Processing of proglucagon to GLP-1 in pancreatic α-cells: is this a paracrine mechanism enabling GLP-1 to act on β-cells?

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Abstract

Proglucagon is cleaved to glucagon by prohormone convertase 2 (PC2) in pancreatic α-cells, but is cleaved to glucagon-like peptide-1 (GLP-1) by PC1 in intestinal L-cells. The aim of this study was to identify mechanisms which switch processing of proglucagon to generate GLP-1 in the pancreas, given that GLP-1 can increase insulin secretion and β-cell mass. The α-cell line, αTC1-6, expressed PC1 at low levels and GLP-1 was detected in cells and in culture media. GLP-1 was also found in isolated human islets and in rat islets cultured for 7 days. High glucose concentrations increased P1 gene expression and PC1 protein in rat islets. High glucose (25 mM) also increased GLP-1 but decreased glucagon secretion from αTC1-6 cells suggesting a switch in processing to favour GLP-1. Three G protein-coupled receptors, GPR120, TGR5 and GPR119, implicated in the release of GLP-1 from L-cells are expressed in αTC1-6 cells. Incubation of these cells with an agonist of TGR5 increased PC1 promoter activity and GLP-1 secretion suggesting that this is a mechanism for switching processing to GLP-1 in the pancreas. Treatment of isolated rat islets with streptozotocin caused β-cell toxicity as evidenced by decreased glucose-stimulated insulin secretion. This increased GLP-1 but not glucagon in the islets. In summary, proglucagon can be processed to GLP-1 in pancreatic cells. This process is upregulated by elevated glucose, activation of TGR5 and β-cell destruction. Understanding this phenomenon may lead to advances in therapies to protect β-cell mass, and thereby slow progression from insulin resistance to type 2 diabetes. Journal of Endocrinology (2011) 211, 99–106

Introduction

Like many prohormones, proglucagon is processed in a cell type-specific manner. In the α-cells of the pancreas, proglucagon is processed to glucagon by prohormone convertase 2 (PC2), but it undergoes alternative processing in the L-cells of the intestine with PC1 catalysing the cleavage to yield glucagon-like peptide (GLP)-1 and GLP-2 (Fig. 1a). Evidence suggests the tissue-specific processing is due to differential expression of PC1 (Tucker et al. 1996) and PC2 (Rouille et al. 1994).

GLP-1 is well known as an incretin hormone and its role in potentiating glucose-stimulated insulin secretion (GSIS) in response to a meal has been clearly elucidated (Edwards et al. 1999). Importantly, GLP-1 may have longer term effects on β-cell neogenesis and proliferation given that the GLP-1 analogue, exendin-4, increases the rate of β-cell regeneration in rats that have undergone a partial pancreatectomy (Xu et al. 1999). GLP-1 has also been shown to reduce β-cell susceptibility to apoptosis following exposure to streptozotocin (STZ; Buteau et al. 2004).

There is increasing interest in GLP-1 as a therapy for type 2 diabetes. However, GLP-1 is degraded by dipeptidyl peptidase IV (DPPIV) and therefore has a very short half-life (Larsen & Holst 2005). Indeed around 90% of the GLP-1 secreted from the L-cells is degraded before it reaches the circulation (Hansen et al. 1999). Thus, it seems unlikely that the majority of GLP-1 secreted from the L-cells acts directly on the pancreas. Studies by Ionut et al. (2006) where they infused GLP-1 and glucose into the systemic circulation and observed only a modest increase in insulin compared with that achieved by a meal, suggest that GLP-1 may be acting at least partially via the vagus nerve to stimulate insulin secretion. However, that begs the question of why there are GLP-1 receptors present on β-cells (De Leon et al. 2006) and it may be that GLP-1 can be generated locally in the pancreas and act directly on β-cells.

Other non-mammal vertebrate species, including fish and frogs produce pancreatic GLP-1 (Irwin 2001) and previous data have shown trace amounts of GLP-1 in the mammalian α-cell, which is increased by high concentrations of glucose (McGirr et al. 2005). However, little is known about the physiological relevance. Animal models have suggested that
whether mechanisms that destroy isolated rat and human islets. We have investigated factors the concentrations of GLP-1 in a pancreatic expression of triplicate wells Gene expression for the convertases (PC1 and PC2), proglucagon and this is known to occur in the L-cells in the intestine. (b and c) (pancreatic (a) Proglucagon is processed by PC2 to mature glucagon in the enteric cell line (GLUTag) in a similar manner to (AR231453) stimulates release of GLP-1 from another agonist for GPR119 is not known, a small molecule agonist Journal of Endocrinology but there is no clear indication of the mechanism (Nie 2000, Thyssen 2006). In considering how GLP-1 secretion could be increased, it is interesting to note that glucose stimulates release of GLP-1 from the L-cells in the intestine (Reimann & Gribble 2002). It is interesting to note that glucose stimulates release of GLP-1 from the L-cells in the intestine (Reimann & Gribble 2002). In considering how GLP-1 secretion could be increased, it is interesting to note that glucose stimulates release of GLP-1 from the L-cells in the intestine (Reimann & Gribble 2002). It is interesting to note that glucose stimulates release of GLP-1 from the L-cells in the intestine (Reimann & Gribble 2002). In considering how GLP-1 secretion could be increased, it is interesting to note that glucose stimulates release of GLP-1 from the L-cells in the intestine (Reimann & Gribble 2002). Total RNA was extracted using Qiagen Mini columns (Qiagen) and its quantity was determined by a NanoDrop spectro-photometer (NanoDrop Technologies, Wilmington, DE, USA). Total RNA of 1 µg was reverse transcribed using TaqMan reverse transcriptase reagents, and random hexamer primers (Applied Biosystems, Foster City, CA, USA) and incubated at 25 °C for 10 min, 48 °C for 30 min and 95 °C for 5 min. QPCR assays were performed on an ABI Prism 7900 TaqMan Sequence Detection system (Applied Biosystems). Primers and probes for the amplification of hypoxanthine-guanine phosphoribosyltransferase (HPRT), PC1 and PC2 were designed using Primer Express 1.5a (Applied Biosystems), sequences are detailed in Table 1. The primers and probes for the remaining genes were TaqMan Gene Expression Assays (based on published sequences (Applied Biosystems; proglucagon, mouse – Mm01269055, rat – Rn00562293; proinsulin,
GLP-1 in the pancreas

Regulation of GLP-1 and glucagon secretion

Cells were seeded at 1 × 10⁵ cells per well and incubated overnight. After preincubation for 30 min in KRH/HEPES (129 mmol/l NaCl, 5.0 mmol/l NaHCO₃, 4.8 mmol/l KCl, 1.2 mmol/l KH₂PO₄, 1.2 mmol/l MgSO₄, 10 mmol/l HEPES, 1.25 mmol/l CaCl₂, 0.1% BSA, pH 7.4) containing 10 mmol/l glucose, the cells were incubated as described. Glucagon was assayed by an enzyme immunoassay (CosmoBio, Tokyo, Japan) (with a detection limit of 20 fmol/ml). Active GLP-1 was measured by ELISA (Linco Research, St Charles, Missouri, USA) (with a detection limit of 10 fmol/ml). The specificity of both peptide assays was evaluated to ensure minimal cross-reactivity between glucagon, GLP-1 and their precursors. The cross-reactivity of GLP-1 in the glucagon assay was 8%. Glucagon was not detected in the GLP-1 ELISA even at 1000 pM, and the cross-reactivity of inactive GLP-11–37 in the GLP-1 assay is < 3% (data not shown).

Regulation of proglucagon processing in isolated islets

Islets of Langerhans were prepared from male Han Wistar rats, as described previously (Ligon et al. 1998). Human islets were obtained from a donor and provided by the United Kingdom Human Tissue Bank (Leicester, UK) and isolated in the Islet Research Laboratory (Worcester, UK) in accordance with current ethical guidelines pertaining to the use of human tissue in research. Islets were incubated in RPMI-1640 media (Invitrogen) containing 10% FCS, 1% PSG and the indicated glucose concentration, at 37°C and 5% CO₂, after which the STZ was removed and replaced with 250 μl culture medium (method adapted from Harb et al. (2007)). Islets were incubated for a period of 48 h with one medium change to remove all contaminants from cell apoptosis. Conditioned medium was collected and analysed for GLP-1 and glucagon. Islet function was assessed by GSIS assay.

Statistical analysis

Experiments were performed at least three times and results are presented as mean ± S.E.M., and P values were determined by the unpaired Student’s t-test. Results were considered significant when P<0.05.

Results

The prohormone convertase, PC1, is expressed in the αTC1-6 pancreatic α-cell line

PC1 and PC2, the proteases known to cleave proglucagon (Fig. 1a), were found in both the αTC1-6 α-cell line and the MIN6 β-cell line (Fig. 1b and c), although expression of PC1 was several hundred fold higher in the β-cells, while PC2 was higher in the αTC1-6 cells. Proglucagon was expressed in the pancreatic α-cell lines, αTC1-6 and αTC1-9 (Fig. 1d), whereas insulin was not expressed but was found in the MIN6 β-cell line (Fig. 1e).
GLP-1 is produced by pancreatic α-cell lines and by human and rodent islets

The αTC1-6 cells secreted both GLP-1 and glucagon into culture medium after only 30 min incubation (Fig. 2a) with approximately tenfold higher concentrations of glucagon (27.3 ± 2.3 fmol/well vs 3.1 ± 0.2 fmol/well, P < 0.001). GLP-1 peptide was also detected in human islets incubated for 24 h under basal conditions. However, the GLP-1 was only present in cell lysates and not in culture medium (Fig. 2b) suggesting it was either not secreted or the concentrations were below the limit of detection under these conditions.

GLP-1 was also detected in extracts of freshly isolated rat islets and concentrations increased from 1.5 to 3.5 fmol/islet after incubation for 3 days in 5 mmol/l glucose (Fig. 2c). The same islets produced glucagon that increased to a greater extent over time in culture (Fig. 2d and e).

PC1 expression is increased by high glucose concentrations in rat islets

PC1 gene expression was markedly increased by incubation with 11 and 25 mmol/l glucose in rat islets cultured for 3 days and expression levels remained elevated by day 7 in the islet cultures with high glucose concentrations (Fig. 3a). The active form of PC1 protein also shows a trend towards increased expression although this was not statistically significant (Fig. 3b). Proglucagon and PC2 mRNA levels did not alter in the rat islets cultured in high glucose over this time course (data not shown).

High glucose stimulates GLP-1 secretion but inhibits glucagon secretion

Glucagon secretion from αTC1-6 cells decreased as glucose concentrations increased from 0.5 to 35 mmol/l (Fig. 4a). A range of incubation times was investigated and 30 min chosen as optimal (data not shown). GLP-1 secretion was stimulated by high glucose (25 mmol/l) whereas glucagon secretion was inhibited (Fig. 4b and c), suggesting that these peptides are released from αTC1-6 cells. KCl, a well-established secretagogue for provoking the release of secretory vesicles from cells, caused an increase in release of both GLP-1 and glucagon (Fig. 4d and e).
Each of the GPCRs can be activated by their specific agonists in a dose-dependent manner in STC-1 cells (data not shown) and this was used to select concentrations of ligands to test on PC1 promoter activity in αTC1-6 cells. Stimulation with a GPR120 agonist caused activation of the PC1 promoter in STC-1 cells but TGR5 and GPR119 ligands had no effect (Fig. 5d). Therefore, we sought to determine if activation of these receptors would upregulate PC1 in αTC1-6 cells. In this α-cell line, TGR5 stimulation with a small molecule agonist (supplied by AstraZeneca) increased PC1 promoter activity to nearly the same extent as forskolin and IBMX, but GPR119 and GPR120 ligands had no effect. Secretion of GLP-1 was stimulated by GPR120 and TGR5 agonists in the αTC1-6

Over the longer term (5 days), expression of the proglucagon gene was not affected by incubation with high glucose (25 mmol/l; Fig. 4f) and there was no change in expression of either PC1 or PC2.

L-cell GPCR ligands can increase GLP-1 release from pancreatic α-cells

In intestinal L-cells, secretion of GLP-1 is thought to be regulated by the GPCRs, GPR120, TGR5 and GPR119, which are activated by specific agonists. We examined expression of these receptors on αTC1-6 and αTC1-9 cells to determine if there was variation in receptor expression and to identify the best cell line for the analysis of PC1 promoter regulation. While the L-cell line, STC-1, had much higher expression of TGR5, αTC1-6 cells expressed more GPR119 and GPR120 than the STC-1 cell line (Fig. 5).

In other endocrine tissues, such as intestine (Dhanvantari et al. 2001) and pituitary (Bloomquist et al. 1991, Li et al. 2001) expression of PC1 can be regulated. Therefore, we investigated whether PC1 promoter activity could be stimulated using transient transfection with a PC1 promoter linked to a luciferase construct. In both the control STC-1 and the αTC1-6 cells, the PC1 promoter is increased by the combination of forskolin and IBMX (Fig. 5d). In the STC-1 cells, the increase in PC1 could affect secretion of other enteroendocrine peptides but in the pancreatic α-cells it is likely to increase GLP-1.
cells, while glucagon secretion was inhibited by all three agonists in the same cells (Fig. 5e and f).

**Damaged islets with decreased β-cell function have increased GLP-1 secretion**

To assess the effect of β-cell damage on GLP-1 secretion from α-cells islets were treated with the β-cell toxin, STZ, for 2 h and then incubated for 48 h before assessing β-cell function (Fig. 6a). Islets treated with 0.3 and 1 mmol/l STZ had a >50% decrease in GSIS when exposed to 10 and 20 mmol/l glucose.

Forty-eight hours after treatment with STZ there was a significant increase in GLP-1 secretion. STZ (0.1 mmol/l) treatment resulted in a 2.5-fold increase in GLP-1 release from islets, while 0.3 mmol/l STZ produced a maximal response with a fourfold increase (Fig. 6b). Over the same concentration range there were no changes in the glucagon release from the islets (Fig. 6c).

**Discussion**

There is increasing interest in using GLP-1 analogues and DPP4 inhibitors as novel treatments for patients with diabetes. In part this is because of the recognised effects of GLP-1 in augmenting insulin release (Buteau et al. 2004). However, there is also data showing that GLP-1 can promote β-cell regeneration (Xu et al. 1999). In this paper, we provide evidence that α-cells from the pancreas can produce GLP-1 and in certain situations there is upregulation of PC1 in α-cell lines that would enable increased cleavage of proglucagon to GLP-1. By using STZ as a mechanism to impair β-cell function in rat islets, we show that GLP-1 secretion by α-cells is upregulated while glucagon secretion is not altered. In the intestinal L-cells, GLP-1 release is stimulated by novel GPCR-related mechanisms. We have found that these receptors are present on α-cell lines and that GLP-1 secretion can be increased by activation of these receptor signalling pathways. Overall, these studies emphasise the importance of alternative processing of proglucagon in pancreatic α-cells and the endogenous mechanisms enabling GLP-1 to have a paracrine effect on β-cell regeneration in the pancreas.

Although glucagon is the major product of the proglucagon gene in the αTC1–6 cell line, the data presented shows that PC1 is expressed and that GLP-1 is produced and detectable in culture medium after only 30 min incubation. This is substantiated by the finding of GLP-1 along with glucagon and oxyntomodulin, using the more qualitative approach of intact-cell mass spectrometry in αTC1–9 cells (Buchanan et al. 2007). In addition, GLP-1 is present in isolated human pancreatic islets cultured for 24 h, raising the possibility that upregulation of this mechanism could provide GLP-1 for paracrine effects. GLP-1 is also found in rodent islets and there is an increase in the GLP-1 concentration when islets are kept in culture with a glucagon secretion inhibitor for 3–7 days. The concentration of GLP-1 detected in the rodent islet was 3.5 fmol/islet, which was comparable to other studies (Thyssen et al. 2006). In the perfused rat pancreas concentrations as low as 50 pmol/l of GLP-1 have been shown to increase insulin secretion (Mojsov et al. 1987). Although this concentration is higher than that detected in this study, this may be reflective of the different concentrations required for exogenous ligand compared with a paracrine effect. As with the human islets, rodent islet GLP-1 could not be detected in the culture medium. This suggests that there could be specific mechanisms to stimulate release of GLP-1 from the α-cells in the islets, although there is also the possibility that GLP-1 is secreted but the levels are below the limit of detection of the current assay. Active GLP-1 (GLP-1 (7–36)), is produced following cleavage of GLP-1 (1–36) by PC1 or furin, which are both expressed in the α-cell. This GLP-1 has previously been found in normal isolated rat islets (Heller & Aponte 1995, Masur et al. 2005). When the islets were cultured ex vivo for 7 days, significant levels of GLP-1 were found at an almost equimolar ratio to active glucagon (Masur et al. 2005).
Significant increases in PC1 expression were also observed when rat islets were cultured in 11 and 25 mM glucose, and this was substantiated by increased PC1 protein. The upregulation of PC1 in islets was not evident after 24 h but was observed after 3 and 7 days in culture. It may be that some increases in PC1 occurred in the islet α-cells, but these changes were obscured by the changes in PC1 that are known to occur in β-cells (Martin et al. 1994, Skelly et al. 1996). Previous studies have shown that high glucose concentrations cause upregulation of PC1, but not PC2 expression, when measured by microarray and luciferase assays in an α-cell model (McGirr et al. 2005). These data suggest that proglucagon processing could be altered to produce increased levels of GLP-1, as a result of increasing expression of PC1 in α-cells.

The increasing concentrations of glucose decreased glucagon as has been shown previously (McKinnon et al. 2006). α-Cell lines have also been shown to produce GLP-1 when exposed to supraphysiological concentrations of glucose (McGirr et al. 2005). However, this study directly compared the effect of high concentrations of glucose on both GLP-1 and glucagon and indicated that there was decreased glucagon with a concomitant increase in GLP-1 secretion. Given that proglucagon is the precursor of both glucagon and GLP1 it is interesting to speculate how glucose could increase secretion of one and decrease secretion of the other. Glucose could theoretically act at the level of synthesis of proglucagon and/or synthesis of either of the processing enzymes and/or release of secretory vesicles. Our hypothesis is that a subset of cells were secreting primarily GLP1 (and the proglucagon fragment glicentin) and other cells were secreting primarily glucagon (and the precursor of GLP-1 (MPGF); see Fig. 1). This could explain why treatment with potassium chloride that causes non-specific release of all secretory granules (which should release peptides from all the cells) results in release of both glucagon and GLP-1. Overall, this study suggests that high glucose concentrations increase PC1 gene expression and stimulate release of GLP-1 from the αTC1-6 cell line.

TGR5, GPR119 and GPR120 are three GPCRs, known to mediate GLP-1 release from L-cells (Hirasawa et al. 2005, Katsuma et al. 2005, Chu et al. 2007). Interestingly, we found that all three receptors were present in αTC1-6 cells, suggesting that signalling through these receptors could occur in the pancreatic α-cells as well as in the intestinal L-cells.

Since it is known that PC1 promoter activity can be regulated by factors that also regulate the hormone, which is cleaved by PC1 (Bloomquist et al. 1991, Jansen et al. 1995), we investigated activation of a transiently transfected PC1 promoter in pancreatic αTC1-6 cells. Incubation with a small molecule agonist of TGR5 caused an increase in PC1 promoter–driven luciferase activity in the αTC1-6 cells to a level similar to that observed with forskolin and IBMX. GLP-1 secretion was stimulated by the activation of TGR5 and GPR120 but not by GPR119. Interestingly, glucagon secretion was not stimulated by any of these agonists. Therefore, GPCR activators can selectively increase GLP-1 release from pancreatic α-cell lines.

It is likely that enabling the α-cell to produce GLP-1 is a physiological response to high glucose concentrations, and may upregulate GLP-1 secretion to regenerate β-cell mass following a metabolic or inflammatory insult. Indeed, Kilimnik et al. (2010), show that PC1 and GLP-1 are present in the pancreatic α-cells of four mouse models of insulin resistance. This is supported by studies where PC1 was up-regulated in α-cells after STZ treatment of rats in vivo (Nie et al. 2000, Thyssen et al. 2006). Wideman et al. (2009) provided elegant evidence that in mice subjected to STZ in vivo, transplantation of α-cells expressing PC1 resulted in improved glucose tolerance. We sought to study this more directly and therefore developed an in vitro model, as described previously (Harb et al. 2007) whereby brief (2 h) treatment of isolated rat islets with STZ resulted in β-cell dysfunction in the form of a reduction in GSIS, glucagon remained to be secreted from the islets, suggesting the α-cells remained functional. If the islets were then left for 48 h there was a rise in GLP-1 secretion that increased with increasing concentrations of STZ. Over the same time frame, the concentration of glucagon in the culture medium remained unchanged. This suggests that disruption of β-cell function is accompanied by an increase in the release of GLP-1. Taken together, these studies provide evidence that increased local production of GLP-1 in the pancreas may occur as a result of impaired β-cell function. The mechanism is unclear but could be because of a loss of tonic inhibition of insulin on GLP-1 production (Ding et al. 2009). Given that β-cells are interspersed with α-cells in the human pancreas (Unger & Orci 2010) this locally produced GLP-1 may have direct paracrine effects on the remaining β-cells to promote regeneration and proliferation. It would be important in future studies to find approaches to upregulating processing of proglucagon to GLP-1 in α-cells in patients with diabetes.

Declaration of interest
Dr D M S and Dr N M W are employed by AstraZeneca. The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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