Glucose-dependent insulinotropic polypeptide signaling in pancreatic β-cells and adipocytes

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ABSTRACT

Glucose-dependent insulinotropic polypeptide (GIP) was the first incretin to be identified. In addition to stimulating insulin secretion, GIP plays regulatory roles in the maintenance, growth and survival of pancreatic islets, as well as impacting on adipocyte function. The current review focuses on the intracellular signaling pathways by which GIP contributes to the regulation of β-cell secretion and survival, and adipocyte differentiation and lipogenesis. Studies on signaling underlying the insulinotropic actions of the incretin hormones have largely been carried out with glucagon-like peptide-1. They have provided evidence for contributions by both protein kinase A (PKA) and exchange protein directly activated by cyclic adenosine monophosphate (EPAC2), and their probable role in GIP signaling is discussed. Recent studies have shown that inhibition of the kinase apoptosis signal-regulating kinase 1 (ASK1) by GIP plays a key role in reducing mitochondria-induced apoptosis in β-cells through protein kinase B (PKB)-mediated pathways, and that GIP-induced post-translational modification of voltage- dependent K^+ (Kv) channels also contributes to its prosurvival role. Through regulation of gene expression, GIP tips the balance between pro- and anti-apoptotic members of the B-cell lymphoma-2 (Bcl-2) protein family towards β-cell survival. GIP also plays important roles in the differentiation of pre-adipocytes to adipocytes, and in the regulation of lipoprotein lipase expression and lipogenesis. These events involve interactions between GIP, insulin and resistin signaling pathways. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2012.00196.x, 2012)

KEY WORDS: Apoptosis, Glucose-dependent insulinotropic polypeptide, Incretin

INTRODUCTION

The hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are released from the intestine in response to nutrient ingestion and stimulate insulin secretion in a glucose-dependent manner, as a result of which they have been classified as 'incretins'[1–5]. Additionally, both hormones play regulatory roles in the maintenance, growth and survival of pancreatic islets, as well as acting in an integrative manner on processes involved in nutrient metabolism, including events underlying the passage of chyme through the gastrointestinal tract, and nutrient digestion, absorption and storage[1–4]. The biological actions of GIP and GLP-1 are terminated by the prolyl endopeptidase, dipeptidyl peptidase-4 (DPP-4)[2,6]. DPP-4 resistant analogs of GLP-1 and DPP-4 inhibitors are two recently introduced classes of type 2 diabetes therapeutics that mimic or potentiate, respectively, actions of the incretins[2,6]. Although these agents have proven to be extremely effective in improving glucose tolerance, it is unlikely that their therapeutic potential has been fully realized, and it is important to elucidate the mechanistic basis underlying incretin actions. In the present review, we focus on the current understanding of the signaling events involved in GIP actions on β-cell function and the adipocyte, with an emphasis on studies carried out in the authors' laboratory.

GIP STIMULATION OF INSULIN SECRETION

The GIP receptor (GIPR) is a member of the class B G protein-coupled receptor family that has now been identified in chordates ranging from fish to mammals[3,7], although little is known about cellular signaling events in lower species. β-Cell responses to GIP are glucose-dependent in rodents[8,9] and humans[10]. There is extensive literature on the mode of action of GLP-1 on the β-cell that has been expertly reviewed[11–15], and information that is relevant to GIP action will be integrated into the current review. Uptake and metabolism of glucose in the β-cell increases the intracellular adenosine triphosphate (ATP)/adenosine diphosphate (ADP) ratio, resulting in closure of ATP-sensitive K^+ (K_{ATP}) channels and membrane depolarization, with consequent activation of voltage-dependent Ca^{2+} channels (VDCC), increases in intracellular Ca^{2+} (iCa^{2+}) and triggering of insulin granule exocytosis[16–18]. Membrane repolarization is mediated by voltage-dependent K^+ (Kv) channels[19] and Ca^{2+}-sensitive K^+ (K_{Ca}) channels. The incretin hormones act at multiple levels within this complex series of events.

Both GIP and GLP-1 have been shown to stimulate adenylyl cyclase (AC) through a stimulatory G protein (Gs) coupled process resulting in increased cyclic adenosine monophosphate (cAMP)[20,21], and this is considered to be the major signaling

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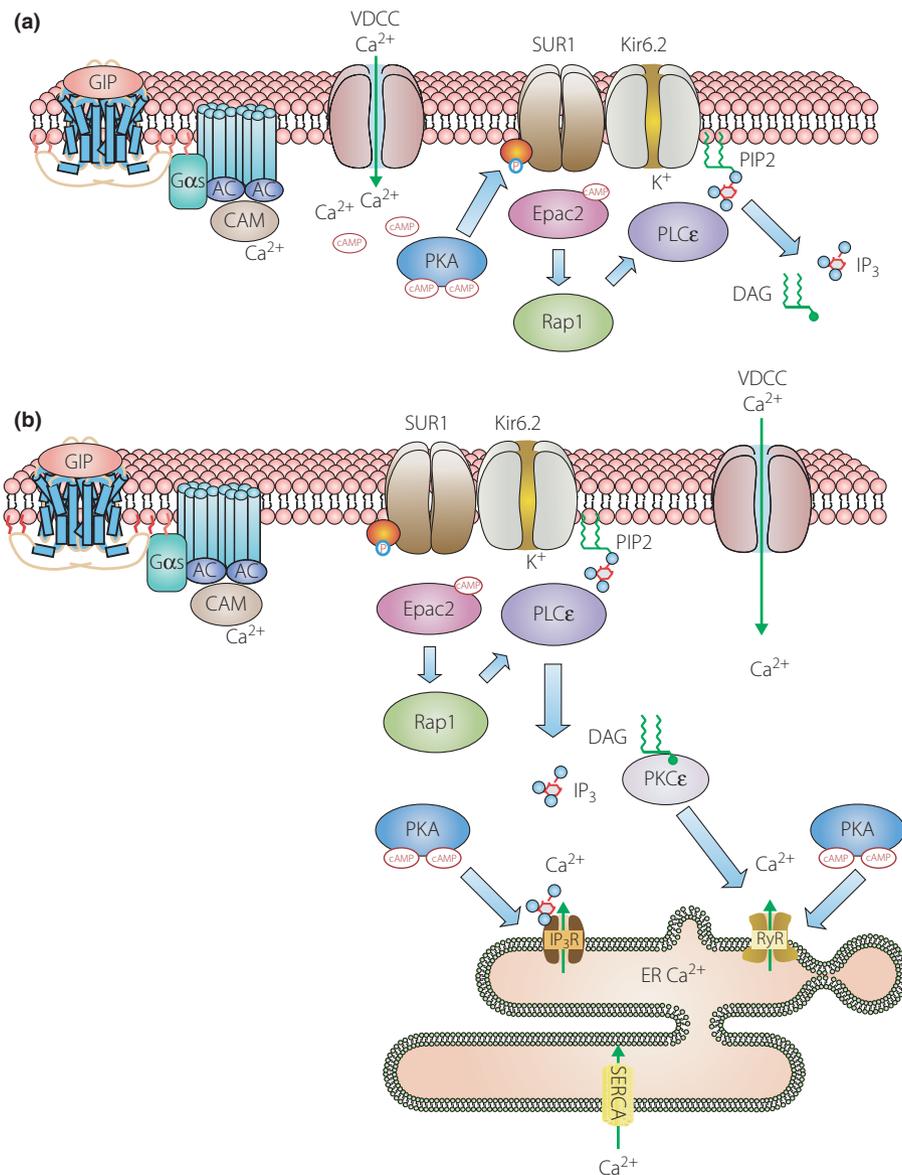
Figure 1 | Signaling pathways proposed to be involved in proximal events in glucose-dependent insulinotropic polypeptide (GIP)-mediated potentiation of glucose-induced insulin secretion. (a) Evidence has been presented supporting roles for both protein kinase A (PKA) and cyclic adenosine monophosphate (cAMP)-activated guanine nucleotide exchange factor (cAMP-GEF)/exchange protein directly activated by cAMP (Epac) in the modulation of adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels. Dissociation of cAMP-Epac2 from sulfonulurea receptor 1 (SUR1) binding has been proposed to activate phospholipase C-ε (PLCε) through Ras-related protein 1 (Rap1), resulting in phosphatidylinositol 4,5 bisphosphate (PIP_2) metabolism and inhibition of adenosine triphosphate-sensitive channel subunit, Kir6.2, membrane depolarization and activation of voltage-dependent Ca^{2+} channels (VDCC). (b) Diacylglycerol-activated PKCε potentiates calcium-induced calcium release through ryanodine receptors and phosphatidylinositol trisphosphate (IP_3) stimulates Ca^{2+} release from IP_3 sensitive endoplasmic reticulum (ER) Ca^{2+} stores. PKA might act to sensitize the Ca^{2+} release channels (based on references 15, 20, 31, 36). AC, adenylyl cyclase; CAM, calmodulin; Gαs, stimulatory G protein α-subunit, IP_3R, inositol trisphosphate receptor; P, phosphate; RyR, ryanodine receptor; SERCA, sarco(endo)plasmic reticulum Ca^{2+}-ATPase.

pathway involved in their potentiation of glucose-induced insulin secretion. Although GIP-stimulated insulin secretion is glucose-dependent, cAMP production and subsequent activation of downstream signaling modules are not[22,23]. As discussed further below, synergistic interaction between glucose and incretin action occurs when Ca^{2+} fluxes are increased through modulation of

K_{ATP} channels and VDCC, as well as through release from the endoplasmic reticulum (Figure 1)[15,17,24]. Type VIII AC has been proposed to act as a 'coincidence detector' of signals from glucose and cAMP[17], as it is activated by both Ca^{2+}-calmodulin and Gαs[25]. Ca^{2+}-calmodulin also modulates the activity of phosphodiesterase (PDE) 1C, and synergistic interactions between Gαs

and Ca^{2+}-calmodulin have been suggested to drive synchronous, in-phase oscillations of cAMP and Ca^{2+} [24,26].

Incretin-induced increases in β-cell cAMP result in the activation of both protein kinase A (PKA) and cAMP-activated guanine nucleotide exchange factor (cAMP-GEF)/exchange protein directly activated by cAMP (Epac) (Figure 1)[15,20,21,27–32]. Epac2A has been identified as the major splice-variant involved in β-cell incretin signaling[32], but there are multiple PKA isoforms present in β-cells and it is unclear as to which contribute to the modulation of insulin secretion. There is also uncertainty over the relative roles played by Epac2 and PKA in events leading to increases in insulin secretion, with K$_{ATP}$ channel closure, Ca^{2+} influx and intracellular mobilization, and processes underlying granule movement and exocytosis, all being potential targets. A number of investigators have carried out sophisticated electrophysiological and imaging studies to identify the pathways targeted by GLP-1 and/or GIP and, as these studies have been comprehensively reviewed[15,21,30,32], only a summary of the main findings will be presented.

GLP-1 was first suggested to modulate β-cell K$_{ATP}$ channel activity through a PKA-mediated pathway[33], and it was later reported that phosphorylation of the β-cell sulfonylurea 1 (SUR1) subunit resulted in K$_{ATP}$ channel closure (Figure 1a)[34]. There is now controversy over the contribution of PKA to K$_{ATP}$ channel regulation[15,32,35], as compelling evidence points to Epac2 as the major factor linking cAMP to K$_{ATP}$ channel closure and increasing Ca^{2+} influx[15,20,31,32]. Under non-stimulated conditions, Epac2 interacts with the nucleotide-binding fold-1 of SUR1 (Figure 1a)[30,36–39]. On binding cAMP, Epac2 dissociates from SUR1[38,39], and activates the GTPase, Ras-related protein 1 (Rap1). Holz *et al.* have proposed that Rap1 stimulates phospholipase C-ε (PLCε)[40], resulting in localized metabolism of plasma membrane phosphatidylinositol 4,5 bisphosphate (PIP$_2$) to phosphatidylinositol trisphosphate (IP$_3$) and diacylglycerol (DAG; Figure 1)[34]. As earlier studies showed that PIP$_2$ reduces the sensitivity of K$_{ATP}$ channels to ATP[41,42], its depletion would be expected to result in potentiated ATP-dependent channel closure and membrane depolarization.

β-Cell stimulation by both GLP-1 and GIP in the presence of elevated glucose has been shown to result in increased Ca^{2+} uptake through VDCC and non-selective ion channels[33,43,44], as well as stimulation of Ca^{2+} release from intracellular stores that, in the case of GLP-1, has been shown to involve Epac2 activation[15,31]. GIP probably activates identical pathways (Figure 1b), although studies on K$_{ATP}$ channel-deficient mice showed that GIP actions on insulin secretion showed a greater dependency on K$_{ATP}$ channels than GLP-1[45]. Phospholipase Cε has been proposed to link Epac and iCa^{2+} fluxes through the activation of endoplasmic reticulum (ER) inositol trisphosphate (IP$_3$) channels and protein kinase Cε (PKCε)-mediated potentiation of calcium-induced calcium release (CICR) through ryanodine receptors (Figure 1b)[15,46], possibly through activation of calcium-calmodulin kinase II[15]. In addition, PKA is capable of sensitizing the intracellular Ca^{2+} release channels to the effects

of IP$_3$ and Ca^{2+} [15]. GIP also activates an islet group VIA Ca^{2+}-independent phospholipase A$_2$ (iPLA$_2$), resulting in increased arachidonic acid (AA) production from membrane lipids[47], and AA has been shown to increase release of Ca^{2+} from intracellular stores, suggesting that it might be coupled to insulin secretion.

β-Cell repolarization involves closure of VDCC, as well as opening of delayed rectifier and A-type Kv channels[18,48]. GIP and GLP-1 both reduce Kv channel currents, prolonging β-cell action potentials and potentiating Ca^{2+} signals[19,48,49]. Kv2.1 is the major delayed rectifier channel in rodent β-cells, playing a dominant role in GLP-1[19], and probably GIP, action. Post-translational modification of Kv2.1 in response to GIP and GLP-1 can modulate channel gating, promote inactivation and increase channel internalization through processes that involve phosphorylation by PKA and PKCζ[19,50,51], and acetylation by cAMP-response element binding protein (CREB) binding protein (CBP; see β-Cell Prosurvival Effects of GIP section)[51]. GIP also stimulates endocytosis of Kv1.4 channels through PKA-dependent phosphorylation[49]. As AA was also recently shown to increase the rate of inactivation of Kv2.1 channels[52], GIP-activation of iPLA$_2$ might also be linked to β-cell repolarization.

In addition to the 'up-stream' events involved in insulin secretion, both incretins exert distal effects on secretory granule exocytosis through PKA-[53] and Epac-[28,30,32,36,46] dependent pathways. Protein kinase A phosphorylates proteins that are components of the exocytotic machinery[21], including α-soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein (α-SNAP) and mammalian uncoordinated homology 13-1 (Munc 13-1)[4]. A number of models have been proposed to explain the role of Epac2 in increasing the probability of granule exocytosis[15,28,54]. Eliasson *et al.*[54] proposed that Epac2 interacts with SUR1 associated with both the secretory granule and the plasma membrane, resulting in activation of a secretory granule chloride channel CIC-3[15,30], granule acidification and priming through a v-type H$^+$-ATPase[54]. In a series of elegant experiments, Seino *et al.*[32] showed that cAMP increases both readily releasable and reserve pools of secretory granules. Epac2A appears to be more important for regulating the readily-releasable pool during the first phase of insulin secretion, whereas PKA might be critical for second phase release[32]. In addition to SUR1 and Rap1, Epac2 also interacts with Ras-related in brain 3 (Rab3)-interactive molecule 2 (Rim2), Piccolo and SNAP-25, and a cAMP–Epac-2–Rim2 complex was shown to play a central role in secretory granule dynamics[20,21]. Although the mechanisms involved in complex formation are still being clarified, dissociation of cAMP–Epac2A from Sur1 promotes Ca^{2+}-dependent heterodimerization of Rim2α and Piccolo, followed by interaction with Rab3A and Munc13-1, core components of the exocytotic apparatus[21,55]. GIP-induced insulin secretion was greatly impaired in Rim2α knockout mice and their isolated islets, establishing its importance in the secretory pathway[55]. Additionally, phosphorylation of snapin by PKA has been shown to be essential for incretin-stimulated assembly of collectrin, SNAP-25 and Epac2[56]. Finally, we recently found that a selective Epac

agonist was capable of activating protein kinase B (PKB; Akt)[57], emphasizing the importance of interaction between kinase pathways. It is currently unclear as to whether this interaction is related to insulin secretion or restricted to β-cell mitogenic and prosurvival effects of GIP (see next section).

β-CELL PROSURVIVAL EFFECTS OF GIP

β-Cell dysfunction and reduced β-cell mass are major factors in the etiology of type 1 diabetes and type 2 diabetes. Whereas autoimmune reactions are responsible for apoptotic loss of β-cells in type 1 diabetes[58], chronic hyperglycemia and hyperlipidemia, elevated cytokines, amyloid deposits, ER stress and other factors contribute to type 2 diabetes[59]. A number of procedures are being investigated for replenishing β-cell mass in diabetes patients, including the production of surrogate β-cells for transplantation and stimulation of residual β-cell proliferation. However, it appears that human β-cell regenerative capacity is limited to around the first three decades of life[60,61]. Therefore, prevention of β-cell loss by inhibiting apoptotic processes is an attractive alternative target.

A role for incretin hormones in maintaining the normal integrity of pancreatic islets was first shown by the observation that GLP-1R$^{-/-}$ mice have elevated levels of β-cell apoptosis[62]. Both GIP and GLP-1 have been shown to exert strong prosurvival effects on β-cells *in vitro*[3,4,11,12]. Additionally, studies on a number of rodent models have shown that GIPR or GLP-1R agonists exert marked anti-apoptotic effects *in vivo*[63–67]. Our laboratory has focused on identifying mechanisms underlying the prosurvival actions of GIP and the following overview summarizes recent findings. GIP shows protective effects on cells that have been subjected to a number of apoptosis-inducing stressors, including high glucose ± fatty acids (glucolipotoxicity), serum depletion and a low glucose environment or treatment with agents that induce genotoxic, mitochondrial or ER stress[57,65,68,69]. In studies on staurosporine-induced apoptosis in insulinoma-1 (INS-1) β-cells, GIP was shown to exert effects at multiple levels in the apoptotic pathway, with reduced mitochondrial translocation of B-cell lymphoma-2 (Bcl-2) associated death promoter (Bad) and Bcl-2 interacting mediator of cell death$_{EL}$ (Bim$_{EL}$) and oligomerization of Bcl-2-associated X protein (Bax), release of cytochrome C and caspase 3 activation[68]. Ultimately, GIP reduces the influence of mitochondria-associated pro-apoptotic Bcl-2 family members, thus restraining the effects of stressors on the β-cell.

Although the pathways linking incretin-induced production of cAMP and activation of PKA and Epac2A with the stimulation of insulin secretion have been extensively studied, the role of cAMP in prosurvival pathways has not been clearly defined. GIP-mediated anti-apoptotic signaling in INS-1 β-cells appears to be strongly dependent on cAMP production, as low concentrations of the adenylyl cyclase inhibitor MDL-12,300A (Santa Cruz Biotechnology, Santa Cruz, CA, USA) ablated the protective effects of GIP on staurosporine-induced cell death[68]. One major pathway by which GIPR- and GLP1-R-mediated

increases in cAMP production promote survival is by increasing expression of anti-apoptotic genes through PKA phosphorylation of CREB at Serine (Ser)133[70–72]. In the case of GIP, *bcl-2* gene expression in INS-1 cells was also found to involve PKA-stimulated dephosphorylation of AMP activated protein kinase (AMPK) and increased nuclear entry of cAMP-responsive CREB coactivator 2 (TORC2)[70]. Incretins activate a number of other genes involved in prosurvival pathways; for example, expression of insulin receptor substrate 2 (IRS2) is stimulated by GLP-1-activated PKA phosphorylation of CREB. Recently, CREB was reported to be responsible for an acute phase of cAMP-dependent gene expression in β-cells, whereas a delayed phase involved induction of IRS2/PKB pathways, activation of mammalian target of rapamycin (mTOR) and increasing hypoxia-inducible factor (HIF) activity[73]. This increase was shown to be associated with altered INS-1 β-cell metabolic activity and improved cell viability[73]. An additional effect of sustained β-cell stimulation by GIP-induced activation of PKB is the phosphorylation and nuclear exclusion of forkhead box protein O1 (Foxo1), that also promotes cell survival as a result of the requirement of nuclear Foxo1 for expression of pro-apoptotic proteins, such as Bax[65]. It remains to be determined whether the HIF pathway also modulates expression of prosurvival bcl-2 family proteins.

Activation of PKB plays a central role in additional anti-apoptotic effects of GIP[65,67,68,74,75]. PKB phosphorylation on Threonine (Thr)308 and Ser473 has been shown to be essential for enzyme activation in many cell types. However, in β-cells, GIP was found to produce rapid increases in PKB activity through a non-PI3kinase-activated pathway in the absence of detectable Thr308 phosphorylation. As 8-(4-chlorophenylthio)-2′-O-methyl cAMP (8′-CPT cAMP), an EPAC-selective agonist, mimicked the effects of GIP, activation appears to be mediated by EPAC2, although it is currently unclear as to whether Rap1 is involved[57,68].

Apoptosis signal-regulating kinase 1 (ASK1) has been shown to operate as a redox sensor that, on exposure to excessive levels of reactive oxygen species (ROS), initiates the mitochondria-mediated apoptotic pathway through activation of p38 mitogen-activated protein kinase (p38 MAPK) and jun N-terminal kinase (JNK). Among the stressors shown to activate ASK1 are oxidative and ER stress, Ca^{2+} overload and receptor mediated inflammatory signals, all of which could contribute to β-cell death. Exposure of INS-1 β-cells transfected with human ASK1 to thapsigargin was found to increase phosphorylation of Thr845 and reduce phosphorylation of Ser83 in ASK1, changes that increase its enzyme activity[76,77], resulting in phosphorylation and activation of p38 MAPK and JNK, through MAPK/extracellular signal-regulated kinase (ERK) kinase (Mek) 3/6 and Mek 4/7, respectively. Activation of PKB with GIP treatment prevented these changes in ASK1 phosphorylation and downstream targets[4,68]. Suppressing ASK1 activation with GIP treatment inhibited apoptosis induced by all apoptosis-inducing agents tested. As a result of the complexity of the system, the
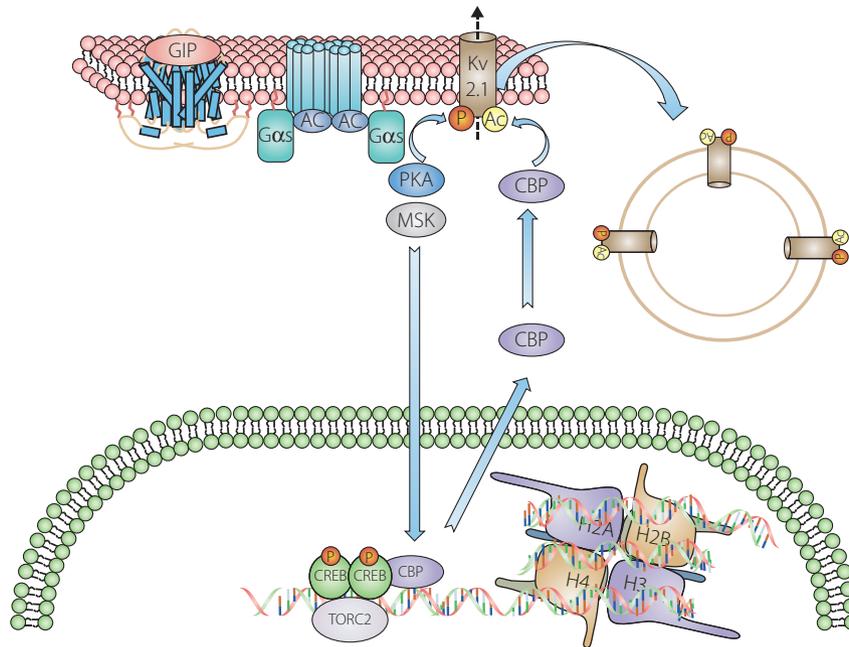
Figure 2 | Proposed signaling pathways by which glucose-dependent insulinotropic polypeptide (GIP) regulates endocytosis of voltage-dependent K$^+$ (Kv)2.1. Binding of GIP to its receptor (GIPR) activates protein kinase A (PKA) and, potentially, mitogen- and stress-activated kinase-1 (MSK-1), resulting in phosphorylation of Kv2.1. Through an unknown mechanism, cyclic adenosine monophosphate-response element binding protein binding protein (CBP) is translocated from the nucleus to the plasma membrane, where it acetylates Kv2.1. Potentiation of Kv2.1 internalization results in reduced K$^+$ fluxes and reduced β-cell apoptosis. AC, adenylyl cyclase; Ac, acetylated; CREB, cAMP-response element binding protein; Gαs, stimulatory G protein α-subunit; P, phosphorylated; TORC2, transducer of regulated cyclic adenosine monophosphate response element binding protein activity.

mechanism of GIP action is currently only open to speculation. In non-stressed cells, ASK1 forms a high molecular weight complex that has been termed the 'ASK1 signalosome'[77], and, in non-stressed cells when ASK1 activity is inhibited, the anti-oxidative protein, thioredoxin (Trx), is a component of the signalosome. If the oxidative state of the cell is greatly increased, the oxidized form of Trx dissociates from ASK1 and tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2) and TRAF6 binding activates ASK1 signaling[77]. Although GIP-stimulated phosphorylation of Ser83 by Akt was clearly involved in inhibiting ASK1 activity, it is also possible that the binding of thioredoxin and/or TRAF2/6 to ASK1 were impacted on. Further details remain to be elucidated.

Additional gene regulatory actions of GIP and GLP-1 involve modification of chromatin structure, thus altering the accessibility of transcription factors to target genes. A number of different post-translational modifications of histone N-termini have been identified, and we showed that both incretin hormones modulate β-cell chromatin structure by increasing acetylation and phosphorylation of core H3 histones[78], with both elevated histone H3 acetyltransferase and reduced histone deacetylase activities contributing to the former[78]. Responses to both incretins involved activation of PKA, and downstream Erk1/2 (p44/42 MAPK) and p38 MAPK signaling modules, ultimately resulting in the activation of mitogen- and stress-activated kinase-1 (MSK-1) and CREB. Histone acetyltransferase (HAT) inhibition

resulted in significant reductions in incretin-stimulated, CREB-activated, *Bcl-2* gene transcription, showing that histone H3 modification plays an important role in the regulation of apoptosis-related proteins[78]. Both GIP and GLP-1 also inhibit ER stress-induced apoptosis[68,79,80] through complex mechanisms[4] that involve both reductions in the apoptotic pathway, leading to caspase activation, and altered expression or activity of a number of proteins involved in the unfolded protein response (UPR), including activating transcription factor 4 (ATF4), binding immunoglobin protein (Bip), CCAAT-enhancer-binding protein homologous protein (CHOP) and growth arrest and deoxyribonucleic acid damage-inducible protein 34 (GADD34)[79,80].

In recent studies, we identified an interesting link between incretin-stimulated post-translational modification of Kv channels and apoptosis (Figure 2). Cell shrinkage (apoptotic volume decrease; AVD) occurs early in the apoptotic process, before DNA fragmentation, cytochrome C release and caspase 3 activation, and it is a prerequisite for completion of programmed cell death. Efflux of K$^+$ is a critical component of the AVD and, as activities of a number of caspases and endonucleases are suppressed at normal intracellular K$^+$ concentrations, decreases in its intracellular concentration result in their activation. In different cell types, several types of K$^+$ channels have been implicated in AVD, including Kv channels. We decided to examine a possible role for the most prevalent rodent β-cell family member, Kv2.1, in the development of apoptosis,

and determine whether regulation of this process by GIP and GLP-1 contributes to their prosurvival effects[51]. INS-1 β-cells, in which Kv2.1 was overexpressed, showed potentiated apoptotic responses to mitochondrial and ER stress, whereas GIP or GLP-1 reduced the potentiation. In studies designed to identify their mode of action, both GIP and GLP-1 promoted phosphorylation and acetylation of Kv2.1 through pathways involving PKA, and/or MSK-1 and HAT (Figure 2). This was associated with reduced cell surface expression of Kv2.1. We subsequently found that GIP and GLP-1 promoted nuclear/cytoplasmic shuttling of CBP, resulting in its interaction with Kv2.1 (Figure 2). Downregulation of CBP ablated incretin-induced acetylation of Kv2.1, suggesting that this HAT is primarily responsible for the acetylation[51]. As ASK1 has been shown to play a key role in the pro-apoptotic modulation of Kv channels[81], it is likely that the GIP-activated pathway leading to phosphorylation of serine 83 in ASK1 interacts with events leading to Kv protein modification.

EFFECTS OF GIP ON ADIPOSE TISSUE

Fat ingestion is a major stimulus for GIP secretion in humans, dogs and rodents[3,82,83], and there is increasing evidence supporting a physiological role for GIP in promoting fat storage. There are two pathways by which GIP impacts on adipocyte metabolism: directly through interaction with GIP receptors on the adipocyte and through stimulation of insulin secretion. GIP infusion has been shown to promote the clearance of chylomicron-associated triglyceride (TG) in dogs[84], and to lower plasma TG responses to intraduodenal fat in rats[85]. However, GIP had no major effect on the rate of removal of intravenously administered TG[86], suggesting that GIP stimulates release of TG from chylomicrons and uptake into adipose tissue. Support for a role for GIP in regulating adipose tissue mass came from rodent studies. Miyawaki et al.[87] first showed that GIPR$^{-/-}$ mice were resistant to obesity when fed a high-fat diet. Mice that were treated with GIPR peptide antagonists[88], vaccinated against GIP[89] or subjected to selective K-cell ablation[90] all showed increased resistance to high-fat feeding-induced obesity[3], showing that GIP normally promotes lipid storage.

Early studies on direct adipocyte actions of GIP showed stimulatory effects on fatty acid (FA) synthesis from acetate in adipose tissue explants[91], increased uptake and incorporation of glucose into lipids[92], as well as enhanced free FA (FFA) incorporation into adipose tissue[93]. Adipocyte lipoprotein lipase (LPL) is responsible for the hydrolysis of TG in circulating chylomicrons, TG-rich lipoproteins and very low-density lipoproteins, resulting in adipocyte uptake of FFA and monoacylglycerol, and the promotion of lipogenesis. GIP was shown to increase LPL enzyme activity, in an insulin-dependent manner, in cultured 3T3-L1 adipocytes, rodent adipocytes and subcutaneous human adipocytes[94–96]. In view of the close correlation between human GIP responses and plasma post-heparin LPL levels[97], it has been suggested that GIP acts on adipocyte storage by matching adipose tissue uptake of FA with the triglyceride load[98].

Suboptimal levels of circulating FFA result in greatly reduced β-cell responsiveness to subsequent glucose stimulation[99]. In vitro studies showed that, in the absence of insulin, GIP stimulates adipocyte TG hydrolysis through PKA activation[100,101], and we suggested that GIP primes β-cells during fasting by releasing adipocyte FFA into the circulation[3,100]. Getty-Kaushik et al.[102] showed that GIP-stimulated increases in glycerol production were accompanied by decreased FFA in perifused adipocytes. They interpreted these responses as reflecting GIP-induced re-esterification from excess FFA[102,103]. A similar response was recently reported in humans, with GIP infusion resulting in small increases in adipose tissue FFA re-esterification[104]. Although, that study was carried out under hyperglycemic conditions. GIP might also have long-term effects on lipid metabolism, as synthesis of pancreatic lipase and colipase were both stimulated by GIP[105], an effect that should increase efficiency of lipid uptake.

In the presence of insulin, GIP signaling appears to play important roles in both the differentiation of preadipocytes and lipogenesis. A complex set of events is involved in preadipocyte to adipocyte development, including growth arrest, increased transcription factor and lipogenic enzyme expression, accumulation of lipid, and the development of sensitivity to regulatory hormones[106]. Expression of the GIPR is extremely low in preadipocytes[103,107,108], but both messenger ribonucleic acid (mRNA) and protein expression increase during differentiation of 3T3-L1 cell[103,108] and human[107] preadipocytes. GIP acted synergistically with insulin to increase neutral lipid accumulation during progression of 3T3-L1 preadipocytes to the adipocyte phenotype[108]. However, it was unclear as to whether synergistic effects of GIP and insulin on GIPR mRNA levels were a result of direct effects on gene transcription or secondary to the progression of differentiation. 3T3-L1 cell differentiation was associated with upregulation of nuclear levels of peroxisome proliferator-activated receptor (PPAR)γ. Treatment with the PPARγ receptor agonists, LY171883 and rosiglitazone, increased GIPR expression in fully differentiated 3T3-L1 adipocytes, whereas the antagonist, GW9662, ablated expression[108]. Acetylation of histone H3/H4 was also increased during differentiation, and both PPARγ and acetylated histone H3/H4 bound to a region of the GIPR promoter containing the peroxisome proliferator response element (PPRE). As RNA interference (RNAi) knockdown of PPARγ in differentiated 3T3-L1 adipocytes greatly reduced GIPR levels, PPARγ appears to be a critical transcription factor in regulating adipocyte receptor expression, but it is unclear as to the role played by insulin.

Evidence has been presented for the involvement of both PPARα[109,110] and PPARγ[111] in the regulation of rodent β-cell GIPR expression, and further studies are required to clarify whether there are cell-selective differences in regulation. Additionally, in earlier studies, it was shown that the GIPR is downregulated in β-cells of obese rodent models of diabetes[109,110,112], but in studies on Vancouver Diabetic Fatty (VDF) Zucker rats, we recently found that, compared with lean controls, GIPR and
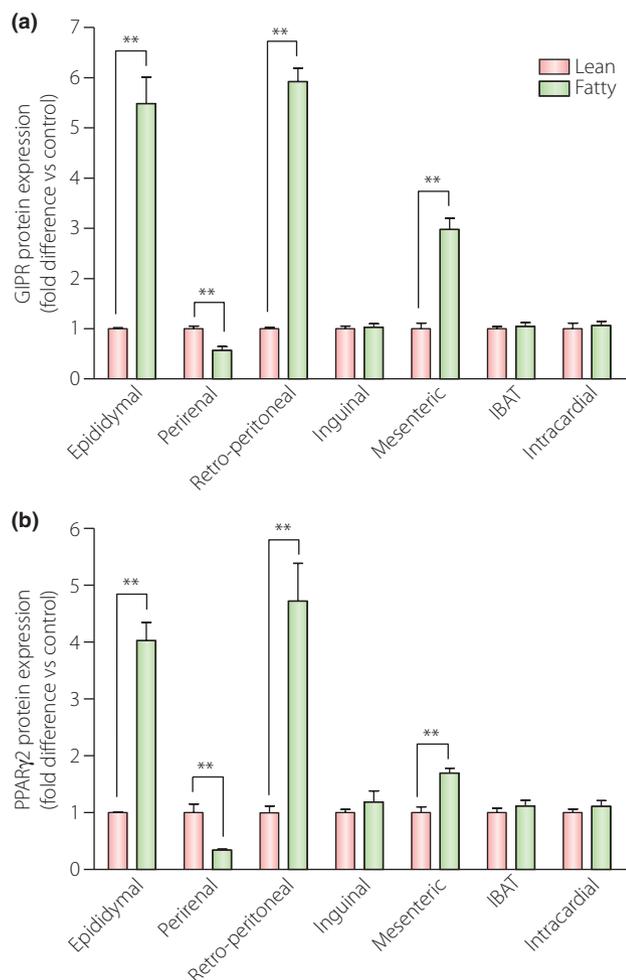
Figure 3 | Glucose-dependent insulinotropic polypeptide receptor (GIPR) and peroxisome proliferator-activated receptor (PPAR)γ protein expression levels in adipose tissue depots from lean and obese Vancouver Diabetic Fatty (VDF) Zucker rats. Tissue was collected from 18-week-old rats and western blot analyses were carried out, with quantification by densitometry ($n = 4$–6 rats). Significance was tested using analysis of variance (ANOVA) with Newman–Keuls post-hoc test. **$P < 0.05$ vs lean control group. IBAT, interscapular brown adipose tissue.

PPARγ protein levels were increased in epididymal and retroperitoneal fat pads, decreased in the perirenal fat depot and unchanged in other fat deposits (Figure 3). In contrast, GIPR expression in subcutaneous adipose tissue from human obese females was reported to be lower than in lean control subjects[113]. However, these results are difficult to compare, because of the different fat depots studied. Additionally, the sensitivity of GIPR expression to the prevailing insulin concentration, the level of adipose tissue insulin resistance and the glycemic status of the subjects/animals could all contribute significantly to the level of GIPR expression.

The pathways involved in GIP-stimulated lipogenesis are proving difficult to define, as a result of interactions between GIP, insulin and adipokine signaling. GIP stimulation of glucose uptake was shown to involve increasing plasma membrane glucose transporter-4 (GLUT-4) levels through a PKB-mediated pathway[103]. Human LPL gene expression is also stimulated by GIP activation of PKB[114], resulting in downstream reductions in LKB1 and AMPK phosphorylation, and increased translocation of TORC2 (cAMP-responsive CREB coactivator 2 [CRTC2]) into the nucleus. Interaction between TORC2 and phospho-CREB results in increased LPL gene expression[114]. Regulation of the phosphorylation state of TORC2 is complex and other members of the AMPK family (salt-inducible kinases [SIK] and MARK2)[115] are also capable of TORC2 phosphorylation, whereas calcineurin and a cAMP-activated pathway induce dephosphorylation[116]. It has not been established as to which of these contribute to GIP-mediated effects. GIP also enhances LPL enzyme activity in cultured 3T3-L1 cells and subcutaneous human adipocytes by non-transcriptional mechanisms[95,96]. In the 3T3-L1 cell line, GIP induces transient activation of p38 MAPK and sustained activation of stress-activated protein kinase (SAPK)/JNK, resulting in the release of resistin that, in turn activates PKB[95]. Somewhat surprisingly, subsequent events mimic those downstream of GIP in the human adipocyte, with decreases in LKB1 and AMPK phosphorylation linked to increased LPL secretion[95]. Human resistin (FIZZ3) shares only moderate sequence homology (~53%) with the mouse peptide[117]. Additionally, FIZZ3/resistin is only weakly expressed in human adipocytes, and monocytes/macrophages are the major sites of FIZZ3/resistin production in adipose tissue[118]. It is currently unknown whether FIZZ3/resistin serves a paracrine function in adipocyte regulation or whether there is an entero–adipokine axis involving GIP and FIZZ3/resistin in humans. However, FIZZ3/resistin has been reported to increase FFA re-esterification[119].

In the majority of *in vitro* studies to date, the effects of GIP on adipogenesis and lipogenesis have been shown to involve synergistic actions with insulin, and there has been significant interest in GIPR antagonists as potential therapies for obesity[88]. However, adipose tissue expansion has been suggested to be an important adaptive response to increased food intake, as it protects against excess fat deposition in other sites, such as liver and muscle[120,121]. GIP could be an important contributor to this response and reducing its effect might not result in the anticipated benefits. Additionally, studies on both transgenic mice[122] and pigs[123] expressing a dominant-negative GIPR showed greatly reduced β-cell mass, with the mice becoming severely diabetic, supporting a critical role for GIP signaling in β-cell development and proliferation.

ACKNOWLEDGEMENTS

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