP-1 activates adenyl cyclase, generating cAMP, which consequently stimulates HSL via PKA, thereby causing the breakdown of triglycerides and the generation of FFAs. The FFAs are converted to LC-CoA, either for oxidation in the mitochondria or for the synthesis of complex lipids (e.g., DAG or PA). Triacsin C interferes with this process by partially blocking the generation of LC-CoA and favoring the accumulation and efflux of FFAs. Support for this model is indicated by the actions of GLP-1 to *1*) decrease $pH_i$, *2*) increase the release of FFAs from the cell, and *3*) stimulate FFA oxidation after overnight labeling of the triglyceride pool. Further support comes from the recent finding that high-fat feeding, which increases islet triglyceride content, leads to an exaggerated response to GLP-1 (32).

The suggestion that lipolysis (with the resulting production of FFAs and LC-CoA) may be involved in the incretin effect of GLP-1 is amenable with our hypothesis that both FFAs and cytosolic LC-CoA and other complex lipids are major effector signals in insulin secretion (12,13). The data also suggest that an important source of these lipid molecules is internal triglyceride stores, the regulation of which has not been previously investigated. The current studies indicate that cAMP may regulate lipolysis, and ultimately FFA and LC-CoA production, via HSL. This may explain why the pancreatic islet contains large triglyceride stores (9,10) and relies on basal FFA oxidation to provide most of its fuel needs (9). These triglyceride stores and their modulation may also play an important role in glucose homeostasis in vivo (14,15,33,34). During the course of this work, similar and more extensive evidence of HSL in islets was published by Mulder et al. (11).

It should be noted that PKA phosphorylation of either purified HSL or HSL in fat cell homogenates causes only a 2- to 3-fold increase in activity, far less than the 20- to 50-fold change in lipolysis in intact cells (35,36). This is attributed to the much greater importance of translocation of HSL to the substrate and changes in the accessibility of the substrate, probably also involving PKA-mediated phosphorylation of perilipin (37). Interestingly, PKA phosphorylation of purified lipase increases its activity only toward certain substrates (e.g., triglyceride and cholesteryl oleate), but not toward the diglyceride substrates that are commonly used in lipase assays and yield the highest rates of hydrolysis by HSL (35,38). Diglyceride substrates were used in the studies

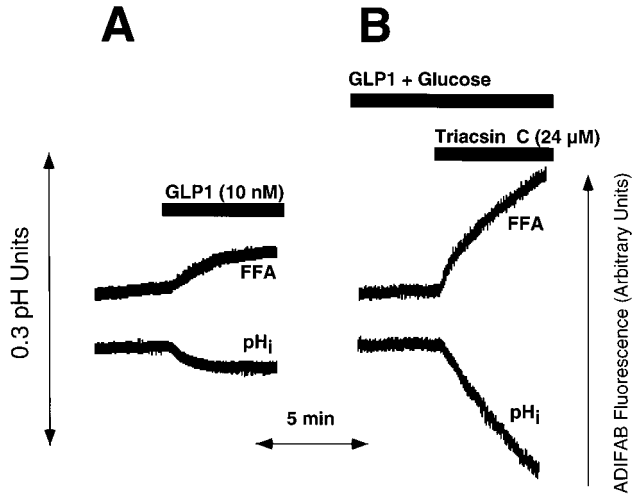


**FIG. 6. The effect of GLP-1 and triacsin C on $pH_i$ and FFA release. Experiments were performed and HIT cells were incubated as described in Fig. 1. In addition, the relative FFA concentration in the medium was monitored with 0.2 μmol/l ADIFAB; an increase in fluorescence indicates an increase in medium FFA. The illustration shown is representative of experiments repeated at least three times.**

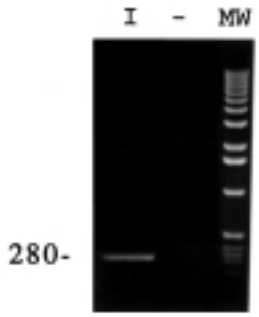


**FIG. 7. Reverse transcription–PCR of rat pancreatic islet RNA (*lane I*) using HSL-specific PCR primers. The products were separated on a 1.2% agarose gel stained with ethidium bromide and photographed with UV transillumination. The figure also shows the negative control lane (–) and the molecular weight marker (MW). The band of the expected size was excised from the gel, then the DNA was extracted and sequenced using Taq cycle sequencing (with the original PCR primers) on an ABI 373 DNA sequencer.**

of HSL-like activity in β-cells by Mulder et al. (11). Although we have confirmed lipase activity in HIT cell homogenates using a diglyceride-based commercial kit, for the above reasons we have not pursued studies of assayed activity in homogenates. Monitoring the FFA release from intact cells, as we have done here, appears to be the more physiologically relevant means of measurement. Admittedly, although the HSL-like enzyme shown to be present in β-cells is the most likely candidate for mediating GLP-1–induced FFA release, it is possible that another lipase (e.g., a $Ca^{2+}$-independent phospholipase) might be involved. Regardless of the identity of the lipase, the important finding for the proposed lipid-mediated pathway of signal transduction in the β-cell is that GLP-1 causes FFA release.

GLP-1 acidified the β-cell under the conditions of our experiments; however, the acidification is unlikely to be important for its incretin action because secretion was not stimulated by GLP-1 in the absence of glucose where the decrease in $pH_i$ was greatest, whereas in the presence of glucose the ability of GLP-1 to acidify was minimal. In contrast, glucose-stimulated secretion is accompanied by alkalinization of the β-cell (20,23). This alkalinization results from an early stimulation of pyruvate transport into the mitochondria (cotransport with a proton), as suggested by observations that blockage of the pyruvate transport with 3-hydroxycyanocinnamate prevented the pH change but not the subsequent glucose-induced rise in $Ca^{2+}$ (20,23). Thus, the changes in $pH_i$ may serve as useful indicators of metabolic events without necessarily playing an important role in stimulus-secretion coupling. In addition, our data indicate that the $pH_i$ change did not act indirectly by causing changes in $Ca^{2+}$ and that agonists that elevated cytosolic free $Ca^{2+}$ did not change the $pH_i$.

The rise in FFA levels caused by GLP-1, as indicated by acidification or FFA efflux from the cell, is insufficient by itself to cause insulin secretion in the absence of glucose. Furthermore, glucose reduced the rate of release of FFAs, and hence probably the cellular level of FFAs, as well as the rate of FFA oxidation; these effects are presumably due to the ability of glucose to promote complex lipid formation and re-esterification. This postulation may suggest that a product formed from FFA but requiring glucose is involved in stimulus-secretion coupling.

Triacsin C was used here to help unmask FFA production by inhibiting both the activation of FFAs to LC-CoA and their further removal by either mitochondrial oxidation or complex lipid formation in the presence of glucose. In theory, triacsin C could be used to distinguish between effects mediated directly by FFAs or by LC-CoA or complex lipids. It has been reported that acutely administered triacsin C largely inhibited oxidation of exogenous FFAs yet had no effect on glucose-stimulated secretion (39); we have confirmed this and, likewise, we have found no consistent effect on the enhancement of secretion by GLP-1 or FFAs (data not shown). On the surface, this might seem to indicate that the FFAs, but not LC-CoA, were involved in the stimulation of secretion. However, the situation is complicated by the existence of pools of LC-CoA and various isoforms of ACS with differing sensitivity to triacsin C (25,40). Indeed, although FFA oxidation is strongly inhibited, the incorporation of FFAs into phospholipids is much less affected by triacsin C (25,39,40). Because glucose itself reduces FFA oxidation, the more relevant path of FFA metabolism for signal transduction is likely to be the formation of complex lipids. We propose that GLP-1 causes the release of FFAs and that this effect can provide more substrate for both FFA oxidation and complex lipid formation, as well as the observed enhanced FFA efflux from the cell; as noted above, high glucose levels tend to direct FFA carbon into the complex lipids, at the expense of the other two possible outcomes.

The potent inhibition of the incretin effect by the lipase inhibitor orlistat (Fig. 8) provides strong evidence for the involvement of PKA-stimulated lipolysis, as indicated in Fig. 9. Orlistat had less effect on glucose-stimulated secretion alone. This does not imply that glucose signaling does not involve a lipid-mediated component, merely that glucose modulation of lipid signals may not arise primarily from direct effects on lipolysis and thus FFA provision for LC-CoA synthesis. Instead, as discussed previously (12), glucose increases the malonyl CoA level, which in turn inhibits carnitine palmitoyl

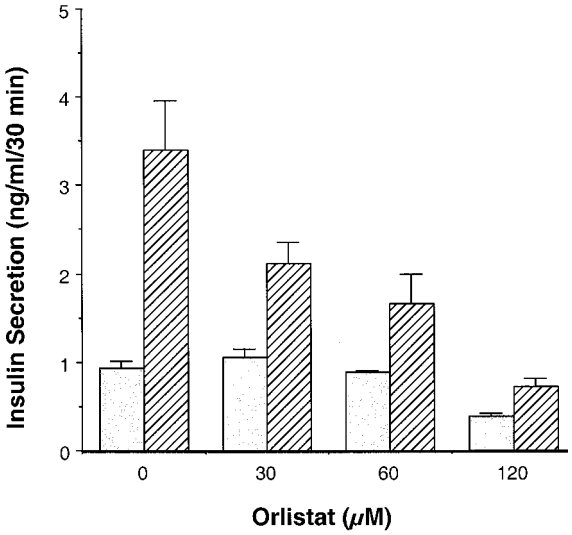


**FIG. 8. The inhibition of the incretin effect of forskolin by orlistat. Cells were preincubated for 30 min with the indicated concentrations of orlistat in the absence of glucose. The media were then changed to ones containing 5 mmol/l glucose with or without 10 μmol/l forskolin, plus the indicated orlistat, and the insulin released was measured after 30 min of incubation. Data shown are means ± SE of 4 wells in a 24-well plate. ▨, Control; ▨, forskolin.**

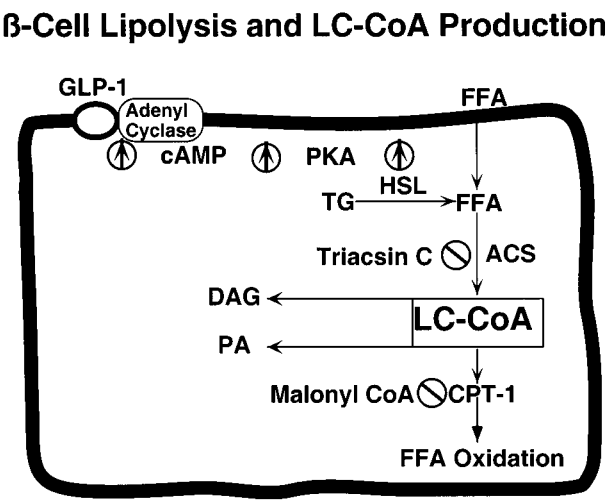


**FIG. 9. Model of the lipolytic pathway in β-cells. The model shows that GLP-1 activates HSL as a consequence of PKA stimulation via cAMP. HSL causes triglyceride breakdown to FFAs. This is followed by activation of FFAs via ACS in a triacsin C–inhibitable step with production of the effector signal LC-CoA. Malonyl CoA prevents the loss of this effector signal (or its products DAG and PA), by blocking its entry and subsequent oxidation in the mitochondria. CPT-1, carnitine palmitoyl transferase-1; TG, triglycerides.**

transferase-1 and reduces mitochondrial uptake and oxidation of LC-CoA. This leads to a rise in cytosolic LC-CoA and, together with the increased production of α-glycerophosphate from glucose, to increases in complex lipid formation.


## ACKNOWLEDGMENTS

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