Alpha cell function in type 1 diabetes

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Abstract

Our understanding of the pathogenesis of type 1 diabetes mellitus has traditionally revolved around the insulin deficiency that follows pancreatic beta cell loss. However, there is an increasing appreciation of defects in other glucoregulatory cells in type 1 diabetes mellitus. Oversecretion of glucagon from pancreatic alpha cells is characteristic of type 1 diabetes mellitus, and modulating these glucagon levels reduces hyperglycaemia. This article reviews alpha cell function in type 1 diabetes mellitus. We examine how its function is controlled and compromised, and review studies that target alpha cell function. Finally, we explore potential approaches to modulating alpha cell function in type 1 diabetes mellitus.

Br J Diabetes Vasc Dis 2014;**14**:45-51

Key words: alpha cell, glucagon, siabetes, type 1, islets of Langerhans, DPP-4, GLP-1, insulin

Introduction

Alpha cells comprise less than 1% of the pancreatic volume and normally about 30% of the pancreatic islets. They are defined immunohistochemically by the presence of glucagon staining, and secretion of glucagon is considered to be their primary role. The glucagon secreting alpha cell and insulin secreting beta cell share a common cell lineage, but differ in their terminal differentiation into their characteristic cell types.[1]

Alpha cells remind us of the enormous contribution that students make to scientific discovery. In 1869, Paul Langerhans identified an island of clear cells in the pancreas whilst still a 22 year old medical student. These were later named the islets of Langerhans and are now known to contain alpha cells. In 1907 another medical student, Michael Lane, discovered there were two distinct cell types in the islets, which he went on to term the 'alpha' and 'beta' cell.[2] Charles Best, whilst working as a medical student for Dr Fredrick Banting, noted that the injection of crude canine pancreatic extracts to diabetic dogs resulted in an early rise in blood glucose, before subsequent hypoglycaemia. Finally, in 1923 Charles Kimball, a biochemistry student, isolated a substance from the alpha cell and named it "glucagon".[3] Despite such a significant discovery, he never received his PhD from the University of Rochester.

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http://dx.doi.org/10.15277/bjdvd.2014.014

Abbreviations and acronyms

DPP	dipeptidyl peptidase
GIP	glucose-dependent insulinotropic peptide
GLP	glucagon-like peptide
HbA1c	glycated haemoglobin
HENAMI	hepato-enteral, neuronal, adipo-muscular, islets of Langerhans
IL-6	interleukin-6
PC	pro-hormorne convertase
PYY	peptide YY

Physiology of alpha cells

Glucagon and glucose regulation

Glucagon increases blood glucose. It does so through targeting a 62kDa G-protein coupled receptor that is expressed predominantly in the liver and kidney, and to a lesser extent in adipose tissue, spleen, heart and gastro-intestinal tract.[4] Stimulation of the glucagon receptor results in glycogenolysis and gluconeogenesis, and suppression of glycogenesis and glycolysis. Both glycogenolysis of the liver and muscle glycogen stores and gluconeogenesis of non-carbohydrate stores release glucose (Figure 1).

Molecular biology of glucagon

The proglucagon gene was identified in 1983, four years after the insulin gene was sequenced. The proglucagon gene encodes for glucagon and GLP-1 and 2 respectively (Figure 2). The proglucagon gene is expressed by pancreatic alpha cells, intestinal L-cells as well as some neuronal cells.[5,6] These cells express

Figure 1. Glycogen and glucose homoeostasis

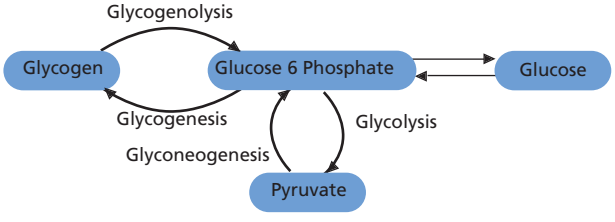
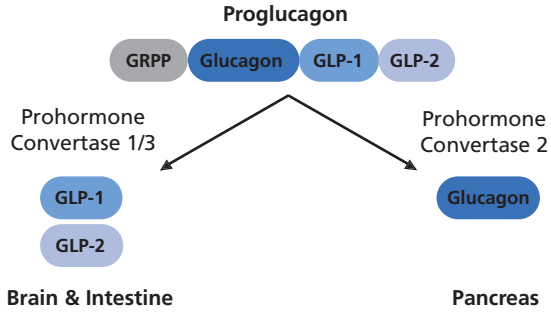


Figure 2. Post-translational processing of the proglucagon peptide. The proglucagon peptide is processed by PC2 in the pancreas to generate glucagon, and by PC1/3 in the gut and nervous system to generate GLP-1 and GLP-2.



different isoforms of the enzyme pro-hormorne convertase (PC) and therefore differentially processes the gene product. PC1/3 is predominantly expressed in the intestinal L-cells and cleaves proglucagon to GLP-1 and GLP-2. PC2 is predominantly expressed in pancreatic alpha cells and the glucagon secreting enteroendocrine cells of the stomach, and cleaves proglucagon to glucagon.[7]

The proglucagon peptide is processed by PC2 in the pancreas to generate glucagon, and by PC1/3 in the gut and nervous system to generate GLP-1 and GLP-2.

Control of glucagon secretion

In health, a rise in blood glucose activates both a glucose dependent and independent inhibition of glucagon secretion from alpha cells. These pathways control blood glucose and regulate the storage and utilisation of energy.

Support for a direct glucose dependent pathway for modulating alpha cell-glucagon secretion comes from observations

Figure 3. The HENAMI – (hepato-enteral, neuronal, adipo-muscular, islet) hormonal network for the control of glucagon secretion

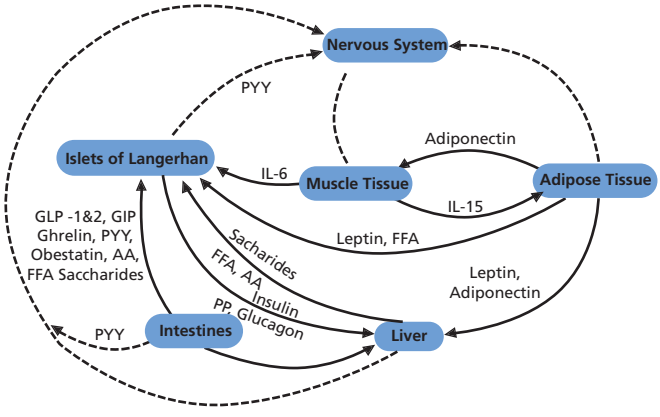
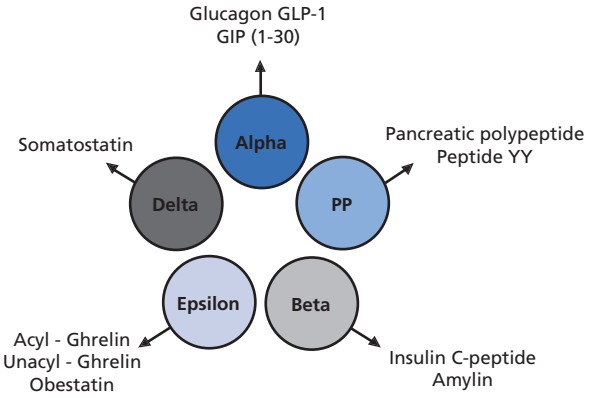


Figure 4. Cells of the islets of Langerhans



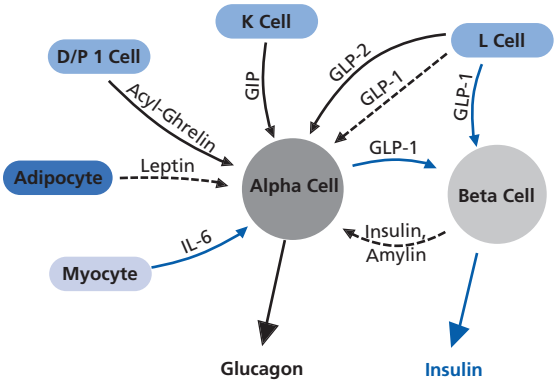
that these cells express ATP regulated potassium channels and glucokinase – both required for glucose sensing.[8] The exact pathways through which glucose modulates glucagon secretion remain to be elucidated. However isolated alpha cells are able to alter their metabolism in response to changes in glucose, and this is associated with changes in cell membrane potential, calcium influx, and exocytosis of glucagon containing secretory granules.[9,10]

The glucose independent pathway for modulating glucagon secretion involves a network of the liver, intestine, nervous, muscular and adipose tissue. We term this the HENAMI network (Figure 3). The islets of Langerhans are a key part of this network and are composed of five distinct types of endocrine cells (see Figure 4).[11-13]

Within the islets, beta cells exert a major paracrine effect (Figure 5) which mediates through insulin, zinc and / or amylin. Insulin directly suppresses glucagon secretion as do amylin and zinc, both co-secreted with insulin.[14]

Alpha cells express somatostatin receptors. Somatostatin is

Figure 5. Paracrine and endocrine control of glucagon secretion by the alpha cell



an inhibitory hormone on somatotrophs and beta cells, and decreases glucagon secretion.[15]

Intestinal L cells secrete GLP-1. GLP-1 inhibits glucagon release from the alpha cells and potentiates glucose induced insulin secretion.[16] Intestinal K cells secrete GIP, which stimulates glucagon release and also protects beta cells from apoptosis.[17] In healthy humans, GIP administration results in dose-dependent glucagon secretion.[18]

The adipocyte hormone leptin inhibits glucagon release and is associated with satiety.[19] Leptin is closely related to IL-6 that is released from muscle tissue in response to vigorous exercise.[20] IL-6 can also stimulate alpha cells to secrete low levels of GLP-1, which stimulates beta cell secretion of insulin and has anti-apoptotic effects on the beta cell.[21] The islets are also heavily innervated by both sympathetic and parasympathetic nerves that are capable of modulating alpha cell function.[22] These effects are mediated via catecholamines, but also through neuropeptides such as neuropeptide Y.

Type 1 diabetes mellitus and the alpha cell

Recent onset of type 1 diabetes mellitus is histo-pathologically characterised by the lymphocytic infiltration of the islets of Langerhans.[23] Histological analysis of pancreata from individuals newly diagnosed with type 1 diabetes mellitus shows specific beta cell loss, with preservation of alpha and other islet cells. Some reports also suggest mild alpha cell hyperplasia at the time of diagnosis.[24]

A primary role for glucagon in the pathology of type 1 diabetes mellitus has been intimated where disruption of glucagon signalling has resulted in protection from diabetes in glucagon receptor knockout mice.[25] In streptozotocin-induced diabetes the severity of the condition is proportional to the number of surviving beta cells and studies have shown that essentially blocking glucagon action improves glycaemic control, thus the mice are protected from diabetes. This is consistent with previous studies of glucagon receptor modulation.[25-28]

The loss of beta cells in type 1 diabetes mellitus and the resulting loss of insulin mediated suppression of glucagon secretion may be expected to result in persistently elevated levels of glucagon. This has been reported in many but not all studies.[29-31] Whilst absolute levels of glucagon may not consistently be elevated, it is clear that these glucagon levels are higher than would be expected for the hyperglycaemia associated with type 1 diabetes mellitus. Furthermore, alpha cell responses to both rising and falling levels of glucose appear compromised in type 1 diabetes mellitus. In health, an increase in glucose, for example following a meal, results in an increase in insulin secretion and either a decrease or no change in glucagon. In type 1 diabetes mellitus, a meal stimulus results in a paradoxical increase in glucagon secretion as demonstrated in children with type 1 diabetes mellitus.[32] The inappropriate increase in meal stimulated glucagon appears to be present at the time of diagnosis, and continues to deteriorate in the following year. It is not clear how longer duration type 1 diabetes mellitus or the presence of complications such as autonomic neuropathy modulates alpha cell behaviour.

The reasons for an inappropriate increase in glucagon in type 1 diabetes mellitus are likely to be multi-factorial. Some studies have reported normal glucagon suppression to an intravenous glucose challenge, whilst an oral challenge results in an inappropriate increase.[33-35] Incretin mediated control of glucagon secretion may therefore be modulated by type 1 diabetes mellitus. It has also been proposed that alpha cells need to sense a rise in insulin to decrease their glucagon secretion, and this rise will not be present in type 1 diabetes mellitus.[36] However studies from type 2 diabetes mellitus suggest the impaired ability of the alpha cell to decrease glucagon secretion in the setting of hyperglycaemia may relate more to defects in the ability of the alpha cell to detect and respond to glucose than the decline in insulin secretion.[37] This is supported by studies of type 1 diabetes mellitus patients early in the natural history of their disease where there are defects in stimulated insulin secretion but not in glucose levels. These patients demonstrate a lack of suppression of glucagon rather than the overt rise detectable in patients later in their natural history.[38] Therefore defects in both the glucose dependent and independent pathways controlling glucagon secretion may contribute to the hyperglycaemia of type 1 diabetes mellitus.

In health, falling glucose triggers glucagon secretion, a phenomenon that is often absent in type 1 diabetes mellitus.[39,40] Furthermore, the greater the loss of beta cell function, the more blunted the glucagon increase with hypoglycaemia.[41,42] This blunted response increases the risk of hypoglycaemia in patients with type 1 diabetes mellitus.

Currently available drugs that target alpha cell function

The GLP-1 pathway

GLP-1 treatment of pancreatic alpha cells reduces glucagon secretion.[43] GLP-1 stimulation of beta cells, and the paracrine effects of the secreted insulin on adjacent alpha cells, also contributes to this reduction in glucagon secretion. Thus, drugs that target the incretin pathway such as GLP-1 agonists and DPP-4 inhibitors exert some of their glycaemic effects through a reduction in endogenous glucagon. These drugs have now established themselves as important agents for the therapy of type 2 diabetes mellitus.

Exploratory trials of the use of GLP-1 modulation in type 1 diabetes mellitus suggest glycaemic benefit (see Table 1).[44-46] Varanasi *et al* showed that the administration of a GLP-1 analogue in individuals with well controlled type 1 diabetes mellitus improved mean fasting glucose and decreased glycaemic excursions. These improvements in glycaemic control were evident after one week of treatment and persisted for the 24-week trial. Kielgast *et al* showed a reduction in HbA1c even in the absence of detectable C-peptide, suggesting that GLP-1 receptor agonism exerts an insulin-independent inhibitory effect on alpha cells. Both these trials showed a reduction in insulin requirements without an increase in hypoglycaemia. There was evidence of weight loss in both studies. Although large trials are under way, GLP-1 analogues are currently unlicensed for use in type 1 diabetes mellitus.

Table 1 Trials of GLP-1 modulation in type 1 diabetes

Study	Number of participants	Study design	Duration of intervention	Intervention	HbA1c(%) effect	Meal stimulated glucagon effect
Varanasi 2011[44]	8 subjects	Open-labelled, cohort trial	24 weeks	Liraglutide variable dose	Baseline vs 24 weeks: 6.5 vs 6.1, p<0.05	N/A
Kielgast 2011[45]	29 subjects	Open-labelled, cohort trial	4 weeks	Liraglutide 1.2mg od	Baseline vs 4 weeks: C-peptide +ve on liraglutide (n=10) 6.6+/-0.3 vs 6.4+/-0.3, p<0.05; C-peptide -ve on liraglutide (n= 9) 7.5+/-0.2 vs 7.0+/-0.1, p<0.05; C-peptide -ve not on liraglutide (n=10) 7.1+/-0.3 vs 6.9+/-0.2, p>0.05	N/A
Raman 2010[46]	8 subjects	Double-blinded, randomized, placebo controlled trial.	Single dose	Exenatide 1.25μg – 2.5μg or placebo	N/A	Exenatide vs placebo p>0.05

Table 2 Trials of DPP-4 inhibitors in type 1 diabetes

Study	Number of participants	Study design	Duration of intervention	Intervention	HbA1c(%) effect	Meal stimulated glucagon effect
Garg 2012[47]	125 subjects	Double-blind, randomized placebo-controlled parallel trial.	16 weeks	Sitagliptin 100mg od or placebo.	Change from baseline: sitagliptin 100mg vs placebo; -0.07 +/- 0.7 vs -0.12 +/- 0.75, p>0.05	4 hrs AUC (pg/ml.min) sitagliptin vs placebo: 84.7 ± 23.6 vs 84.9 ± 35.3, p>0.05
Farngren 2012[48]	28 subjects	Double-blind, randomized, placebo-controlled crossover trial.	4 weeks	Vildagliptin 50mg bd or placebo.	Difference between 4 weeks: vildagliptin vs placebo; -0.32 +/- 0.09%, p<0.005	2hr AUC (nmol/l.min) vildagliptin vs placebo: 2.4 +/- 0.2 vs. 2.6 +/- 0.2, p<0.05
Ellis 2011[49]	20 subjects	Double-blind, randomized, placebo-controlled crossover trial.	4 weeks	Sitagliptin 100mg od or placebo.	Difference between 4 weeks: sitagliptin vs placebo; -0.27 +/- 0.11%, p<0.05	N/A
Foley 2008[50]	12 subjects	Double blind, randomised, placebo-controlled, crossover trial.	4 weeks	Vildagliptin 100mg bd or placebo.	N/A	1hr AUC (mcg/l.min) vildagliptin vs placebo: -0.2+/- 0.6 vs 1.9+/- 0.6, p<0.05

AUC = area under the curve; N/A = not applicable

DPP-4 inhibitors

DPP-4 is an enzyme that degrades endogenous GLP-1, and inhibiting DPP-4 potentiates the incretin pathways. These drugs are well established for therapy in type 2 diabetes mellitus but are currently unlicensed for type 1 diabetes mellitus. However, recent studies show promise for this class of drug in treating type 1 diabetes mellitus. A four week study of vildagliptin treatment in 28 participants with type 1 diabetes mellitus successfully reduced their meal stimulated glucagon levels. A 16-week intervention study of sitagliptin in 125 participants with type 1 diabetes mellitus showed not only improvement in glucagon levels, but also a reduction in HbA1c (see Table 2).[47-50] Whilst larger trials are awaited, this class of therapy remains unlicensed for type 1 diabetes mellitus.

Amylin

Pramlintide is an amylin analogue licensed in the USA as an adjunct therapy to insulin in type 1 diabetes mellitus and type 2 diabetes mellitus. Amylin is co-secreted with insulin from beta cells, and is therefore absent in long standing type 1 diabetes mellitus. In a randomised controlled trial pramlintide 60g given three or four times a day for a year decreased HbA1c by ~0.3%, but another randomised controlled trial was unable to detect any benefit at 6 months (Table 3). Pramlintide can significantly decrease post-meal glucagon levels in type 1 diabetes mellitus and trials have also shown that pramlintide reduces glucose variability by delaying gastric emptying.[51-55]

Table 3 Trials of the amylin analogue pramlintide in type 1 diabetes mellitus

Study	Number of participants	Study design	Duration of intervention	Intervention	HbA1c(%) effect	Meal stimulated glucagon effect
Chase 2009[51]	9 subjects	Single blinded, randomised placebo controlled, crossover trial.	Single dose	Pramlintide 15μg or pramlintide 30μg or placebo	N/A	3hr AUC (pg/ml.hr) pramlintide 15mcg vs pramlintide 30mcg vs placebo: 4+/-7 vs 5+/-7 vs 35+/-9
Edelman 2006[52]	230 subjects	Double-blinded, randomized, placebo controlled trial.	29 weeks	Pramlintide 30-60μg tds or placebo	Change from baseline: pramlintide vs placebo; -0.5% vs -0.5%, p>0.05	N/A
Ratner 2004[53]	304 subjects	Double-blinded, randomized, placebo controlled trial.	52 weeks	Pramlintide 60μg tds or pramlintide 60μg od or placebo	Change from baseline: pramlintide tds vs pramlintide od vs placebo; -0.29% vs -0.34% vs -0.04%, p<0.05	N/A
Levetan 2003[54]	18 subjects	Open-labelled, cohort trial.	4 weeks	Pramlintide 30μg tds	N/A	3hr AUC (pg/ml.hr) baseline vs 4 weeks: 55+/-44 vs 7+/-38, p<0.05
Whitehouse 2002[55]	342 subjects	Double-blinded, randomized, placebo controlled study	52 weeks	Pramlintide 30-60μg od or placebo	Change from baseline: pramlintide vs placebo; -0.39% vs -0.12%, p<0.01	N/A

AUC = area under the curve; N/A = not applicable

Novel drugs that target the glucagon pathway and alpha cell function

An exciting potential for modulating the glucagon pathway lies in therapies that act directly on glucagon receptor signalling. As previously described, disruption of glucagon signalling ameliorates the hyperglycaemia of insulin deficient diabetes in animal studies. It was originally shown by Peter Flatt in 1979 that administration of glucagon antibodies modulated glucose levels[56] – another first for a student - and more recently administration of glucagon receptor blocking antibodies to non-diabetic monkeys reduced blood glucose level in a dose dependent manner.[57] The antibody was also given in higher doses to ob/ob mice and was successful in suppressing their hyperglycaemia.[58] Early clinical trials in patients with type 2 diabetes mellitus show promise.[59,60] Therefore therapies that modulate glucagon receptor signalling could potentially be used to treat the hyperglycaemia of type 1 diabetes mellitus. Potential limitations to this approach are early findings from studies in rodents that glucagon receptor modulation is associated with islet hyperplasia and disturbance in lipid metabolism in rodent studies,[61] clearly issues that need further investigation.

Novel dual GLP-1/glucagon receptor and GLP-1/GIP receptor targeted peptides have been shown to treat hyperglycaemia in mice.[62-64] In animal studies, circulating glucagon is decreased following therapy with ghrelin antagonists, and with leptin agonists.[65,66] These therefore have therapeutic potential, and the leptin analogue metreleptin has now entered phase 1 clinical trials in patients with type 1 diabetes mellitus.[67]

A group of novel drugs that target the GPR119 receptor have shown promise in protecting mouse alpha and beta cells from streptozotocin-induced apoptosis[68] and may therefore improve glycaemic control through preservation of these cells.

Tocilizumab, an IL-6 receptor monoclonal antibody, improved glycaemic control when given for the therapy of rheumatoid arthritis in patients with type 2 diabetes mellitus.[69] Acute changes in IL-6 may act through increasing gut expression of GLP-1. Thus, there may be future scope in using the IL-6 receptor in regulating alpha cell secretion of glucagon and improving glycaemic variability.

Conclusion

Type 1 diabetes mellitus is a chronic autoimmune disease associated with a significant risk of long-term vascular complications. The risk of these complications is reduced by good glucose control. Functioning alpha cells as well as functioning beta cells are key to glycaemic homeostasis. The loss of beta cell function in type 1 diabetes mellitus results in unrestrained hyperglucagonaemia and thus to hyperglycaemia.

There are significant aspects of glucagon physiology that remain to be understood. In particular, the natural history of alpha cell function in type 1 diabetes mellitus needs to be fully eluci-

Key messages

- Glucagon secretion is controlled by glucose plus a 'network' of hormones, and plays a critical role in the regulation of blood glucose
- GLP-1 agonists, DPP-4 inhibitors and amylin analogues modulate glucagon levels and have therapeutic potential in type 1 diabetes mellitus
- Novel agents are in development that directly influence glucagon receptor signalling.

dated. Meanwhile, a number of agents that modulate alpha cell function show therapeutic promise for type 1 diabetes mellitus. Currently, DPP-4 inhibitors and GLP-1 agonists utilised in type 2 diabetes mellitus may improve glycaemia in type 1 diabetes mellitus. They require large clinical trials to confirm safety and efficacy to examine their potential for routine clinical use.

Conflict of interest None
Funding sources None
Contributions DSH performed the literature search and wrote most of the article. PN reviewed and redrafted the article and supervised DSH.
Acknowledgements We thank Prof Patrik Rorsman and Dr Reshma Ramracheya at Oxford University for their invaluable help in putting this article together.

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