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REVIEW

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Alpha cell function in health and disease: influence of glucagon-like peptide-1

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Abstract Although there is abundant evidence that hyperglucagonaemia plays a key role in the development of hyperglycaemia in type 2 diabetes, efforts to understand and correct this abnormality have been overshadowed by the emphasis on insulin secretion and action. However, recognition that the incretin hormone glucagon-like peptide-1 (GLP-1) exerts opposing effects on glucagon and insulin secretion has revived interest in glucagon, the neglected partner of insulin, in the bihormonal hypothesis. In healthy subjects, glucagon secretion is regulated by a variety of nutrient, neural and hormonal factors, the most important of which is glucose. The defect in alpha cell function that occurs in type 2 diabetes reflects impaired glucose sensing. GLP-1 inhibits glucagon secretion in vitro and in vivo in experimental animals, and suppresses glucagon release in a glucose-dependent manner in healthy subjects. This effect is also evident in diabetic patients, but GLP-1 does not inhibit glucagon release in response to hypoglycaemia, and may even enhance it. Early clinical studies with agents acting through GLP-1 signalling mechanisms (e.g. exenatide, liraglutide and vildagliptin) suggest that GLP-1 can improve alpha cell glucose sensing in patients with type 2 diabetes. Therapeutic approaches based around GLP-1 have the potential to improve both alpha cell and beta cell function, and could be of benefit in patients with a broad range of metabolic disorders.

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Abbreviations AGR_{arg}: acute glucagon response to intravenous arginine bolus · CCK: cholecystokinin · DPP-4: dipeptidyl peptidase IV · GIP: glucose-dependent insulinotropic polypeptide · GLP-1: glucagon-like peptide-1 · PG_{50}: plasma glucose level at which 50% of the maximal suppression of the AGR_{arg} is observed

Introduction

Thirty years ago, Unger and Orci proposed a 'bihormonal hypothesis' to explain the origin of hyperglycaemia in diabetes [1]. They cited convincing evidence that, in addition to relative or absolute hypoinsulinaemia, relative hyperglucagonaemia is essential in the pathogenesis of diabetes mellitus. Since that time, and despite further evidence implicating alpha cell dysfunction in the development of type 2 diabetes, beta cell function and insulin resistance remain central to most discussions concerning the pathogenesis and treatment of this disorder. However, it should be recognised that the increased 'demand' for insulin imposed by insulin resistance can be countered by reducing glucagon secretion, as well as by increasing the secretion of insulin.

We will review normal alpha cell function and its clinical measurement, and will examine the abnormalities of alpha cell function that occur in type 2 diabetes. The influence of the incretin hormone glucagon-like peptide-1 (GLP-1) on alpha cell function in vitro and in experimental animals, healthy humans and diabetic patients will then be discussed. We conclude with a survey of recent studies describing the acute and chronic actions of potential new therapeutic agents that use GLP-1 signalling mechanisms in the treatment of type 2 diabetes.

Normal alpha cell function

As with the beta cell, regulation of alpha cell function is complex, with a range of nutrient, neural and hormonal influences upon glucagon secretion. Glucose is perhaps the most important physiological regulator of alpha cell function, and profoundly suppresses glucagon secretion. In the perfused rat pancreas, threshold, half-maximal and maximal alpha cell responses to glucose occur at approximately 2.5, 5.0 and 10.0 mmol/l glucose [2]. Following an overnight fast, plasma glucagon rises in humans if glucose falls below a threshold of approximately 3.8 mmol/l [3] and decreases progressively as plasma glucose rises above the normal range [4].

Most amino acids stimulate glucagon release, but the relative potency of individual amino acids varies between species [5]. Arginine is the most effective amino acid in man, and is often employed in provocative tests of both alpha cell and beta cell function [6]. NEFA and ketones also influence glucagon secretion. Although a physiological role of lipids is not established, NEFA and ketones have been reported to suppress glucagon secretion in vitro [7] and administration of lipid has been shown to decrease plasma glucagon levels in man [8].

The autonomic nervous system can exert a profound influence on glucagon secretion, and activation of either branch stimulates glucagon release [9, 10]. As detailed in Table 1, numerous hormones (many of which are also neurotransmitters) also affect glucagon release. Insulin suppresses glucagon secretion via a local endocrine effect [11]; somatostatin, by a paracrine effect [12]. Thus, alpha cells are exposed to very high concentrations of insulin and somatostatin, and there is some evidence to suggest that insulin mediates glucose inhibition of glucagon secretion [13] and that withdrawal of this local insulin 'tone' mediates the effect of hypoglycaemia on glucagon release [14]. There are, however, other mechanisms that influence hypoglycaemic stimulation of glucagon release [15, 16].

Table 1 Factors regulating glucagon secretion

Stimulatory	Inhibitory
Amino acids [5]	Glucose [2]
Sympathetic nerves [9]	NEFA [8]
Parasympathetic nerves [10]	Ketones [7]
Adrenaline (epinephrine) [112]	Insulin [113]
Oxytocin [114]	Somatostatin [115]
Vasopressin [116]	Secretin [117]
GIP [118]	GLP-1 [54]
PACAP [119]	Carbohydrate meal [18]
GRP [120]	
CCK [121]	
VIP [122]	
Protein meal [18]	
Stress [20]	
Hypoglycaemia [3]	

GRP gastrin-releasing peptide; *PACAP* pituitary adenylate cyclase-activating polypeptide; *VIP* vasoactive intestinal polypeptide

Stress hormones such as adrenaline stimulate glucagon secretion, and activation of both branches of the autonomic nervous system contributes to the glucagon response, particularly when hypoglycaemia is profound [17].

Each of the factors listed above act in a coordinated fashion to regulate glucagon secretion. Accordingly, in healthy subjects, glucagon levels increase in response to a high-protein meal and decrease in response to a high-carbohydrate meal or oral glucose [18], thereby minimising fluctuations in plasma glucose levels. During stress (e.g. hypoxia [19] or hyperthermia [20]), where it is advantageous to mobilise fuels, glucagon levels increase and, particularly in the case of hypoglycaemic stress, there are several other mechanisms that elevate glucagon levels [15] and protect against severe and possibly life-threatening consequences.

Glucose and a variety of hormones and substrates act in a coordinated manner to regulate glucagon secretion. Normal alpha cell function serves to protect against hypoglycaemia and to minimise prandial glucose excursions.

Clinical measures of alpha cell function

The development of the insulin RIA introduced an unprecedented degree of specificity and sensitivity for the measurement of insulin [21], and was soon followed by a glucagon RIA [22]. The existence of larger molecular weight species of glucagon-like immunoreactivity of extrapancreatic origin made quantification of pancreatic glucagon difficult until specific antisera such as 30 K allowed the development of sensitive and specific assays for the 3,500 molecular weight species [23]. Even today, pancreatic specific assays yield a rather wide range of 'basal' glucagon values, e.g. 30–60 ng/l (~8.6–17.2 pmol/l) in healthy subjects. This highlights the importance of comparing values only within a single study and using a single assay system. Since many pancreatic-specific assays 'read' a large molecular weight interference factor as glucagon (this is unrelated to glucagon and remains constant [24]), changes from baseline provide a more reliable assessment of in vivo glucagon secretion than basal fasting glucagon levels.

The most commonly used provocative test of alpha cell function involves administration of arginine, usually as an intravenous bolus of 5 g, which results in a maximum stimulation [6, 25]. The acute glucagon response to arginine (AGR_{arg}) is a convenient measure of alpha cell responsiveness and is usually expressed as either the incremental AUC for 10 min or the mean increment above the pre-stimulus baseline from 2–5 min following arginine administration. Although other non-glucose secretagogues, such as isoproterenol, are also used in provocative tests of beta cell function [26], bolus administration of isoproterenol is less effective than arginine in stimulation of acute glucagon release in humans [27]. Acute insulin-induced hypoglycaemia has also been used to assess alpha cell

function in humans [28], although arginine stimulation tests clearly pose less risk and are more routinely employed.

Just as glucose modifies the beta cell response to non-glucose stimuli, it also strongly influences the alpha cell response to other provocative stimuli. Hyperglycaemic clamps using variable-rate glucose infusions can therefore be combined with arginine stimulation tests to characterise alpha cell function more fully. Figure 1 shows results from a study in which the AGR_{arg} was measured at basal glucose and at two levels of hyperglycaemia [25]; the glucagon response to arginine falls as glucose levels are increased.

Assessment of the AGR_{arg} at several glucose levels allows more rigorous definition of the influence of glucose on arginine-stimulated glucagon release, and quantification of alpha cell function can be derived from the data obtained. The minimum AGR_{arg} (seen at plasma glucose levels above ~25 mmol/l in healthy subjects [29]) and the slope of the linear portion of the curve provide information on the responsiveness of the alpha cell to glucose-induced suppression, and the PG_{50}, the glucose level at which half-maximal suppression occurs, is a measure of alpha cell sensitivity to glucose. It should be noted that the PG_{50} may be underestimated if a minimum AGR_{arg} is not achieved at the highest glucose level tested.

Abnormalities of alpha cell function in diabetes

As with beta cell dysfunction in type 2 diabetes, most—and perhaps even all—of the abnormalities of alpha cell function seen in this disorder may be considered to reflect an impairment of glucose sensing. Absolute fasting plasma levels of glucagon may or may not be higher in patients with type 2 diabetes than in non-diabetic subjects, but fasting hyperglucagonaemia is clearly present in the context of ambient glucose levels, and this contributes to the increased rate of hepatic glucose output seen in patients with type 2 diabetes [30–32]. Another manifestation of

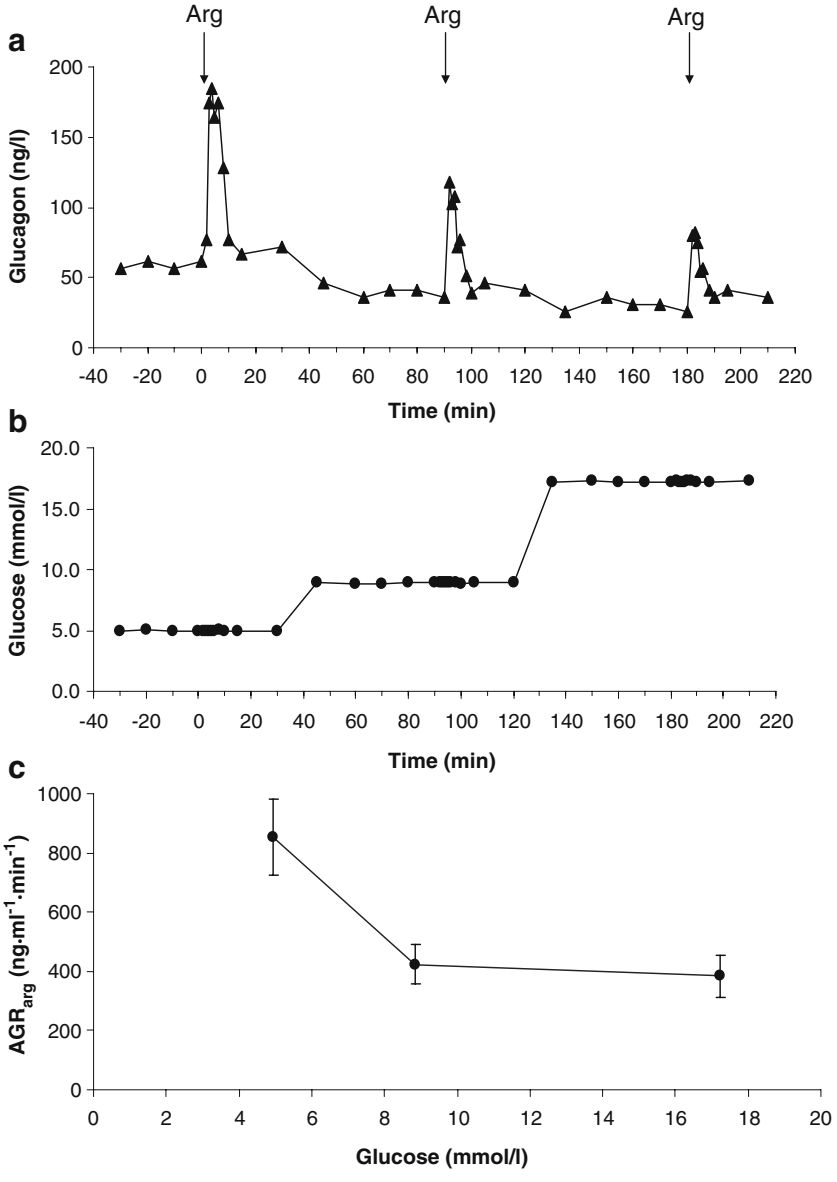

Fig. 1 Plasma levels of glucagon (**a**) and glucose (**b**) and the acute glucagon response to intravenous arginine bolus (AGR_{arg}=10 min AUC) (**c**) during stepped hyperglycaemic clamps in healthy volunteers. The data in the upper two panels are from a single representative subject, while the data in the bottom panel are presented as means±SEM, *n*=16. Adapted from [25], Copyright 1982 American Diabetes Association. From Diabetes, Vol. 31, 1982; 489–495. Reprinted with permission from the American Diabetes Association

impaired glucose sensing by the alpha cell is the loss of hyperglycaemia-induced suppression of glucagon release. The magnitude of the reduction in plasma glucagon levels in response to glucose infusion [33], an oral glucose load [34] or a high carbohydrate meal [18] is considerably less in patients with type 2 diabetes than in those with NGT. In fact, as illustrated in Fig. 2, glucose may elicit a paradoxical stimulation of glucagon secretion in diabetic patients, particularly in those with more advanced disease [34]. It has also been suggested that the alpha cell is resistant to insulin [35]; however, it is difficult to distinguish between impaired glucose sensing and alpha cell insulin resistance.

The glucagon response to the stimulatory effect of arginine is higher in patients with type 2 diabetes than in non-diabetic subjects [36]. However, in view of the concurrent hyperglycaemia, it is difficult to distinguish hyper-responsiveness to arginine from faulty glucose sensing. Indeed, as illustrated in Fig. 3, the plasma glucose level required for half-maximal suppression of the AGR_{arg} is substantially higher in diabetic subjects than in normal, glucose-tolerant subjects, suggesting that the sensitivity of the alpha cell to the suppressive effects of glucose is decreased [29].

One of the most clinically important aspects of alpha cell dysfunction in diabetes is a failure to respond appropriately to hypoglycaemia. A marked impairment is well documented in patients with type 1 diabetes [37] and those with long-standing type 2 diabetes [38], although the degree of impairment in patients with 'mild' type 2 diabetes is controversial [39], as are the relative contributions of altered pancreatic glucose sensing and autonomic failure [40]. Nonetheless, impaired alpha cell glucose sensing can result in inadequate glucagon secretion in response to decreases in plasma glucose, and thereby increased risk of severe hypoglycaemia, and in excessive glucagon secretion in the fasting [41] and postprandial states [42], which contribute to the development and progression of hyperglycaemia in type 2 diabetes.

Many of the manifestations of alpha cell dysfunction described above have been reported in subjects with IGT [6, 43, 44]. Furthermore, a prospective study of postmenopausal women found that the AGR_{arg} at a clamped plasma glucose of 14 mmol/l was an independent predictor of glucose intolerance [45]. Such findings strongly support the bihormonal hypothesis [1] and attest to the importance of pancreatic glucagon. Accordingly, when GLP-1 was reported to augment insulin secretion and suppress glucagon in man [46], this newly discovered peptide became the focus of intense and continued research, holding promise for the treatment of type 2 diabetes.

There are clear defects in alpha cell function in patients with type 2 diabetes, including relative glucagon hypersecretion at normal and elevated glucose levels, and often an impaired response to hypoglycaemia. Thus, alpha cell dysfunction in diabetes may be described as inadequate glucose sensing.

GLP-1

GLP-1 is a 30-amino acid peptide produced by L cells in the lower intestine by alternative processing of the gene encoding proglucagon [47]. The structure of GLP-1 was originally deduced from cDNA cloning of the gene encoding preproglucagon (GLP-1 [1–37]). However, GLP-1 [1–37] is inactive, requiring N-terminal truncation of amino acids 1–6 for activation. The biologically active forms of GLP-1 are GLP-1 [7–37] and GLP-1 [7–36] amide, previously known as insulinotropin [48], glucagon-like insulinotropic peptide (GLIP) [49] and truncated GLP-1 [50]. This latter designation is unhelpful, since inactivation of GLP-1 [7–37] and GLP-1 [7–36] amide occurs through further N-terminal truncation by the enzyme dipeptidyl

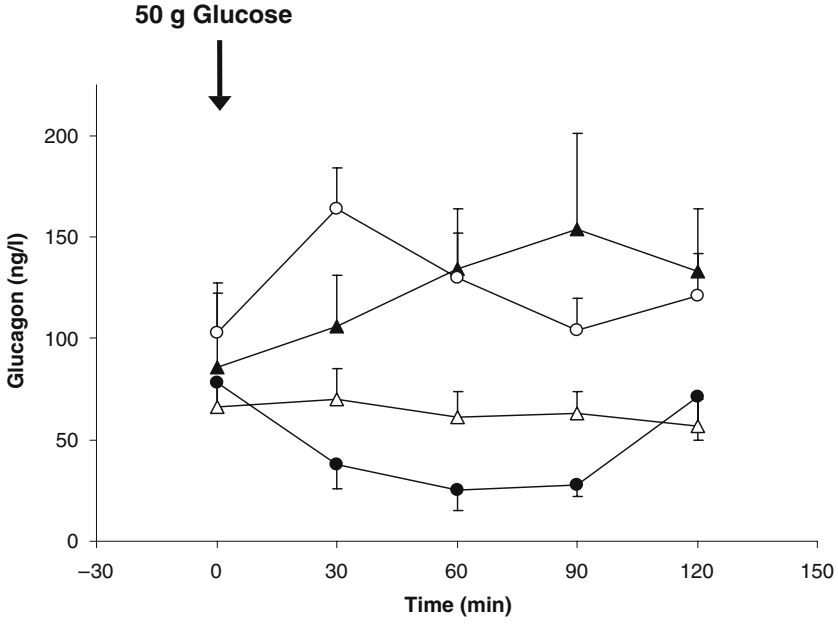

Fig. 2 Plasma glucagon levels during 50 g OGTTs in subjects with NGT (*closed circles*, *n*=6) and patients with type 2 diabetes classified as mild (fasting blood glucose <6.7 mmol/l, *open triangles*, *n*=11), moderate (6.7–11.1 mmol/l, *closed triangles*, *n*=8) or severe (>11.1 mmol/l, *open circles*, *n*=14). The data are presented as means±SEM. Adapted from [34]

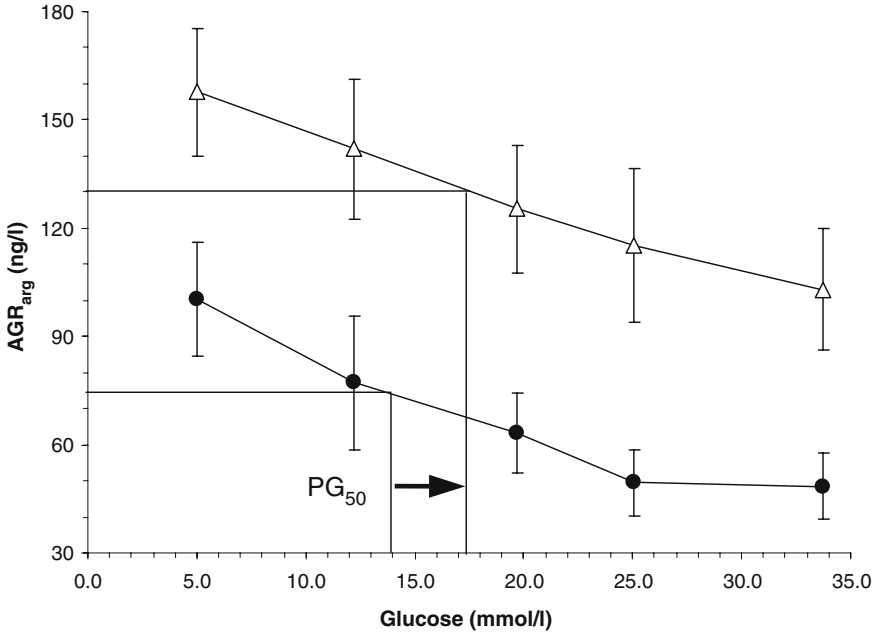

Fig. 3 Acute glucagon response to intravenous arginine bolus (AGR_{arg} = mean level from 2–5 min) during stepped hyperglycaemic clamps in subjects with NGT (*closed circles*) and in patients with type 2 diabetes (*open triangles*). The data are presented as means±SEM, n=8 in each group. Adapted with permission and republished from [29]

peptidase IV (DPP-4), yielding GLP-1 [9–37] and GLP-1 [9–36] amide, which have also been referred to as truncated GLP-1. For the purposes of this paper, and consistent with recommendations arising from the first international symposium on GLP-1, the term GLP-1 will be used to designate either of the biologically active peptides, GLP-1 [7–37] and GLP-1 [7–36] amide.

GLP-1 is released from the gut in response to food intake and its most well-known physiological function is as an incretin [46], i.e. promoting assimilation of ingested nutrients via glucose-dependent stimulation of insulin release. The clinical potential of GLP-1-based therapies has received much attention and is the topic of several recent reviews, which have primarily focused on the beta cell [51–53]. However, GLP-1 also suppresses glucagon release [54], and this action may be as important as its insulinotropic effect in terms of its therapeutic properties.

Effects of GLP-1 on alpha cells in vitro

Although somewhat controversial [55], displaceable binding of GLP-1 to pancreatic cells that test positive for glucagon immunoreactivity has been reported [56], suggesting the existence of GLP-1 receptors on pancreatic alpha cells, and thus a direct effect of GLP-1 on glucagon secretion. Although GLP-1 augments Ca^{2+}-dependent exocytosis in rat pancreatic alpha cells [57], GLP-1 has been shown to inhibit glucagon release from the perfused rat pancreas [58–61], isolated rat islets in static incubations [62], the perfused canine pancreas [60, 63], the perfused porcine pancreas, the perfused porcine nonantral stomach [64] and cultured human islets [65]. The glucagonostatic effect of GLP-1 does not appear to be mediated by a local endocrine effect of insulin, since glucagon release is inhibited even when insulin is unstimulated [61, 64]. This is supported by the observation that GLP-1 also inhibits glucagon release from a glucagon-secreting cell line [66].

The influence of ambient glucose on the effects of GLP-1 on glucagon secretion in vitro is not altogether clear. Many studies use a relatively low glucose level (~3 mmol/l) in the perfusate or culture media, often with arginine or a mixture of amino acids to establish a high baseline secretory rate and thus a broad dynamic range. Under these conditions it has been shown that GLP-1 exerts an inhibitory effect on glucagon release [65, 67]. The effect of GLP-1 on alpha cell glucose sensing in vitro has not been assessed. One study with the GLP-1 agonist exenatide (previously known as exendin-4; a naturally occurring peptide isolated from salivary secretions of *Heloderma suspectum*) did examine glucagon release from the perfused rat pancreas in response to a square wave decrease in glucose level from 11 to 3.2 mmol/l, with or without GLP-1 infusion, and found a small suppressive effect of GLP-1 [68]. However, the alpha cell response to the glucose decrement alone was very modest. It would be of interest to assess the effect of either a glucose decrement on a background of GLP-1 with a perfusion system, or a full glucose dose response with or without co-incubation with GLP-1 in a static system.

In vitro studies show that GLP-1 or agonists can exert powerful inhibitory effects on glucagon secretion. The effects that have been described would generally be of benefit in the treatment of diabetes. More rigorous examination of the influence of GLP-1 on alpha cell glucose sensing will be needed to determine if therapies based on GLP-1 signalling mechanisms will actually improve alpha cell function vis-à-vis suppressing glucagon secretion under all circumstances.

Effects of exogenous and endogenous GLP-1 on alpha cell function in experimental animals

The following sections will discuss the effects of exogenously administered GLP-1 or agonists such as exenatide and liraglutide (previously known as NN2211; a long-acting derivative of GLP-1 that is resistant to the actions of DPP-4) and will examine the effects of DPP-4 inhibitors such as vildagliptin (previously known as LAF237) and valine pyrrolidide, which act by increasing plasma levels of endogenously released GLP-1 and possibly other substrates such as glucose-dependent insulinotropic polypeptide (GIP). These sections will consider information regarding GLP-1 antagonists and neutralising antibodies that may provide information on the physiological role of endogenous GLP-1.

In normal mice fed an unrestricted diet, intravenous administration of GLP-1 had no influence on basal plasma glucagon levels but suppressed the acute stimulatory effect of co-injection of cholecystokinin (CCK) or the cholinergic agonist carbachol [69]. In normal rats, intravenous infusion of GLP-1 in the fed state reduced plasma glucagon, while glucose decreased from 7.3 to 5.6 mmol/l and insulin levels increased markedly but transiently. In contrast, in rats fasted for 48 h, the same rate of GLP-1 infusion increased plasma glucagon, and glucose fell from 5.0 to 4.4 mmol/l following transient stimulation of insulin. Thus, GLP-1 suppressed glucagon during modest hyperglycaemia, but did not inhibit the increase of glucagon in response to incipient hypoglycaemia [70].

In minipigs rendered glucose-intolerant with either nicotinamide or streptozotocin, intravenous injection of the GLP-1 derivative liraglutide was found to exaggerate plasma glucagon suppression induced by the hyperglycaemic clamp; however, glucagon increased promptly during the rapid decrease in glucose levels that followed termination of the clamp. Liraglutide therefore suppressed glucagon secretion during hyperglycaemia in these minipigs with IGT, but did not impair the response to falling glucose [71]. A physiological role of endogenous GLP-1 in the

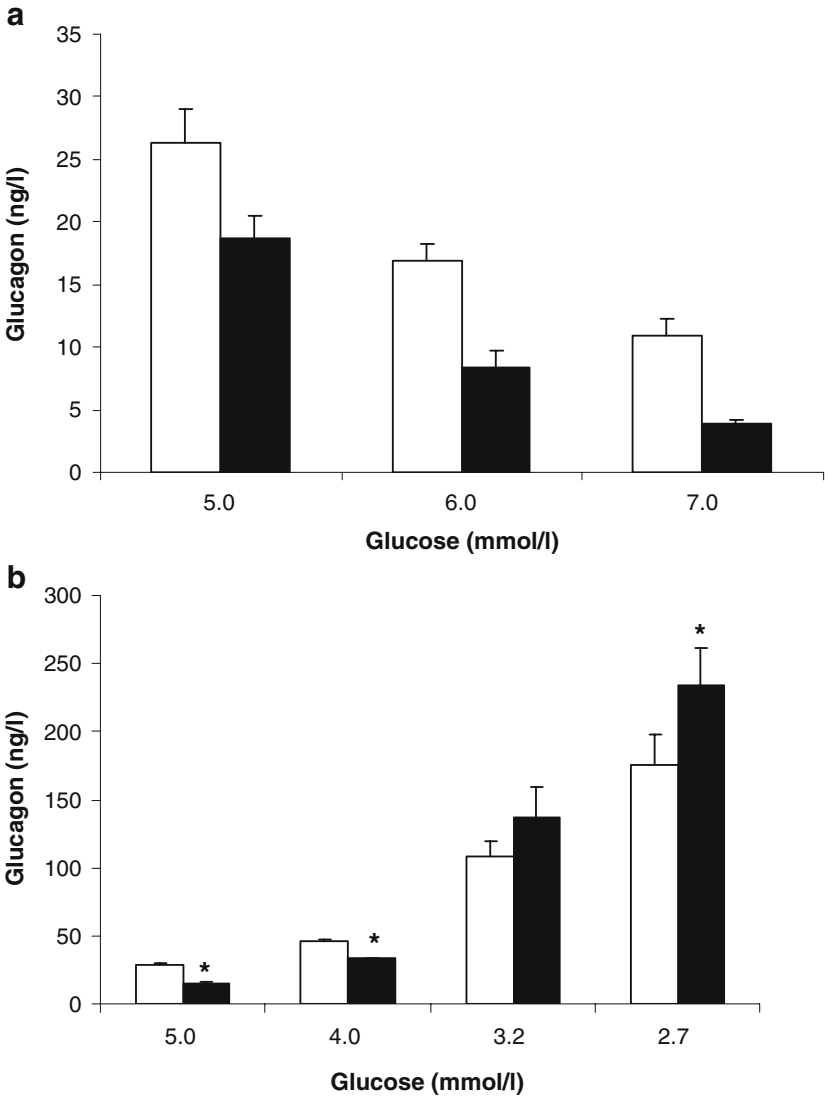

Fig. 4 (**a**) Plasma glucagon levels during stepped hyperglycaemic clamps in healthy volunteers during intravenous infusion of placebo (*open bars*) or GLP-1 (*closed bars*). The data are presented as means± SEM, n=8. Reprinted from [82] with permission from Elsevier. (**b**) Plasma glucagon levels during stepped hypoglycaemic clamps in healthy volunteers during intravenous infusion of placebo (*open bars*) or exendin-4 (*closed bars*). The data are presented as means±SEM, n=11. *p<0.05 vs placebo. Adapted from [83], Copyright 2004 American Diabetes Association. From *Diabetes*, Vol. 53, 2004; 2397–2403. Reprinted with permission from the American Diabetes Association

regulation of alpha cell function may be inferred from a study in healthy baboons using a peptide antagonist (exendin-[9–39]) [72]. In animals fasted overnight, plasma glucagon and glucose levels were higher during infusion with the antagonist than during a control saline infusion. Plasma insulin did not differ significantly between studies, suggesting that endogenous GLP-1 tonically restrains glucagon release.

Many recent studies have explored the effects of DPP-4 inhibition in experimental animals; plasma glucagon was measured in two of these studies. In the first study, 12 weeks of daily treatment with the DPP-4 inhibitor P32/98 did not affect fasting glucagon levels in the Vancouver diabetic fatty rat [73]. In the second study, acute administration of the DPP-4 inhibitor NVP-DPP728 suppressed the glucagon $AUC_{(0–90\ min)}$ by ~70% following a mixed meal in normal dogs. The difference between active and placebo treatment did not reach statistical significance, probably because of low sample size and substantial variability in response [74].

There are surprisingly few reports on the effects of GLP-1 agonists or antagonists on glucagon levels in experimental animals. Although some studies found no effect of GLP-1 on glucagon, most of the limited evidence available is consistent with the concept that GLP-1 improves alpha cell glucose sensing.

Effects of exogenous and endogenous GLP-1 on alpha cell function in healthy humans

From the time of its discovery [75] and the first reports that it could stimulate insulin secretion [48], GLP-1 was considered a candidate incretin hormone. Kreymann et al. studied the potential contributions of GLP-1 and GIP to the incretin effect in healthy volunteers; GLP-1 raised insulin and suppressed basal glucagon when infused to mimic postprandial levels, whereas GIP increased glucagon [46]. Many subsequent studies have measured plasma glucagon, but few have focused on alpha cell function.

Subcutaneous injection of GLP-1 produced dose-related and parallel decreases in fasting plasma glucose and glucagon in healthy individuals [54]. GLP-1 infusion suppressed glucagon levels by ~28% during glucose infusion to maintain steady-state euglycaemia. Insulin infusion in the same volunteers during steady-state euglycaemia had a less suppressive effect, suggesting that the effects of GLP-1 on plasma glucagon levels are not mediated exclusively by insulin; however, it is not clear whether local insulin levels were similar during the two protocols [76]. In studies examining the effects of GLP-1 infusion during a standardised mixed meal, GLP-1 markedly [77] or completely [78–80] suppressed the meal-induced increase in glucagon and essentially abolished prandial glucose excursions. In another study, however, GLP-1 had no effect on the degree of suppression of glucagon induced by intravenous glucose, although GLP-1 did significantly reduce basal glucagon [81].

The effects of GLP-1 or exenatide on glucagon levels during stepped hyperglycaemic and hypoglycaemic clamps provided the first information about GLP-1 receptor activation and alpha cell glucose sensing in human subjects. As illustrated in Fig. 4a, GLP-1 infusion during stepped hyperglycaemic clamps suppressed plasma glucagon during euglycaemia and had a progressively larger effect as glucose increased [82]. As illustrated in Fig. 4b, exenatide infusion suppressed plasma glucagon during euglycaemia, did not affect glucagon during moderate hypoglycaemia, and significantly increased plasma glucagon during more severe hypoglycaemia [83]. In a similar study of stepped hypoglycaemic clamps during GLP-1 infusion, GLP-1 suppressed glucagon levels during euglycaemia but had no detectable effect on plasma glucagon during hypoglycaemia [84]. Taken together, these findings demonstrate that GLP-1 suppresses glucagon secretion at normal and elevated glucose levels but does not inhibit, and may even modestly augment, glucagon secretion induced by hypoglycaemia.

As in non-human primates, infusion of the GLP-1 antagonist exendin-[9–39] amide in healthy volunteers fasted overnight both blocked the glucagonostatic (and the insulinotropic) effects of exogenous GLP-1 and raised plasma glucagon in the absence of exogenous GLP-1 [85]. Plasma glucose increased, but insulin remained unchanged. This suggests that endogenous GLP-1 tonically restrains glucagon secretion in humans, and may indicate that GLP-1-based therapies could influence fasting, as well as postprandial, glucose.

GLP-1 and its agonists suppress glucagon secretion at euglycaemic and hyperglycaemic levels in healthy volunteers, but do not do so at hypoglycaemic levels. Thus, unlike other known modulators of glucagon release, GLP-1 appears to enhance alpha cell glucose sensing.

Acute effects of exogenous and endogenous GLP-1 on alpha cell function in diabetes

Gutniak and colleagues demonstrated the glucagonostatic effect of GLP-1, both in patients with type 2 and type 1 diabetes [49] (Fig. 5) This study showed that the ‘diabetic’ alpha cell is responsive to GLP-1 despite its inability to respond appropriately to glucose, and that the effects of GLP-1 are not mediated by endogenous insulin, since they were evident in patients with type 1 diabetes. Several studies have confirmed that GLP-1 given as a subcutaneous injection [86, 87], a buccal tablet [88] or an intravenous infusion [89–91] before a mixed meal effectively prevents inappropriate meal-induced glucagon release and greatly reduces prandial glucose excursions in patients with type 2 diabetes. A dose-related reduction in meal-stimulated glucagon release by subcutaneous injection of GLP-1 was also demonstrated in patients with type 1 diabetes [92]. Since GLP-1 can slow gastric emptying, particularly at supraphysiological concentrations [90], these studies do not rule out the possibility that inhibition of gastric emptying (and,

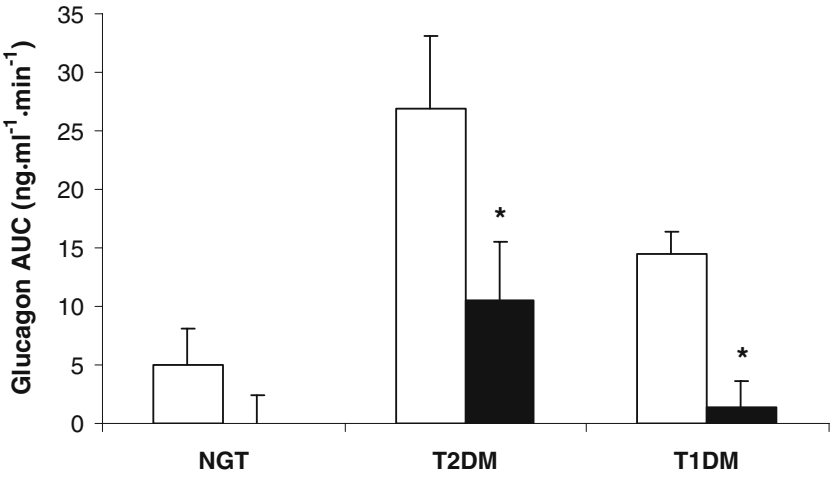

Fig. 5 The plasma glucagon $AUC_{(0–210\ min)}$ during a mixed meal in subjects with NGT (n=8), type 2 diabetes (T2DM, n=8) or type 1 diabetes (T1DM, n=8) during intravenous infusion of saline (*closed bars*) or GLP-1 (open bars). The data are presented as means±SEM. *p< 0.005 vs saline. Adapted from [49]

accordingly, a reduction of the stimulus for glucagon secretion) contributes to the observed effects. Further study will be needed to establish the relative contributions of delayed gastric emptying and effects on the alpha cell to the reduction in postmeal glucagon levels that occurs in response to agents that act through GLP-1 signalling mechanisms.

Studies of GLP-1 infusions in the fasting state have established that GLP-1 can directly inhibit glucagon release in patients with diabetes, irrespective of any effect on gastric emptying (Fig. 6) [93]. It should also be noted that glucagon levels rapidly increased as glucose levels approached the normal range, despite ongoing GLP-1 infusion, suggesting that GLP-1 may improve abnormal alpha cell glucose sensing in type 2 diabetes. The direct suppressive effect of GLP-1 infusion on fasting glucagon levels has further been demonstrated in patients with type 1 diabetes [94] and in patients with advanced type 2 diabetes after 'sulphonylurea failure' [95].

GLP-1 not only influences alpha cell function in individuals with diabetes, but is arguably more effective in patients with type 2 diabetes than in healthy subjects. Nauck and colleagues compared the influence of GLP-1 in patients with type 2 diabetes and in non-diabetic subjects [96]. As expected in healthy subjects, oral glucose or hyperglycaemic clamp suppressed plasma glucagon levels, whereas patients with type 2 diabetes showed a transient paradoxical increase in glucagon following oral glucose and failed to suppress during the hyperglycaemic clamp, thus illustrating the failure of alpha cell glucose sensing in diabetic subjects. Infusion of GLP-1 during the hyperglycaemic clamp had a much larger suppressive effect on plasma glucagon in patients than in healthy volunteers. Similar findings were reported by Vilsbøll et al. [97]. In both of these hyperglycaemic clamp/GLP-1 infusion studies, the absolute glucagon levels observed in patients with type 2 diabetes were either equal to or lower than those observed in healthy individuals, once again suggesting that GLP-1 can improve glucose sensing by the alpha cell, or that GLP-1 'allows' glucose to suppress glucagon release in diabetic patients.

Perhaps the clearest demonstration of the influence of GLP-1 on alpha cell function in diabetic patients was provided by a study in which arginine-stimulated glucagon responses were examined at basal glucose levels and during hyperglycaemic clamps at 14 and 28 mmol/l glucose. GLP-1 infusion significantly enhanced glucose-induced suppression of both prestimulus and arginine-stimulated glucagon levels at each glucose concentration (Fig. 7). As discussed above, an index of alpha cell sensitivity to glucose can be obtained from the glucose level at which half-maximal suppression (PG_{50}) of glucagon levels (Fig. 7a) or response (Fig. 7b) occurs. The PG_{50} for prestimulus glucagon averaged 15.5±1.0 mmol/l when saline was infused and 11.0±0.8 mmol/l during GLP-1 infusion (p<0.001). Similarly, the PG_{50} for AGR_{arg} was 15.5±0.7 mmol/l during saline and 12.1±0.8 mmol/l during GLP-1 (p<0.001). Such findings suggest that GLP-1 infusion shifts the glucose dose–response curve to the left, i.e. sensitizes the alpha cell to the suppressive effects of glucose [98]. It may be noted that the magnitude of the decrease in the PG_{50} for the AGR_{arg} in response to GLP-1 to is very similar to the increase in this parameter estimated from mean data comparing patients with type 2 diabetes with healthy volunteers (cf. Fig. 3). Accordingly, it may be speculated that a GLP-1-based therapy may be capable of correcting the defect in alpha cell glucose sensing that is characteristic of type 2 diabetes.

At present, only limited data are available with respect to the acute effects of GLP-1 agonists other than the native peptide on alpha cell function in diabetic patients. A recent study demonstrated that subcutaneous injection of exenatide decreased fasting plasma glucagon in patients with type 2 diabetes in a dose-related manner and eliminated meal-stimulated glucagon release [99]. In contrast, subcutaneous injection of the acylated derivative of GLP-1, liraglutide, did not affect fasting glucagon or glucagon during graded glucose infusions in patients with type 2 diabetes [4]. However, in another study, bedtime injection of liraglutide modestly decreased postmeal glucagon [100].

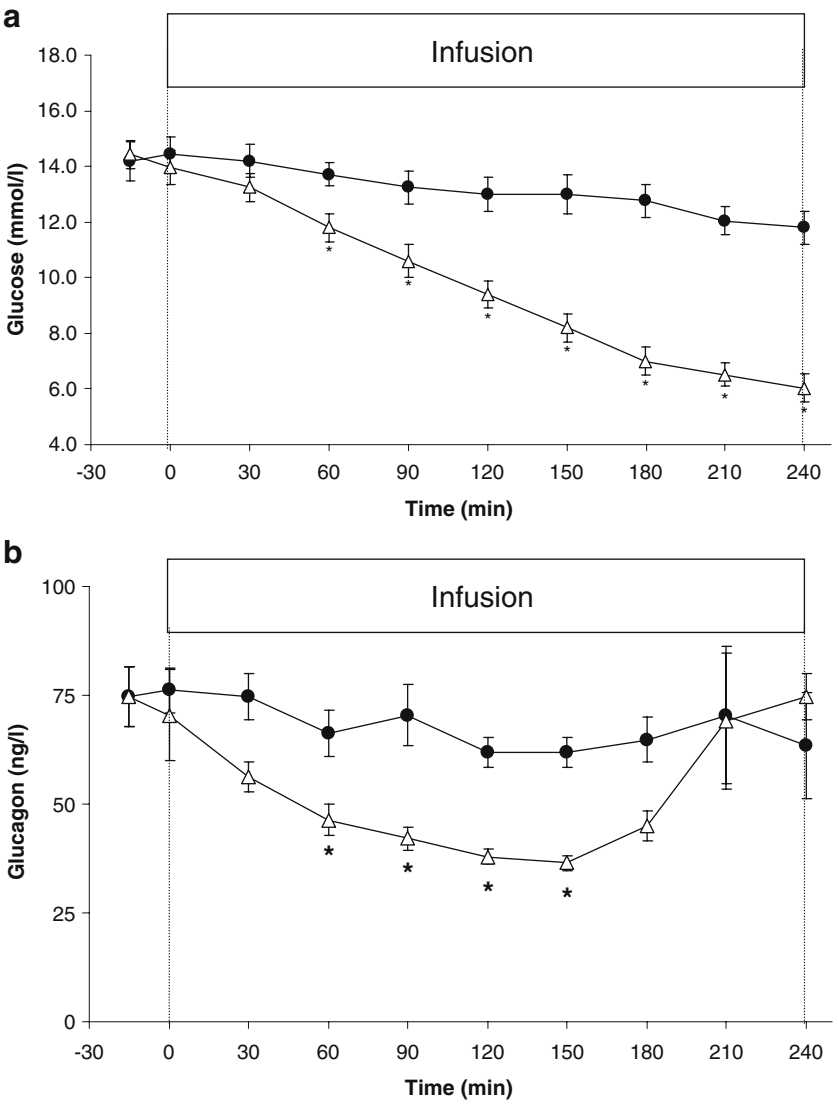

Fig. 6 Plasma levels of glucose (**a**) and glucagon (**b**) in overnight-fasted patients with type 2 diabetes during intravenous infusion of placebo (*closed circles*) or GLP-1 (*open triangles*). The data are presented as means± SEM, n=10. *p<0.05 vs placebo. Reprinted from [93] with kind permission of Springer Science and Business Media

GLP-1 directly suppresses glucagon secretion in patients with type 2 diabetes, independently of any effects on insulin or gastric emptying. The glucagonostatic effect of GLP-1 is at least as pronounced in diabetes as in health, but the alpha cell response to incipient hypoglycaemia is not suppressed by GLP-1. GLP-1 appears to improve alpha cell function, although further studies are needed to establish if this peptide can normalise the defects in alpha cell glucose sensing that are characteristic of type 2 diabetes.

Chronic effects of exogenous and endogenous GLP-1 in patients with type 2 diabetes

Zander and colleagues reported the first longer-term study of GLP-1 based therapy in patients with type 2 diabetes [101]. They found that a 6-week continuous subcutaneous infusion of GLP-1 did not affect fasting or 8-h mean plasma glucagon, as compared with saline infusion, but there was a tendency for glucagon to be lower after 1 and 6 weeks of treatment, and fasting and postmeal glucose were substantially reduced. The authors concluded from this that GLP-1 improved alpha cell sensitivity to prevailing glucose levels. In contrast, in a 3-month study in elderly patients switched from oral therapy to continuous GLP-1 infusion, as compared with those remaining on their previous oral therapy, found no effect of GLP-1 on glucagon, and the authors interpreted this as a loss of effect on glucagon with chronic treatment [102].

Another study explored the effects of 1 week of treatment with liraglutide on measures of alpha cell function in patients with type 2 diabetes. Liraglutide did not affect fasting glucagon, but decreased the 24-h glucagon AUC, which included three standardised meals, and the decrease following a protein-rich dinner was particularly large. Treatment with liraglutide reduced glucagon levels relative to placebo (by ~17%) during a hyperglycaemic (15 mmol/l) clamp, and the glucagon AUC during an arginine stimulation test performed during the hyperglycaemic clamp was also reduced (by ~16%) [103]. This outcome contrasts with some earlier negative results with acute administration of liraglutide (see above) and suggests that this GLP-1 derivative can affect alpha cell function during chronic treatment. Similarly, an 8-week study of once-daily injection of

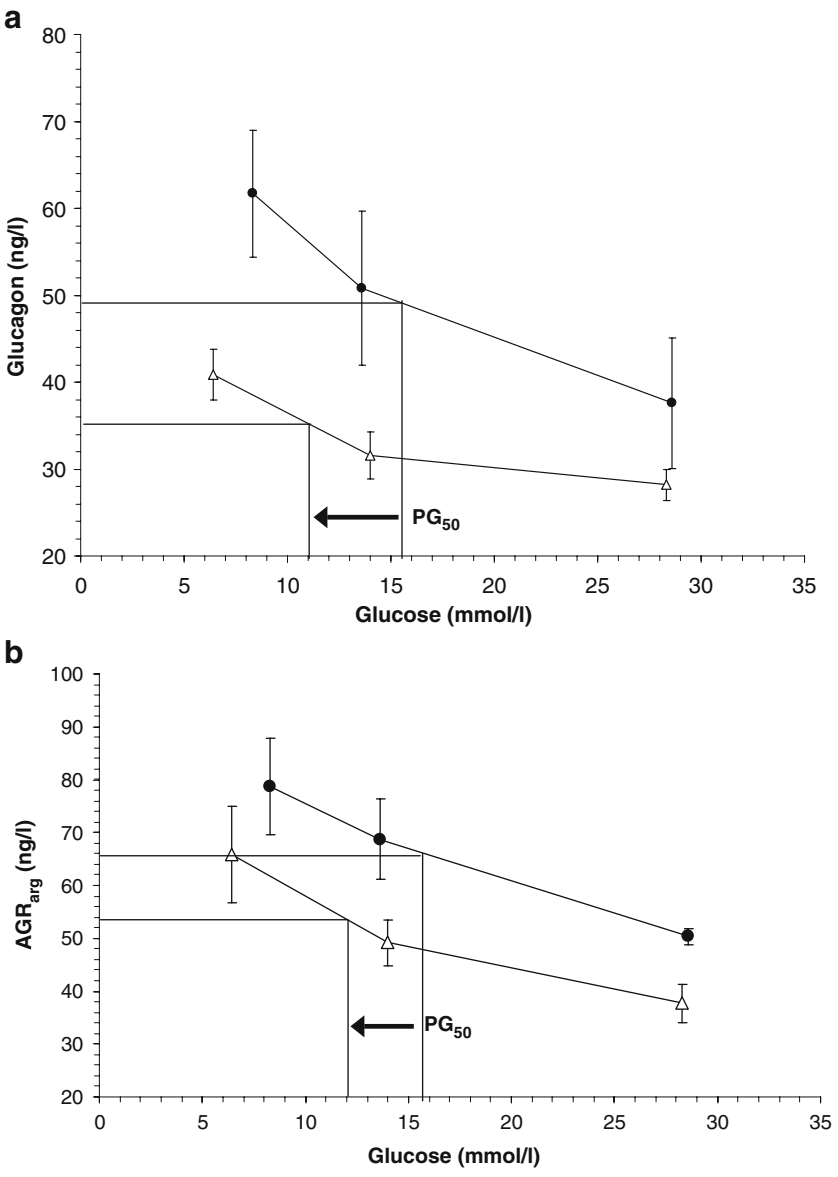

Fig. 7 Prestimulus plasma glucagon levels (**a**) and the acute glucagon response to intravenous arginine (AGR_{arg}=mean level from 2–5 min) (**b**) during stepped hyperglycaemic clamps in patients with type 2 diabetes during infusion of saline (*closed circles*) or GLP-1 (*open triangles*). The data are presented as means±SEM, n=6. Adapted with permission from [98]

liraglutide in patients with type 2 diabetes reported that glucagon levels were reduced during meals, although no data were shown [104]. However, in a 12-week dose-ranging study of once-daily injection of liraglutide in patients with type 2 diabetes, no dose had a statistically significant effect on glucagon levels [105]. Taken together, the available information on liraglutide cannot as yet support firm conclusions regarding this compound and glucagon secretion, although it seems likely that, like GLP-1 and exenatide, liraglutide exerts glucagonostatic effects.

No glucagon data were reported in recent reports of a 4-week study of exenatide added to metformin or sulphonylurea treatment [106] or of a 30-week study of exenatide added to sulphonylurea treatment [107]. Earlier studies of a 48-h infusion of GLP-1 in patients during ongoing pioglitazone [108] or metformin [109] treatment did indicate a glucagon-lowering effect of the native peptide in combination with oral agents. Hence, it may be expected that GLP-1-based therapies will maintain their effectiveness when combined with existing oral agents.

As mentioned above, an alternative approach to enhancing the therapeutic effects of GLP-1 is to block the rapid degradation of endogenous GLP-1 by DPP-4 and thereby increase circulating levels of biologically active GLP-1. As with other small molecule DPP-4 inhibitors, vildagliptin has good oral availability; therefore, unlike the native peptide and peptide agonists, it does not require parenteral administration. Treatment with vildagliptin over 4 weeks in drug-naïve patients with type 2 diabetes increased circulating levels of intact GLP-1, and decreased plasma glucagon and glucose during a standardised meal challenge [110]. A similar effect of vildagliptin was recently reported in patients with type 1 diabetes [111], suggesting that the effect on the alpha cell did not require endogenous insulin.

The ability of GLP-1 agonists to suppress glucagon secretion in patients with type 2 diabetes appears to be maintained with chronic (up to 8 weeks) treatment. Treatment with a DPP-4 inhibitor over a 4-week period also reduced meal-stimulated glucagon levels.

Future directions and therapeutic implications

Although much remains to be learned about GLP-1 and alpha cell function, current evidence suggests that GLP-1-based therapies will be effective at improving glycaemic control in patients with a broad range of metabolic disease, at least in part due to their ability to improve, perhaps even normalise, alpha cell function. The terminology in this area is evolving, and we suggest that the term 'incretin mimetic' is appropriately applied to GLP-1 agonists and derivatives such as exenatide and liraglutide, while the term 'incretin enhancer' may be useful to distinguish the mechanism of action of DPP-4 inhibitors such as vildagliptin from agents that act directly on target tissues.

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Duality of interest.

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