Glucagon-like peptide 1 and fatty acids amplify pulsatile insulin secretion from perifused rat islets

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Glucose-induced insulin secretion from isolated, perifused rat islets is pulsatile with a period of about 5–10 min, similar to the insulin oscillations that are seen in healthy humans but which are impaired in Type II diabetes. We evaluated the pattern of enhancement by the potent incretin, glucagon-like peptide 1 (GLP-1). GLP-1 increased the amplitude of pulses and the magnitude of insulin secretion from the perifused islets, without affecting the average time interval between pulses. Forskolin and the phosphodiesterase inhibitor isobutylmethylxanthine had the same effect, suggesting that the effect was due to elevated cAMP levels. The possibility that cAMP might enhance the amplitude of pulses by reducing phosphofructo-2-kinase (PFK-2) activity was eliminated when the liver isoform of PFK-2 was shown to be absent from β-cells. The possibility that cAMP enhanced pulsatile secretion, at least in part, by stimulating lipolysis was supported by the observations that added oleate had a similar effect on secretion, and that the incretin effect of GLP-1 was inhibited by the lipase inhibitor orlistat. These data show that the physiological incretin GLP-1 preserves and enhances normal pulsatile insulin secretion, which may be essential in proposed therapeutic uses of GLP-1 or its analogues.

Key words: cAMP, lipolysis, orlistat, phosphofructo-2-kinase.

INTRODUCTION

Insulin is released in a pulsatile fashion *in vivo* in man and animals and *in vitro* from the perfused pancreas and from groups of perifused islets [1]. The oscillatory pattern of secretion is impaired in patients with Type II diabetes [2–4]. Also, near relatives of Type II diabetic patients display abnormal insulin oscillations in the presence of normal glucose tolerance [5]. Such findings have led to the appreciation of the importance of secretory patterns and the mechanisms that regulate pulsatile insulin secretion.

The gut-derived peptide glucagon-like peptide 1 (GLP-1) increases glucose-induced insulin secretion from β-cells [6], but only when glucose concentrations are above the normal glycaemic level of approximately 5 mM [7,8]. Although the therapeutic advantages of GLP-1 are appreciated, the mechanisms through which GLP-1 elicits these effects are not completely characterized. The two *in vivo* active forms of GLP-1 (7–37 and 7–36 amide fragments) bind to β-cell receptors coupled to G proteins and cause an increase in cAMP content [9]. GLP-1 also increases intracellular Ca^{2+}, possibly due to either the rise in cAMP [10] or its ability to augment glucose-induced closing of ATP-dependent K^+ channels [11].

Although the peptide hormone–cAMP signalling cascade is well understood, the mechanisms by which cAMP enhances insulin secretion are not well established. Stimulatory glucose itself increases intracellular cAMP levels in islets in a dose-dependent manner in parallel with its ability to enhance insulin secretion [12–16]. Forskolin [17] and isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor [16], also increase cAMP levels in islets [14,18] and enhance glucose-induced insulin secretion. cAMP and glucose together increase intracellular Ca^{2+} concentrations by augmenting Ca^{2+} influx. However, the ability of cAMP to increase intracellular Ca^{2+} was found to account for only 19% of its effect, since cAMP had a small effect on Ca^{2+} transients, but a large effect on insulin secretion [19]. This argues that other signalling mechanisms are involved.

In the present study, we show that GLP-1 and other cAMP-elevating agents, such as forskolin and IBMX, enhance the secretory pulses in perifused islets, rather than merely elevating the non-oscillatory or baseline component. We have proposed that oscillatory glucose metabolism in the β-cell may underlie the insulin oscillations [1]. Therefore, one possible explanation for this amplification of pulsatile secretion by cAMP-elevating agents is altered regulation of the glycolytic oscillator, phosphofructo-1-kinase (PFK-1). The naturally occurring fructose 1,6-bisphosphate [F(1,6)P_2] analogues, glucose 1,6-bisphosphate and fructose 2,6-bisphosphate [F(2,6)P_2], can block glycolytic oscillations [20,21]. A drop in F(2,6)P_2 might promote the autocatalytic feedback activation of PFK-1 by F(1,6)P_2 that is the basis of the oscillations. cAMP could modulate F(2,6)P_2 levels via activation of protein kinase A (PKA) and phosphorylation of the bifunctional enzyme phosphofructo-2-kinase (PFK-2). Phosphorylation of PFK-2 increases its phosphatase activity and inhibits its kinase activity, resulting in a decrease in F(2,6)P_2 levels. However, there are several isoforms of PFK-2, and only the liver isoform is so affected by PKA phosphorylation [22].

A second possibility is suggested by our observations that GLP-1 rapidly released non-esterified ('free') fatty acids (FFA) from cellular stores and stimulated FFA oxidation in HIT clonal β-cells [23]. Similar changes were observed with forskolin, suggesting that stimulation of lipolysis was a function of PKA activation in β-cells. Indeed, hormone-sensitive lipase (HSL) has been found in β-cells [23,24]. Furthermore, triacsin C, an

Abbreviations used: FFA, non-esterified ('free') fatty acids; F(1,6)P_2, fructose 1,6-bisphosphate; F(2,6)P_2, fructose 2,6-bisphosphate; GLP-1, glucagon-like peptide 1; HSL, hormone-sensitive lipase; IBMX, isobutylmethylxanthine; LC-CoA, long chain acyl-CoA; PFK-1, phosphofructo-1-kinase; PFK-2, phosphofructo-2-kinase; PKA, protein kinase A; RT, reverse transcriptase.

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inhibitor of the conversion of FFA to its metabolically active form, long chain acyl-CoA (LC-CoA), enhanced the GLP-1-stimulated efflux of FFA from the β-cell. We have proposed that LC-CoA and complex lipids synthesized from LC-CoA may be important signalling molecules involved in stimulus–secretion coupling [25,26]. Hence, GLP-1 may exert its incretin effect by stimulating lipolysis and enhancing the production of these lipid products.

A portion of this work was presented at the 1995 annual meeting of the European Association for the Study of Diabetes (September 12–16, Stockholm, Sweden) [35].

MATERIALS AND METHODS

Islet preparation

Pancreatic islets were isolated from male Wistar or Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) weighing 125–200 g. Minced pancreata were digested with collagenase (Sigma, St. Louis, MO, U.S.A.) as described previously [27], and islets were isolated by sedimenting at 2000 *g* through histopaque (Sigma) at 4 °C. Islets were washed three times in Hanks salt solution (Sigma) supplemented with glucose, BSA and sodium bicarbonate, pH 7.4 [27], to remove the histopaque. Groups of islets were then transferred to Petri dishes with 2 ml of RPMI 1640 tissue culture medium (Gibco, Grand Island, NY, U.S.A.) with 11 mM glucose and 10 % (v/v) fetal bovine serum (Hyclone Laboratories, Logan, Utah, U.S.A.) and incubated overnight at 37 °C with 95 % air and 5 % CO_2.

Islet perifusion

Groups of 50–100 islets were sandwiched between two layers of Cytodex-3 microcarrier beads (Sigma) in a column of 1 cm diameter and 1.3 cm height. They were perifused at 37 °C as described previously [28] with a gassed (5 % CO_2/95 % O_2) Hepes-buffered Krebs salt solution containing 119 mM NaCl, 4.6 mM KCl, 1 mM $MgSO_4$, 0.15 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 20 mM Hepes (pH 7.4), 0.05 % (w/v) BSA (fraction V; Sigma) and the designated concentrations of glucose and other additions as indicated. The islets were preperifused for 30 min with 3 mM glucose before collection of samples. Eluted fractions were then collected every 30 s. The column and media entering the column were maintained at 37 °C using water jackets or a temperature-controlled cabinet. Each sample was assayed for insulin by radioimmunoassay by following the assay protocol for rat insulin distributed by Linco Research Inc. (St. Louis, MO, U.S.A.). Insulin secretion time course data are presented as 3-point running averages of the raw data. Insulin secretion data from islets were normalized and expressed as pM insulin per μg of islet protein using a conversion factor of 0.34 μg protein/islet [28].

PFK-2 isozyme transcript generation by reverse transcriptase (RT)-PCR

Pancreatic islets were isolated from 200 g male Sprague–Dawley rats using pancreatic duct infiltration with collagenase and Histopaque gradient separation [29]. Total RNA was isolated using the guanidinium isothiocyanate/phenol method (Gibco BRL) and was reverse transcribed for 10 min at 25 °C and 50 min at 42 °C using random hexamers. Rat liver cDNA was prepared in a similar manner after tissue homogenization. After inactivation of the reverse transcription reaction at 70 °C for 15 min, RT-PCR was performed using the following primers: liver PFK-2-specific exon 1 sense primer, 5′-atcaacgatcgtcgttctctg-3′; muscle PFK-2-specific exon 1 sense primer, 5′-gtctgtaaatgcaagagagcc-3′; shared muscle and liver PFK-2 antisense primer, 5′-acctgctgctgtgactacag-3′; heart specific sense and antisense primers, 5′-gtaaggatgagaaggaacagc-3′ and 5′-cttagaaacatgtaccagagtg-3′ respectively [30]. Primer sequences were compared with available GenBank data using the FastA program in the GCG analysis suite (Genetics Computer Group, WI, U.S.A.) to ensure specificity of match. The accession number for the muscle specific exon 1 with the common liver muscle isoform gene is X15579. The accession number for the liver specific exon 1 with the common liver muscle isoform gene is Y00702. The accession number for the heart gene is S53338. 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. Negative control reactions lacking cDNA were amplified simultaneously with the experimental samples. Experimental samples were also processed in the absence of RT 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. The predominant bands were excised from the gel, the DNA extracted (Qiagen) and sequenced using Taq cycle sequencing with the original PCR primers, on an ABI 373 DNA sequencer.

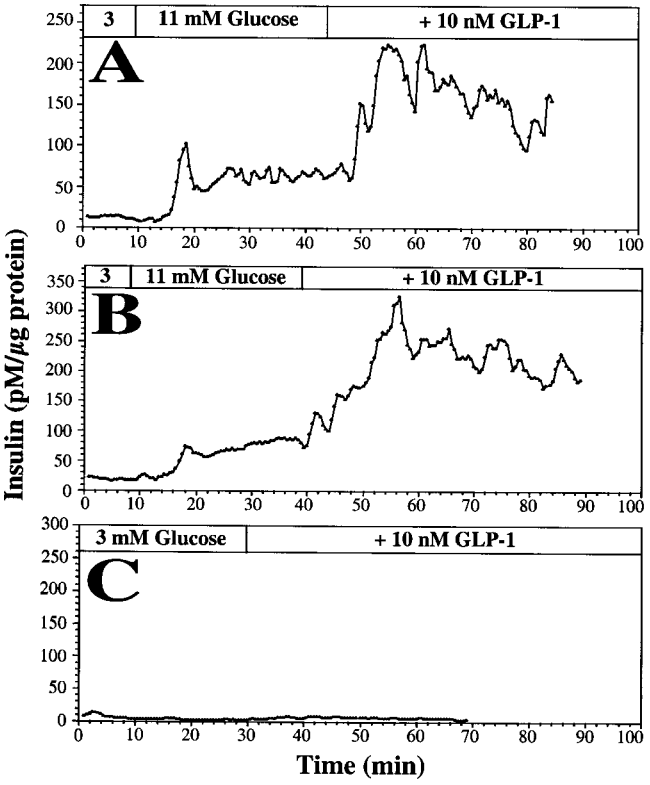

Figure 1 Effect of GLP-1 on pulsatile insulin secretion from perifused islets

Wistar rat islets were perifused with the indicated glucose concentrations (shown at the top of each panel; 3 stands for 3 mM, the basal glucose concentration), and 10 nM GLP-1 was added as indicated. (**A**) The effect of GLP-1 in the presence of a stimulatory concentration of glucose is illustrated; similar results were seen in 9 experiments. (**B**) A rare result where there was little stimulation by 11 mM glucose; nevertheless there was a large stimulation when GLP-1 was added. (**C**) The lack of effect of GLP-1 at basal glucose ($n = 3$) is shown.

Materials

GLP-1 and IBMX were from Sigma. Forskolin (Research Biochemicals International, Natick, MA, U.S.A.) was dissolved in DMSO. Orlistat (Xenical; Roche) capsule material was

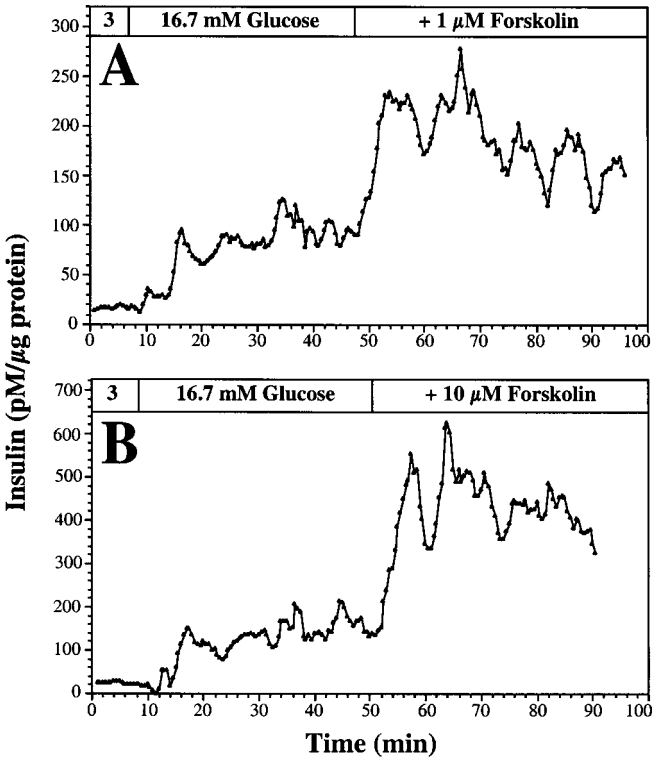

Figure 2 Effect of forskolin on pulsatile insulin secretion from perifused islets

Wistar rat islets were perifused with the indicated glucose concentrations (shown at the top of each panel; 3 stands for 3 mM, the basal glucose concentration) to which either 1 μM (**A**) or 10 μM (**B**) forskolin was added at the time indicated. Similar results were seen in a repeat experiment at each forskolin concentration.

extracted with ethanol to make a nominally 500 mM stock solution.

Mathematical evaluation of pulsatile secretion

Pulses in insulin secretion were detected and analysed as described previously [28], using Cluster analysis [31] of the raw (non-averaged) data. Essentially, this method determines significant rises and falls and thereby determines the location of peaks and valleys, and secondarily the average height of peaks above baseline ('amplitude') and the average peak-to-peak time interval ('period'). The significance of differences in these parameters was determined by Student's t test. The advantage of a pulse detection program like Cluster is the ability to discern such pulsatile behavior when the pulses are not completely regular, as is often the case in biological systems.

RESULTS

Effect of GLP-1 on pulsatile insulin secretion

Figure 1(A) illustrates the pattern of insulin secretion from islets isolated from Wistar rats and perifused with the indicated glucose concentrations. Glucose-stimulated insulin secretion was pulsatile with a 5–8 min period (average peak-to-peak time interval), as found previously [28]. Addition of GLP-1 substantially increased average secretion (3 ± 0.4 fold, $P < 0.0001$, $n = 10$) and pulse amplitude (2 ± 0.4 fold, $P < 0.02$, $n = 9$), without significantly altering the average time between peaks (6 ± 1 versus 7 ± 1 min). In a rare experiment that did not display pulsatile

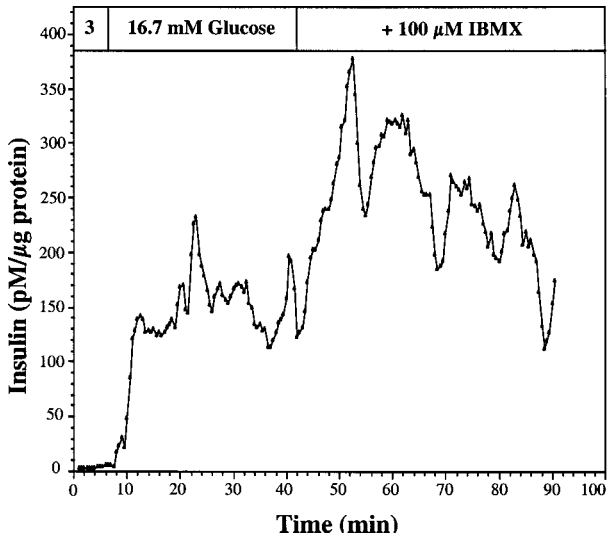

Figure 3 Effect of IBMX on pulsatile insulin secretion from perifused islets

Wistar rat islets were perifused with the indicated glucose concentrations (shown at the top of the panel; 3 stands for 3 mM, the basal glucose concentration) to which 100 μM IBMX was added at the time indicated. Similar results were seen in a repeat experiment.

secretion in response to glucose alone (1 out of the 10), addition of GLP-1 induced pulsatile secretion (Figure 1B). GLP-1 had no effect on basal (3 mM glucose) insulin secretion (Figure 1C). Similar results were seen with islets isolated from Sprague–Dawley rats (results not shown).

Effect of forskolin and IBMX on pulsatile secretion

Since GLP-1 is thought to exert its effects via activation of adenylyl cyclase, we next tested whether other agents that increase cAMP, such as forskolin or the phosphodiesterase inhibitor IBMX, would have similar effects on pulsatile secretion. Forskolin increased average insulin secretion about 2-fold, and pulse amplitude 3- to 5-fold, above that obtained with stimulatory glucose alone, without affecting pulse frequency (Figure 2). IBMX caused a 2-fold increase in average insulin secretion and at least a 2-fold increase in pulse amplitude without altering pulse frequency (Figure 3).

Islet expression of PFK-2 isoforms

To test whether the enhancement of pulsatile secretion, caused by GLP-1 and other agents which elevate cAMP levels could be due to inhibition of PFK-2 to lower F(2,6)P_2 levels, the expression of PFK-2 isoforms in the rat pancreatic islet was assessed using RT-PCR. The liver PFK-2 isoform is the only one yet cloned that is known to be inhibited by phosphorylation by PKA [22]. Using a primer specific for the unique 5′-end of the liver isoform and an antisense primer for the shared muscle and liver sequence, we amplified a DNA fragment of the expected size (529 bp) from the liver cDNA (Figure 4). There was no amplification product in the pancreatic islet lane. The pancreatic islets did express transcript for the muscle and heart isoforms, as indicated by amplified DNA fragments of the expected sizes of 394 bp and 1437 bp respectively (Figure 4); however, the muscle isoform lacks a PKA phosphorylation site, and the heart isoform is activated rather than inhibited by PKA-mediated phosphorylation [22]. The identities of the amplified fragments from the islet and liver were confirmed by DNA sequencing. The positions of the primers for the muscle isoform are 63–83 and 457–437 of

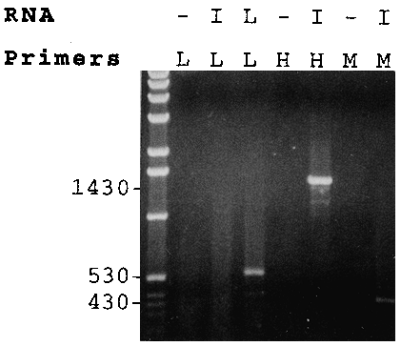

Figure 4 Absence of liver-type PFK-2 in isolated islets

RT-PCR was performed on total RNA from rat liver (L) and pancreatic islets (I) using oligonucleotide PCR primers specific for either the liver (L), muscle (M) or heart (H)-type PFK-2. The products were separated on a 1.2% agarose gel, stained with ethidium bromide and photographed with UV transillumination. The photograph is representative of four different experiments all of which gave the same results.

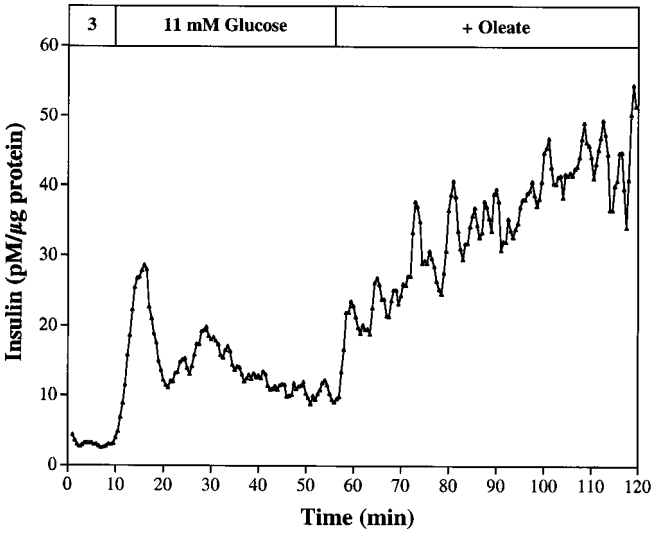

Figure 5 Effect of oleate on glucose-stimulated insulin secretion from perifused islets

Sprague-Dawley rat islets were perifused with the indicated glucose concentrations (shown at the top of the panel; 3 stands for 3 mM, the basal glucose concentration). During the high glucose perifusion, 0.1 mM oleate complexed to albumin (0.1% total) was added at the indicated time. The Figure is representative of an experiment that was repeated three times; oleate addition caused a 2-fold increase in average secretion and at least a 2-fold increase in pulse amplitude.

the accession number X15579 sequence, and for the liver isoform they are 72–91 and 600–580 of Y00702. The positions of the amplification primers for the heart gene are 388–408 and 1824–1803, and those of the sequencing primers are 388–408 and 1323–1303, of the accession number S53338 sequence. The sequence was identical to the published sequence in 98.6% of 720 bases sequenced on a single sequencing run with each primer. That these islet transcripts for the heart and muscle PFK-2 isoforms are β-cell in origin is suggested by the fact that similar expression patterns were generated when using RNA from a clonal pancreatic β-cell line, INS-1 (results not shown). These results indicated that GLP-1 and other cAMP-elevating agents do not modulate glycolytic flux and secretion via inhibition of PFK-2.

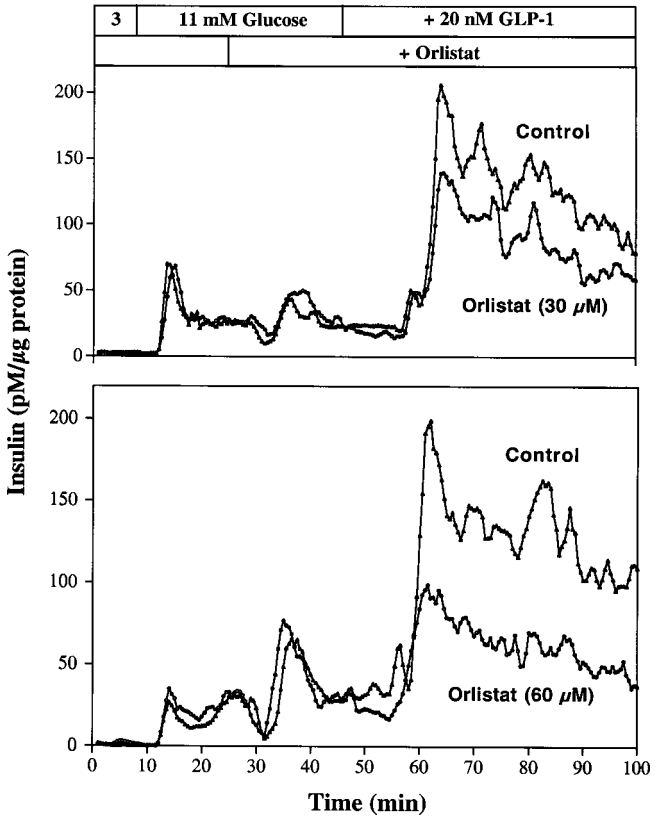

Figure 6 Inhibition of GLP-1 incretin action by orlistat

Sprague-Dawley rat islets were perifused with the indicated glucose concentrations (shown at the top of the upper panel; 3 stands for 3 mM, the basal glucose concentration) and GLP-1 in parallel columns. In one of the columns, orlistat was added at the indicated time; an equivalent amount of ethanol vehicle was added to the control. The experiment with 60 μM orlistat was repeated with similar results.

Role of lipolysis in GLP-1 incretin action

Another metabolic effect of cAMP elevation is the stimulation of lipolysis to release FFA, which we have observed in clonal β-cells stimulated with GLP-1 [23]. We therefore tested the effect of exogenously added fatty acid on the secretion pattern. Like GLP-1, acute addition of oleate (as a complex with BSA) amplified glucose-stimulated pulsatile insulin secretion (Figure 5). On the other hand, prior addition of orlistat (tetrahydrolipstatin), a lipase inhibitor [32–34], substantially reduced the action of GLP-1 (Figure 6). Orlistat at a concentration of 60 μM caused about a 50% inhibition of the GLP-1 stimulated secretion, whereas 30 μM caused about a 30% inhibition.

DISCUSSION

The studies presented here demonstrate that the incretin GLP-1 not only increases the average insulin secretion by perifused pancreatic islets, but increases the amplitude of glucose-stimulated pulsatile insulin secretion without affecting the pulse frequency. While these *in vitro* experiments were underway [35], Porksen et al. [36] have shown *in vivo* that GLP-1 administration increases the insulin pulse mass. This may also provide an explanation for the higher pulse mass observed *in vivo* with glucose ingestion compared with infusion, since glucose ingestion causes GLP-1 release by the gut [37]. This effect of GLP-1 on amplitude/pulse

mass may be of physiological importance, since it has been demonstrated that pulsatile insulin is more effective on target tissues [1].

In the *in vivo* study by Porksen et al. [36], because of the added complexities of recirculation and kinetics of insulin disappearance, the raw data for insulin levels were subjected to deconvolution analysis based on a mathematical model, in order to obtain the parameters of the pulsatile secretion. In the *in vitro* perifusion system used in the present study, such complicating factors are avoided; furthermore, the direct effect of GLP-1 on the islet is unambiguously corroborated. In addition, we show that two other agents that raise cAMP, forskolin and the phosphodiesterase inhibitor IBMX, enhanced pulsatile secretion in a manner similar to GLP-1, suggesting a cAMP/PKA-mediated action. We then examined two possible actions: inhibition of the liver isoform of PFK-2, and activation of HSL.

The rationale for examining PFK-2 was based on our proposal that oscillations in glucose metabolism and the ATP/ADP ratio may underlie oscillatory insulin secretion [1], and the demonstration that F(2,6)P_2 can inhibit glycolytic oscillations and the generation of a high ATP/ADP ratio [21]. However, we found that the PKA-inhibitable, liver isoform of PFK-2 was not present in islets or INS-1 clonal β-cells, suggesting that the effects of GLP-1 or cAMP on pulsatile insulin secretion were not due to a primary effect on oscillatory glycolysis. The brain isoform of PFK-2 was recently identified in islets; however, this isoform lacks a PKA phosphorylation site [38]. Although PFK-1 itself can be phosphorylated by PKA, this phosphorylation causes little change in activity, at least for mammalian isoforms. Since the ability of cAMP to increase intracellular Ca^{2+} accounts for only a small part of its effect [19], there must be other mechanisms through which cAMP enhances insulin secretion. The studies presented here support a role for lipolytic stimulation in the enhanced pulsatile insulin secretion which occurs in response to elevations in cAMP.

It has long been recognized that acute addition of FFA can enhance glucose-stimulated insulin secretion, analogous to the incretin effect of GLP-1. It is demonstrated here that FFA (oleate) can, like GLP-1 and other agents which elevate cAMP levels, enhance pulsatile secretion. In support of the concept that FFA might mediate the effects of these incretins, we have shown in HIT clonal β-cells that GLP-1 increases lipolysis, as indicated by intracellular acidification and the release of FFA monitored with ADIFAB, a fluorescently-labelled fatty acid binding protein [23]. Furthermore, this lipolytic effect was amplified by triacsin C, an inhibitor of FFA activation to LC-CoA by acyl-CoA synthetase. Islets do contain significant amounts of triacylglycerol [39,40] which upon hydrolysis can produce LC-CoA (from FFA) and diacylglycerol, both of which have been proposed to increase insulin secretion [25,41]. Islets also contain HSL [23,24], which can be activated by PKA-mediated phosphorylation. As discussed in [23], the effect in the intact cell probably involves translocation and substrate availability, which may well involve other PKA-phosphorylated proteins such as perilipin, since changes in apparent HSL activity in the intact (fat) cell are much greater than that which can be assayed in homogenates or purified preparations. Hence GLP-1, and other agents which elevate cAMP levels, could be enhancing pulsatile secretion by stimulating lipolysis. This proposal is strongly supported by the observation in the present study that the lipase inhibitor orlistat potently inhibited this incretin effect of GLP-1 (Figure 6). The degree of inhibition seen in perifused islets, about 50 % by 60 μM orlistat and about 30 % by 30 μM orlistat, is similar to the 70 % and 50 % inhibition of forskolin-stimulated secretion by these orlistat concentrations in batch-incubated HIT clonal β-cells [23]. There may also have been some inhibition of glucose-stimulated secretion developing in the perifused islets with 60 μM orlistat (Figure 6); little, if any, such inhibition was seen when 30 μM orlistat was used. In the batch-incubated HIT cells there was no inhibition of glucose-stimulated secretion at concentrations of orlistat up to 60 μM, but some inhibition at 120 μM. These conclusions are also supported by a recently published study [42] showing inhibition of IBMX-stimulated secretion (and also glucose-stimulated secretion) in batch incubated islets by the anti-lipolytic agent 3,5-dimethylpyrazole.

It is generally recognized that GLP-1 largely signals through cAMP in the pancreatic β-cell [43]. GLP-1- and forskolin-stimulated effects were inhibited by the cAMP antagonist Rp-cAMPS in single cells [11,44,45]. Capacitance measurements of exocytosis in single β-cells indicated two phases of action of cAMP: a PKA-dependent accelerated mobilization of insulin granules to the readily releasable pool, and a PKA-independent increase in release probability [43,44]. Recent work in mouse islets [46] has reported both PKA-dependent and PKA-independent actions of cAMP/GLP-1 on insulin secretion, each amounting to about half the total effect, with the PKA-independent action attributed to the cAMP binding protein cAMP-GEFII (Epac 2), that complexes with Rim2 (Rab3-interacting molecule 2) to affect exocytosis. The 50 % inhibition seen with the lipase inhibitor in the present work may thus represent a substantial inhibition of the PKA-dependent action.

It has been our hypothesis that LC-CoA, and/or complex lipids derived from it, participate in the fuel stimulus–secretion coupling in the pancreatic β-cell [25,41]. As noted above, glucose raises cAMP levels somewhat, although far less than do GLP-1 or forskolin, and may signal through lipolysis to some extent. This may explain the partial inhibition of glucose-stimulated secretion seen at higher concentrations of orlistat [23]. The importance of lipolysis in this regard is further underlined by recent studies of HSL-null mice that showed deficient insulin secretion in response to glucose both *in vivo* and in isolated islets [47]. However, the major mechanism by which glucose is likely to raise cytosolic LC-CoA levels is through a rise in malonyl-CoA, which then inhibits carnitine acyltransferase I and thus mitochondrial transport and oxidation of LC-CoA [25,41]. Glucose-induced insulin secretion is indeed associated with inhibition of FFA oxidation, increased FFA esterification, and complex lipid formation by pancreatic β-cells. It is noteworthy that this mechanism is of course still dependent on the provision of FFA by lipolysis or uptake, which may account for the loss of glucose-stimulated secretion in islets from HSL-null mice or when lipolysis is inhibited pharmacologically.

Antinozzi et al. [48] reported no effect of triacsin C on glucose-stimulated secretion and concluded that LC-CoA and lipids derived from it were probably not important to the stimulus–secretion coupling process. However, as discussed previously [23], a conclusive negative result is difficult to defend because of the existence of different pools of LC-CoA and other lipids, and differing sensitivity to triacsin C of acyl-CoA synthetase isoforms and of the synthesis of different classes of lipids. We also found that triacsin C did not appear to inhibit the incretin action of GLP-1 in the present studies. However, it should also be noted that diacylglycerol can be formed directly in lipolysis, and that this component of the GLP-1 effect, if significant, would not be inhibitable by triacsin C in any case.

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