Action of Incretins on the Pancreatic Alpha Cell

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Abstract (5-10 Lines)

T2D is a bihormonal disorder characterised by hypoinsulinaemia and hyperglucagonaemia. The incretin GLP-1 is therapeutically attractive as, in addition to augmenting glucose-stimulated insulin secretion, it is also able to suppress glucagon secretion from alpha cells. However, the mechanism by which GLP-1 is able to modulate glucagon secretion has not been fully established. The observation that the GLP-1 receptor is expressed at extremely low levels on alpha-cells has led some groups to postulate that the effects of GLP-1 may be mediated by paracrine mechanisms. Conversely, other groups have shown that GLP-1 is able to directly regulate glucagon secretion in isolated alpha-cells, discounting the role of islet cross-talk. Further work needs to be performed to fully understand the mechanism of GLP-1 action on alpha cells. Specifically it will be important to determine if GLP-1 can mediate its effects via another receptor on alpha-cells or whether its abundantly-occurring metabolite, GLP-1 (9-36) is also able to affect glucagon secretion.

Keywords (5-10)

Glucagon

Alpha-cells

Glucagon-like peptide 1 (GLP-1)

Glucose-dependent insulinotropic peptide (GIP)

Introduction

T2D is often characterised as the inadequate beta-cell response to increased insulin resistance in peripheral tissues. However, there is strong evidence to suggest that glucagon secretion from pancreatic alpha-cells is also dysregulated in subjects with this disorder. T2D should therefore be viewed as a bihormonal disease characterised by both hypoinsulinaemia and hyperglucagoneamia and the contribution of glucagon to the pathological disease state should not be overlooked.

Glucagon is a hyperglycaemic hormone which, following secretion from the pancreatic alpha-cell, is able to increase hepatic glucose output via suppression of glycolysis and stimulation of gluconeogenesis and glycogenolysis. In healthy subjects, glucagon secretion from alpha-cells is stimulated by hypoglycaemia and suppressed during hyperglycaemia, thus working synergistically with insulin to regulate blood glucose levels within a narrow physiological range (4-6mmol/L). In patients with T2D however, perturbations in the regulation of glucagon secretion have been observed. For example, patients with T2D exhibit elevated plasma glucagon levels in the fasting state whilst demonstrating impaired suppression of glucagon secretion in response to glucose (1). Importantly glucagon dysregulation has been shown to contribute to the hyperglycaemic phenotype (1). Of interest, patients with T2D have demonstrated a delayed suppression of glucagon secretion in response to an oral glucose challenge whilst suppression after an intravenous infusion mimicked that seen in healthy individuals (1). This suggests that factors secreted via the oral ingestion of nutrients may be vital to the regulation of glucagon secretion.

Gastrointestinal factors which are secreted in response to nutrient intake to modulate glucose homeostasis are referred to as incretins. Incretins ubiquitously enhance glucose-stimulated insulin secretion (GSIS) in pancreatic beta-cells but can modulate differential effects on the alpha cells. Thus, glucose-dependent insulinotropic peptide (GIP), released from K cells of the duodenum and proximal jejunum in response to the rate of nutrient absorption, *stimulates* glucagon secretion (2). Conversely, glucagon-like peptide 1 (GLP-1), released in a bi-phasic manner from L cells of the distal ileum, has a potent *inhibitory* effect on glucagon secretion. Consequently, analogues of GLP-1,

rather than glucagon-enhancing GIP, have attracted therapeutic interest. To date they are the only available therapeutics which are able to both lower blood glucose levels by stimulating insulin secretion as well as suppress glucagon secretion in patients with type 1 and 2 diabetes (3, 4).

Evidence from both in vitro and in vivo studies has demonstrated the efficacy with which GLP-1, and its analogues, can suppress glucagon secretion. Thus, GLP-1 infusion in healthy male subjects caused an inhibition of glucagon secretion at both low and high doses. In these subjects plasma glucose levels were lowered as a result of a 30% reduction in hepatic glucose production as opposed to glucose clearance, suggesting a direct role of GLP-1 on glucagon secretion in vivo (5). Similarly, additional in vivo studies have utilised the GLP-1 receptor (GLP1R) antagonist exendin 9-39, as a means of blocking the actions of GLP-1. In healthy volunteers administration of an oral glucose challenge reduced plasma glucagon levels as expected. However, following a glucose challenge in subjects treated with the antagonist exendin 9-39, glucagon suppression was lost and a modest increase in glucagon levels was noted (6).

These findings clearly demonstrate that GLP-1 is able to modulate alpha-cell function and glucagon secretion in vivo. However, these original studies have not addressed whether the glucagon-suppressing actions of GLP-1 are mediated by a direct action on the alpha-cell, or via a paracrine mechanism. It is well established that GLP-1 stimulates insulin and somatostatin secretion from the pancreatic beta and delta cells respectively (7). Both of these hormones can directly suppress glucagon secretion and therefore it has been postulated that GLP-1 mediated suppression of glucagon secretion may be caused via a paracrine interaction.

However, there is some compelling evidence suggesting that GLP-1-mediated glucagon suppression occurs via a direct (autocrine) effect on the pancreatic alpha cells and not as a result of a paracrine mechanism. In this chapter we will discuss evidence for and against this theory, as well as highlighting some of the experimental approaches which have been employed to date. In addition we will also focus on the technical challenges faced when elucidating the underlying mechanisms of GLP-1 action on alpha-cells and discuss the questions which remain unanswered in this field.

Review of the field

GIP

Although both GLP-1 and GIP contribute approximately 80% of the insulin secretory response to oral glucose, the action of GLP-1 and GIP on glucagon secretion differs. Unlike GLP-1, which inhibits glucagon secretion, GIP exerts a stimulatory response on glucagon release (2). GIP binds to GIP receptors (GIPRs) on the alpha cells and stimulates the secretion of glucagon via increases in intracellular cAMP (8). Recent studies have reported local GIP production in mouse and human alpha-cells, by means of immunohistochemistry and measurement of GIP secretion in response to various secretagogues. The functional role of local GIP production by islet alpha-cells is unclear, however one suggestion is that constitutive GIP secretion from alpha-cells under stimulatory conditions, e.g. during fasting, can prime the beta-cells for improved subsequent GSIS (9).

In T2D, the ability of GIP to enhance GSIS is lost, possibly due to chronic desensitisation or downregulation of the GIPR (9). This finding, coupled with the observation that GIP stimulates glucagon secretion, has rendered GIP therapeutically unattractive. Therefore the remainder of this chapter will focus on the other major incretin hormone, GLP-1, and its effects on glucagon secretion.

GLP1R expression on pancreatic alpha-cells

As mentioned above, the observation that GLP-1 mediates an effect on glucagon secretion in-vivo has led many groups to investigate the mechanism of its action. To this end, attention has naturally focussed on determining whether the classical GLP-1 receptor, GLP1R, is expressed on alpha cells.

GLP1R is a member of the glucagon receptor family of G-protein coupled receptors (GPCRs). The receptor was initially cloned from a rat pancreatic cDNA library in 1992 but has since been shown to be expressed in multiple human tissues including lung, heart, and the GI tract (7). Expression of

GLP1R in pancreatic alpha-cells has proven more difficult to establish and multiple experimental approaches have been utilised to address this fundamental question.

In 1991, Orskov and colleagues used radiolabelled GLP-1 (^{125}I-GLP-1) to assess binding capability to islet endocrine cells. This study demonstrated that although GLP-1 was able to bind to alpha and delta cells, binding was stronger to insulin-producing cells, indicating higher levels of GLP1R in beta-cells (10). Similar patterns of GLP1R expression were observed by Heller et al. in 1995. A three-fold higher GLP1R expression level was observed in beta-cells compared to alpha or delta cells as detected by means of GLP1R radiographic density. Interestingly, in this study measurements were performed at only two hours after islet isolation. When performing the same experiments 12 or 24 hours post-culture, a three-fold reduction in the signal was observed in all cell types, suggesting that islet culture can significantly alter the expression of the GLP1R (11).

In 1996, Moens and colleagues examined GLP1R mRNA and protein expression levels in rodent alpha and beta-cells. Although beta-cells reproducibly demonstrated GLP1R expression at both the RNA and protein levels, no GLP1R was identified on alpha-cells (12) . Similarly, a study by Tornehave and team failed to demonstrate expression of the GLP1R mRNA or protein in alpha cells. Using in-situ hybridisation and double and triple immunofluorescence, GLP1R colocalisation was restricted to insulin-containing cells in rodent and human islets. To confirm the specificity of the methodology, tissue sections from GLP1R knockout animals were stained, confirming total lack of GLP1R expression in tissues known to be positive for the receptor (13).

More recent approaches have utilised antibody-independent methods to determine the expression profile of the receptor in islets. A study in 2010 by De Marinis et al used a transgenic mouse model expressing the Venus fluorescent protein under the proglucagon promoter and islets isolated from these animals were flow-sorted to obtain a pure preparation of alpha cells. Comparative mRNA expression analysis demonstrated that both alpha and beta-cells express GLP1R, but levels in alpha cells were only 0.2% of those detected in beta-cells (14). Similarly, using a novel transgenic mouse model in which GLP1R-Cre mice were crossed with fluorescent reporter strains, offspring were generated that expressed fluorescent markers in target tissues that currently, or during development expressed GLP1R (15) . This study reported GLP1R expression in only 9.5% of the fluorescence activated cell sorted alpha cells. Considerable effort has also been invested in

generating a more specific antibody against the GLP1R. Recently, Pyke and others have reported the generation of a monocloncal antibody which has been extensively tested in both primate and human tissues. Thus, when tested in normal monkey and human pancreas almost total co-localisation of GLP1R and insulin was observed, although expression was also thought to be present in a very small subset of non-beta cells (16).

To date, the techniques employed to determine GLP1R expression have mostly relied upon antibodies that have been neither sensitive nor specific. Gene expression studies using quantitative real-time PCR (RT-PCR) have also raised concerns regarding the reliability of the data generated. Messenger RNA expression analyses are performed by generating amplicons far smaller than the entire full-length transcripts. It should be noted that cells are often able to produce multiple non-coding mRNAs from a single DNA sequence. These transcripts may be detected by the same primers which detect the full-length mRNA, thus providing no means of discerning between the coding and non-coding transcripts. For example, analysis of GIPR expression revealed approximately 64 GIPR mRNA transcripts in human adipose tissue, of which only two contained an open reading frame that could give rise to a fully functioning, membrane-spanning receptor protein (17).

In summary the conflicting findings reported in the literature may arise as a result of several factors ranging from the use of freshly-isolated versus cultured islets, the use of rodents in contrast to humans, experiments performed on single cells or fixed sections or the use of non-specific antibodies. However, the current transgenic animal model, coupled with data from a highly sensitive antibody analysis, suggest that GLP1R is expressed on alpha-cells but at levels considerably lower than those expressed in beta-cells.

GLP-1 evoked inhibition of glucagon secretion via paracrine mechanisms?

As discussed above, the initial controversy regarding GLP1R expression on alpha cells, with some studies having failed to observe any expression at all, led to the suggestion that GLP-1 regulates glucagon secretion via paracrine interractions in the islet. As GLP1R expression is abundant in beta and delta cells, and given the islet architecture and potential cross-talk between different cell types, it is possible that insulin and/or somatostatin and their derivatives could influence the actions of GLP-1 on alpha cells. Interestingly however, infusion of exogenous GLP-1 in patients with type 1 diabetes, who were unable to secrete significant amounts of C-peptide (equivalent to endogenous

insulin), resulted in significant reduction in glucagon secretion. The suppression in glucagon levels was comparable to that seen in T2D patients, who were able to also produce a 20-fold greater increase in plasma insulin and C-peptide, suggesting that the GLP-1 effect is independent of insulin secretion (18). Likewise De Heer et al., have demonstrated inhibition of glucagon secretion by GLP-1 at a low glucose concentration, at which basal secretion of insulin was very low or undetectable (19).

Having discounted a paracrine effect from insulin, De Heer et al proposed that the inhibition of glucagon secretion may be exerted via somatostatin. Somatostatin receptors (SSTRs) are present on the alpha cell, predominantly in the form of subtype 2 (SSTR2). In a perfused rat pancreas, De Heer and colleagues investigated the importance of somatostatin on GLP-1-mediated glucagon suppression via two independent methods. Firstly, SSTR2 was blocked using a selective antagonist, PRL-2903, whilst in an additional set of experiments the effects of somatostatin were prevented by immunoneutralisation with a somatostatin antibody. In the presence of GLP-1, glucagon secretion was suppressed whilst addition of the SSTR2 antagonist alone enhanced glucagon secretion. However, co-application of the SSTR2 antagonist and GLP-1 was able to reverse the inhibitory effects on glucagon secretion. In fact, levels of secretion remained equivalent to those observed when treated with SSTR2 alone. In light of these findings De Heer and colleagues reported that GLP-1 mediated its effects on glucagon secretion via somatostatin. Interestingly however, the authors were unable to completely replicate their findings via somatostatin immunoneutralisation. In the presence of a somatostatin antibody, GLP-1 was still able to suppress glucagon secretion, although levels were attenuated compared to control experiments (19).

Direct Mechanism of GLP-1 action

Consistent with other studies, several pieces of evidence from our own group indicate that the inhibitory effect of GLP-1 on glucagon secretion may not exclusively result from a paracrine somatostatin-mediated mechanism (14). Firstly, in isolated mouse islets we have shown that at in the absence of glucose, when insulin and somatostatin secretion does not occur, GLP-1 can still inhibit glucagon secretion by ~50% (Figure 1B). Second, GLP-1 is able to retain its inhibitory properties in the presence of CYN154806, a somatostatin receptor 2-specific antagonist targetting the somatostatin receptors in alpha cells. Third, GLP-1 inhibits glucagon secretion in dispersed mouse alpha cells whereby cell-to-cell communication is minimal or non-existent, thus ruling out any potential paracrine interaction (14).

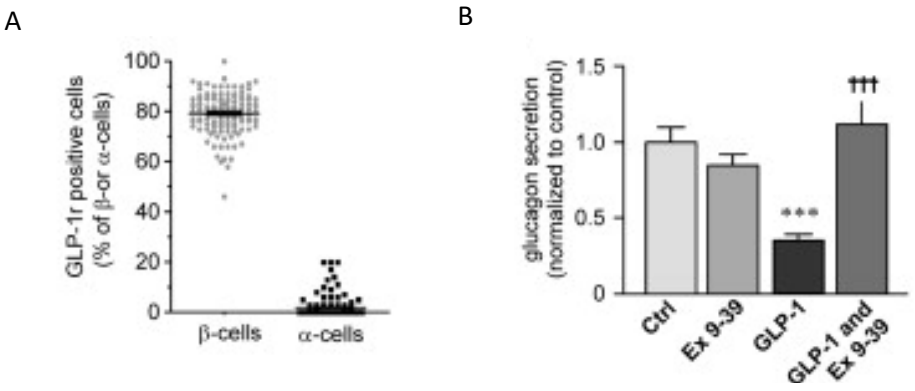

Figure 1.GLP1R expression and GLP-1 activity in mouse islets. A; Immunocytochemistry was performed for GLP1R, glucagon and insulin in mouse islets. The number of beta-cells (insulin positive) and alpha-cells (glucagon positive) which were also positive for GLP1R are shown (left graph). B; Glucagon secretion was determined at 1mM glucose (Ctrl), in the presence of 100nM GLP-1 and/or 1µM exendin 9-39 (14).

Therefore we have proposed that GLP-1 acts directly on the alpha-cell to inhibit glucagon secretion. Key to this model is the low GLP-1 receptor density on the pancreatic alpha cells (Figure 1A). GLP-1 binds the low number of GLP-1 receptors and causes a proportional activation of Gs-coupled membrane receptors, which leads to a small increase in intracellular cAMP production. Subsequent activation of PKA leads to PKA-dependent phosphorylation of P/Q-type Ca^{2+} channels (20), inhibition of their activity, and reduced exocytosis of glucagon-containing secretory granules. This is in contrast to adrenaline, which activates 100- to 1000-fold more G_s-coupled membrane receptors and thereby produces a larger increase in intracellular cAMP. Activation of the low- affinity cAMP sensor Epac2 binding, by the increase in intracellular cAMP concrentration, leads to activation of L-type Ca^{2+}channels and increased Ca^{2+} influx leading to enhanced secretion of glucagon. Interestingly, the differential effects of GLP-1 and adrenaline could be mimicked by low (nanomolar) and high (micromolar) concentrations of the adenylyl cyclase activator forskolin. Thus, low concentrations of forskolin inhibited glucagon secretion in contrast to high concentrations of the activator, which increased glucagon secretion (14).

Although both glucose and GLP-1 mediate their inhibitory effects on glucagon secretion via the P/Q-type Ca^{2+} channel in mice, GLP-1 effects occur independently of the K_{ATP} channel. For example, use of the K_{ATP} channel activator, diazoxide, blocked the inhibitory actions of glucose but was not able to antagonise the effects of GLP-1. This suggests that glucose modulates P/Q-type Ca^{2+} channels by an indirect mechanism mediated by changes in the alpha cell membrane potential resulting from

closure of the K_{ATP} channels, whilst GLP-1 inhibits glucagon secretion by a more direct effect on Ca2+-channel activity and exocytosis (14) (Figure 2).

These detailed cell-physiological studies must now be validated to the same extent in human tissue. Preliminary work from our own group suggests that in isolated human islets, GLP-1 is able to directly mediate glucagon suppression. This effect is independent of the action of somatostatin, and can be blocked using the GLP1R antagonist, exendin 9-39 (Ramracheya and Rorsman, unpublished).

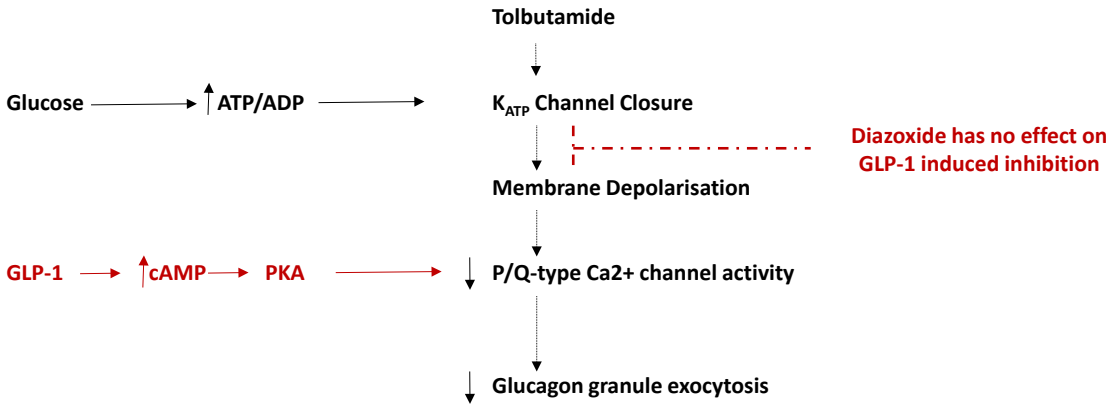

Figure 2. Glucose and GLP-1-mediated suppression of glucagon secretion in mouse islets. The effects of glucose on glucagon secretion are mediated via a K_{ATP} dependent mechanism. These effects can be blocked by diazoxide, a K_{ATP} channel activator. In contrast, GLP-1 inhibits glucagon secretion by PKA mediated inhibition of the P/Q-type calcium channel, bypassing the K_{ATP} channel.

Introduction to Key Challenges

Elucidating the mechanism by which GLP-1 modulates glucagon secretion from the pancreatic alpha cell is fraught with several challenges, one of which is methodological. Currently there is an urgent need for a more robust detection system for many of the hormones and receptors involved in understanding the mechanisms of action of incretins.

In particular, although it has been more than 50 years since the glucagon radioimmunoassay (RIA) was developed, in general this field is still in its developmental stage and refinement of a robust and specific detection/assay system for glucagon, GLP-1 and GLP1R is urgently needed. Current limitations on methodological approaches, namely sensitivity, specificity, reproducibility and reliability, may be contributing to different and often conflicting observations reported in the

literature. To tackle this urgency, there is a current NIH call for the improvement of particular methods to help address such issues and a few such methods will be discussed here.

Development of specific methods for GLP1R detection

As mentioned previously, many of the controversies surrounding GLP-1-mediated glucagon suppression to date have stemmed from inconclusive assessment of GLP1R expression. Although variations may arise from different experimental approaches, there is also a certain amount of concern regarding reliability of commercially-available detection methods. For example, pre-validated antisera detected non-specific bands when used on lysates from GLP1R knockout mice whilst utilisation on lysates overexpressing GLP1R failed to detect any immunoreactivity (17). In a more recent study, three commercially available GLP1R antibodies were used to determine GLP1R expression in positive (normal mouse lung) and negative (mouse lung from GLP1R knockout mice) control samples. The authors were not able to confirm the specificity or sensitivity of any of these antibodies (17). Therefore there is clearly a need to develop more extensively validated detection methods for the GLP1R before we can fully understand its true expression profile. Although Pyke and colleagues have taken steps forward with the generation of their monoclonal antibody this is likely to be an extremely challenging task due to the high levels of homology between GPCRs and their low cellular density (16).

Developing More Specific Antibodies for GLP-1 and Glucagon

One of the key challenges facing researchers at present stems from the fact that both GLP-1 and glucagon are synthesised from the same precursor protein, proglucagon. This 158 amino acid peptide is synthesised in both intestinal L cells and the pancreatic alpha-cells. However, cell-specific cleavage events generate protein profiles unique to each cell type (7). For example, cleavage by prohormone convertase 2 (PC2) in alpha cells generates glucagon and major proglucagon fragment (MPGF) whilst predominant cleavage by PC1/3 in intestinal L cells generates GLP-1, GLP-2 and Glicentin, which can itself be processed to GRPP and oxyntomodulin (Figure 3).

Glucagon and GLP-1 are often detected in human serum or plasma. L cells and alpha-cells are able to process the proglucagon peptide in tissue-specific manners before secretion. However, the

generation of multiple proteins from one precursor peptide dictates the need for careful antibody selection to minimise cross-reactivity. Accurate quantification of plasma glucagon levels may be determined by antibodies targeted to the N-terminus (amino acid 33) or to the C-terminus (amino acid 61). Unfortunately N-terminal glucagon antibodies may also detect intestinally-produced oxyntomodulin whilst C-terminal glucagon antibodies can detect proglucagon 1-61. Similarly with GLP-1, antibodies directed to the N-terminus may bind to MPGF secreted from the alpha-cells whilst C-terminal antibodies will not be able to distinguish between the active (7-36) and inactive (9-36) forms of the metabolite. Therefore antibodies targeted to one end of the peptide are unlikely to generate specific results when testing in plasma. One strategy would be to develop reliable and reproducible sandwich assays based on antibodies targeting both ends of the peptides.

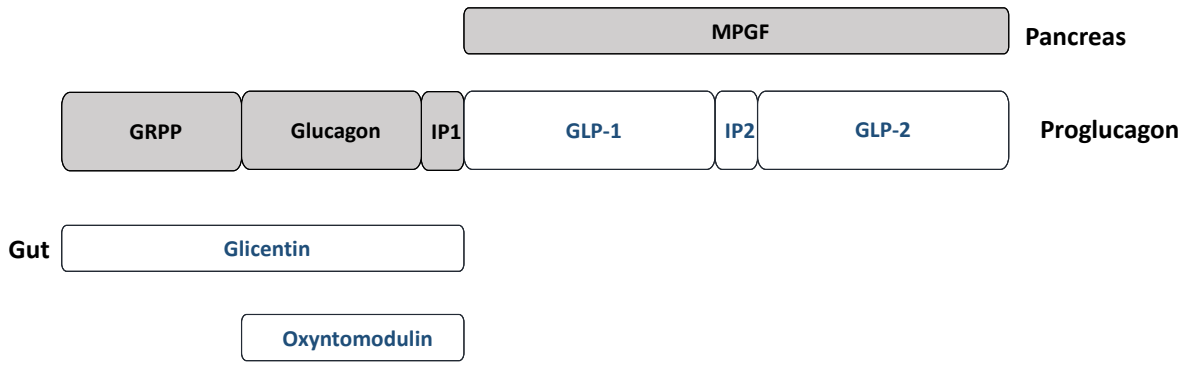

Figure 3. Structure of the proglucagon gene and tissue specific expression profiles. Within the pancreas, processing of the proglucagon gene by prohormone convertase 2 leads to the generation of glicentin-related polypeptide (GRPP), glucagon and the major proglucagon fragment (MPGF). Alternatively, processing within the gut by prohormone convertases 1/3 leads to the generation of GLP-1, GLP-2 and Glicentin, which can itself be cleaved to form oxyntomodulin.

Rodent Islets versus Human Islets

To date, much of the research into GLP-1-mediated glucagon suppression has been performed in rodent islets due to scarcity of human tissue. However, it is now well established that there are a number of key differences between rodent and human islets. For example, human islets contain fewer beta-cells (55% of total cells) compared to rodent islets (77%), but in contrast possess a higher percentage of alpha-cells (21). Not only do the two species contain varying proportions of endocrine cells but their architecture has also been found to differ greatly. Thus, rodent islets contain a core of beta-cells with an alpha-cell mantle, whilst human islets exhibit a trilaminar plate configuration. In humans, beta-cells exist between two enriched alpha cell layers, but this structure is able to fold into a configuration in which almost all endocrine cells are in contact with the microvasculature,

maintaining both homologous beta-cell interactions and heterologous alpha-beta cell connections (22). In addition, a number of components of key signalling cascades have been shown to differ between the two species. Thus, the threshold for glucose-induced stimulation of insulin secretion is as low as ~3 mM in human islets whereas glucose stimulation first becomes detectable at 6 mM in mouse islets. There are also many differences in the complement of voltage-gated ion channels and their density between human and rodent islets (reviewed in (23)). These significant differences in the composition, structure and function of islets indicate that they are regulated differently and it is important to consider these confounding factors when directly extrapolating data from one species to another. With a view to understanding the pathology to develop novel therapies of clinical diabetes it is therefore imperative that observations made in animal models are validated in human studies.

Introduction to Major Gaps in the Field

Despite global efforts to elucidate the role of GLP-1 on glucagon release, there remain several major gaps in this field.

Gap #1: Is there a second receptor for GLP-1?

As discussed in this chapter, a vast amount of work has been performed to elucidate the expression of the GLP1R in pancreatic alpha-cells. Despite evidence clearly demonstrating a direct role of GLP-1 on alpha cells, current highly-sensitive techniques have shown receptor expression levels to be extremely low within these cells, totalling less than 1% of that observed in the pancreatic beta cell. This phenomenon is also observed in other tissues throughout the body. For example, there is clear evidence of GLP-1-mediated effects on the liver, skeletal muscle and adipose tissue and yet localisation of the GLP1R in these tissues has remained inconclusive. This begs the question as to whether there may be an additional receptor via which GLP-1 is able to mediate its actions. GLP1R belongs to the glucagon receptor family of GPCRs. These receptors share similar conformations and are activated by ligands which are all derived from the same proglucagon precursor protein. It may therefore tempting to speculate that a certain degree of crosstalk exists between these structurally-similar ligands and receptors. In fact, one protein produced from the proglucagon precursor, oxyntomodulin, has already been shown to act as a partial agonist at the GLP1R (24). Exploration of

additional receptors for the GLP-1 ligand in future may provide additional insights into its mechanism of action on pancreatic alpha-cells.

Gap #2: Evidence of a local GLP-1 system in pancreatic islets?

One of the most striking aspects of GLP-1 is that only a small proportion of the active peptide secreted from the intestinal L-cells is able to reach the pancreas, and yet GLP-1 has considerable functional activity on islets in vivo. This has led many groups to speculate as to whether there may be additional synthesis of GLP-1 locally within the islets which could act in an autocrine manner to modulate islet function. A recent study by Marchetti and colleagues has used a combination of functional, molecular and immunohistochemical methods to address this question in human islets (25). They have convincingly shown that a small percentage (25%) of glucagon-positive cells co-stained with GLP-1 and that intact islets were able to secrete GLP-1 in response to a high glucose challenge. Islet GLP-1 content constituted a surprisingly large fraction of glucagon (25%) but it remains to be determined whether this represents biologically-active GLP-1 or whether this signal is due to unspecific interaction with proglucagon components (it remains possible that the antigenic epitope is recognised by the GLP-1 antibody). Moreover, when condition media containing secreted GLP-1 was applied to an islet preparation in the presence of 11mM glucose, GSIS was enhanced, an effect which could be blocked by exendin 9-39. Importantly GLP-1 did not localise to any insulin or somatostatin-positive cells which suggests that the local GLP-1 islet production is restricted to alpha cells (25). However, the molecular mechanisms underlying GLP-1 synthesis and secretion in alpha cells remain to be elucidated. In the future it will be important to determine how the proglucagon precursor peptide is processed to generate both GLP-1 and glucagon and whether these hormones, with opposing biological functions, are packaged into distinct granules secreted in response to different stimuli. Moreover, it will also be pertinent to determine whether islets are able to synthesise and secrete dipeptidyl peptidase 4 (DPP-IV), the enzyme responsible for the cleavage of active GLP-1, and elucidate the role and activity of this enzyme in the islet milieu.

Gap #3: Is there a role for GLP-1(9-36) in physiology?

The active form of GLP-1, GLP-1 (7-36), has an exceptionally short half-life. Within minutes of entering the systemic circulation, GLP-1 (7-36) is rapidly degraded to GLP-1 (9-36) by the enzyme DPP-IV. Given that this breakdown product accounts for approximately 80% of systemic GLP-1, it has been argued whether this metabolite has any biological significance. Interestingly however, in

healthy human subjects, administration of GLP-1 (9-36) was shown to have no effect on insulin response to glucose, glucose disappearance rate or glucose effectiveness in comparison to the active form of the incretin which, in contrast, elevated each of these glucose homeostatic parameters (26). These findings have been replicated by numerous groups and consequently GLP-1 (9-36) has not merited any in-depth investigation as a potential regulator of glucose homeostasis and has since been deemed a 'biologically-inert' metabolite.

However, there is now emerging evidence from more recent studies suggesting that GLP-1 (9-36) may be associated with a number of biological roles. Thus, intravenous administration of GLP-1 (9-36) to anaesthetised pigs was shown to enhance glucose disposal by a mechanism independent of insulin secretion. Although the authors were unable to define the precise mechanism by which the effects were mediated, the study clearly demonstrated the effectiveness of GLP-1 (9-36) to act as an anti-hyperglycaemic hormone (reviewed in (7)). Studies have also been performed to elucidate the effects of GLP-1 (9-36) on peripheral tissues. During a euglycaemic clamp, administration of GLP-1 (9-36) to obese subjects resulted in the need for glucose infusion as a result of a 50% inhibition of hepatic glucose production (27). To further investigate the effects of GLP-1 (9-36) on the liver, and to rule out a direct effect of insulin on gluconeogenesis, an additional study was performed in which isolated mouse hepatocytes were exposed to GLP-1 (9-36) ex vivo. In the absence of insulin, the metabolite was still able to suppress gluconeogenesis in hepatocytes confirming a biological role for the peptide (28). In addition, recent studies have also identified a role for GLP-1 (9-36) in cardiac function. GLP-1 (9-36) is able to act as a vasodilatory agent evoking cardioprotective effects in hearts isolated from GLP1R knockout mice and application of a DPP-IV inhibitor exhibited suppression of these cardioprotective effects (29) .

Therefore, in contrast to earlier reports, there is sufficient compelling evidence to redeem GLP-1 (9-36) as physiologically relevant. Consistent with the earlier study, we have confirmed that GLP-1 (9-36) does not affect glucose-induced insulin secretion but our preliminary data suggests that it is able to inhibit glucagon secretion in human islets with an efficacy as high as that of GLP-1(7-36) (Ramracheya et al., unpublished). Although the molecular mechanisms by which this metabolite can suppress glucagon secretion remain to be established, fully understanding its role in glucagon secretion may lead to improved development of anti-diabetic agents. This, however, raises the argument that, if the breakdown product is essential for regulating glucagon levels, what will be the potential implications of DPP-IV inhibition therapy on its role?

Conclusion

GLP-1 is able to directly suppress the secretion of glucagon in-vivo making it a useful therapeutic treatment strategy. However despite years of research, there remains a lack of consensus on the underlying mechanisms of GLP-1 action on alpha-cells still. Many of the conflicting reports in the literature are likely to have arisen due to the difficulties in assaying this highly homologous protein and the utilisation of non-specific antibodies. In the future, with the advent of more highly-sensitive methodologies, it is hoped that these fundamental questions will be addressed. In addition, further characterisation of the local islet production of GLP-1 and biological significance of GLP-1 (9-36) may lead to the development of novel treatment strategies for hyperglycaemia.

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