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Invited review

Extrapancreatic glucagon: Present status

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ABSTRACT

Pancreatic alpha cells are generally considered the only source of glucagon secretion in humans. In the 1970s several groups investigating totally pancreatectomised animals reported that glucagon-like immunoreactive material could be detected in the gastrointestinal tract and reopened the question of an extrapancreatic source of glucagon proposed in 1948 when a hyperglycaemic substance was found in the gastrointestinal tract of dogs and rabbits. Nevertheless, over the years, controversy about the existence of extrapancreatic glucagon has flourished as it proved difficult to accurately measure fully processed 29-amino acid glucagon. Recent advances in analytical methods have increased sensitivity and specificity of glucagon assays and, furthermore, technical advances in mass spectrometry-based proteomics have made the detection of low-abundant peptides, such as glucagon, in human plasma more accurate. Here we review new data on extrapancreatic glucagon secretion in the context of historical data and recent analytical breakthroughs. Furthermore, the source, regulation and potential physiological role of extrapancreatic glucagon are discussed and ongoing challenges and knowledge-gaps are outlined.

Contents

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1. Introduction

The 29-amino acid peptide hormone glucagon is considered of fundamental importance for maintaining normal fuel balance as it stimulates ketogenesis and release of glucose to the circulation when blood glucose concentrations fall below normal physiological levels. As decade's of reporting glucagon-like immunoreactivity from the gut turned out to include a range of gut peptides derived from the precursor of glucagon, proglucagon, pancreatic alpha cells in the islets of Langerhans have generally been considered the only source of glucagon secretion in humans. However, recent developments in analytical methods have increased sensitivity and specificity of glucagon assays and technical advances in mass spectrometry-based proteomics have made the detection of low-abundant peptides, such as glucagon, in human plasma possible. This has procured new evidence of circulating 'pancreatic' 29-amino acid glucagon in totally pancreatectomised patients [1], and recently it was shown how oral glucose administration in these patients gives rise to robust elevations in plasma glucagon concentrations approximating those observed during hypoglycaemic conditions in healthy subjects. These findings have reopened the question of an extrapancreatic source of glucagon and we are now facing the challenge of mapping and understanding the regulation and physiological as well as pathophysiological implications of extrapancreatic glucagon. In this article we review current knowledge on extrapancreatic glucagon in the context of historical data, recent analytical progress, anatomy, physiology, pathophysiology and future perspectives.

2. Glucagon down history

Since the discovery in 1889 by Minkowski and Mering that removal of the pancreas immediately resulted in diabetes it has been evident that the pancreas is of crucial importance for the maintenance of blood glucose homeostasis. Ligation of the pancreatic duct did not result in these metabolic manifestations and it was concluded that one or more pancreatic factors secreted to the systemic circulation could be responsible [2]. This finding was followed by studies showing how intravenous infusion of pancreatic tissue extracts in diabetic animals resulted in reductions of blood glucose levels and decreased glycosuria [3]. Ultimately, these findings led to the isolation of insulin in 1921 by Banting and Best [4] - one of the most important discoveries in medical history. Interestingly, Banting and Best's studies elucidated an initial brief hyperglycaemic response before the well-known glucose-lowering effect after administration of crude pancreatic extracts in depancreatised dogs. A finding that initially did not receive much attention but in 1922 Kimball and Murlin succeeded in isolating the hyperglycaemic substance from insulin and showed that administration of this pancreatic preparation had a potent hyperglycaemic effect when administered in depancreatised dogs [5]. They reasoned that the pancreas contains a second hormone with effects on glycaemic levels, and named this the "glucose agonist", hence the name glucagon. Subsequent work showed that the hyperglycaemic effect of this still unidentified substance, was mediated through a glycogenolytic effect on the liver and for the following many years it was re-named as "the hyperglycaemic-glycogenolytic (H-G) factor". In 1948 Sutherland and de Duve isolated small amounts of purified H-G factor from the pancreas of different species, including humans, and showed that the H-G factor was preserved still when the pancreatic acinar and beta cells were injured by alloxan, leading to the hypothesis that the pancreatic alpha cells were the origin of the H-G factor [6,7]. Subsequent studies using the chemical substances synthalin A and cobalt to ablate the pancreatic alpha cells showed a decrease in the pancreatic content of the H-G factor, confirming the pancreatic alpha cells as the source of the pancreatic H-G factor [8]. Interestingly, Sutherland and de Duve also found a H-G factor in the upper three-fourths of the gastric mucosa, the duodenum and ileum of dogs, and in the stomach of rabbits [6]. These findings seemed to be species specific as the gastric mucosa of other animals (pig, sheep and cattle) contained none (or only traces) of the H-G factor. In the 1950s the pancreatic H-G factor was purified and crystallised at Eli Lilly and Co., and subsequently the 29-amino acid sequence of glucagon (the name glucagon was in these years re-introduced) was determined [9,10] leading to the successful development of medical use of glucagon in the treatment of severe hypoglycaemic reactions to insulin [11,12]. In 1959 the first radioimmunoassay (RIA) for the detection of glucagon was developed [13]. This made it possible to describe how glucagon concentrations changed in response to fuel shortage or abundance and glucagon became known as the gluco-regulatory counterpart of insulin [14]. Subsequently, it became evident that glucagon concentrations are elevated in patients with diabetes in the fasting state as well as postprandially [15], and the renowned "bihormonal hypothesis" was put forward by Unger and Orci stating that the combination of insulin deficiency and hyperglucagonaemia is essential for diabetic hyperglycaemia [16]. Since then glucagon has been recognised as a multifaceted hormone with important physiological effects beyond glucose metabolism - including effects on lipid and protein metabolism, regulation of food intake and body weight, and with positive chronotropic and inotropic effects on the heart [17]. The advent of the glucagon RIA also resulted in the findings of a wide range of peptides structurally related to glucagon, produced in extrapancreatic tissues, especially the gut, with glucagon-like immunoreactivity [18]. These peptides should later turn out to play important roles in human physiology, but at the same time they posed a problem for the measurement of 'true' 29-amino acid glucagon. Not until years later it was shown that the glucagon-like peptides, like glucagon, all came from the same precursor, namely, proglucagon [19,20].

3. Proglucagon and enteroglucagons

After the introduction of the inaugural glucagon RIA, it was demonstrated in 1968 that the material secreted from the gut following oral glucose ingestion (but not secreted during intravenous glucose infusion) displaying glucagon-like immunoreactivity was different from true pancreatic glucagon based on its biologic activity and immunochemical and electrophoretic properties [21,22]. This enhanced the need

for finding antisera specific for pancreatic type glucagon, and, in the following years many alleged "specific" antisera with low cross-reactivity between pancreatic glucagon and gut glucagon-like immunoreactivity were developed. However, the actual specificity of these antisera was soon after questioned. A review by Holst in 1978 gives an outline of the knowledge, including assay systems and extraction and separation methods of the extrapancreatic glucagons of that time [23]. Further studies on the synthesis of glucagon suggested that glucagon was a product of a large precursor molecule, proglucagon, present in the pancreatic alpha cells and in the small intestine [24,25]. From immunoprecipitations of proteins extracted from pancreatic islets it was possible to identify proteins of different molecular size, a 18 K Da protein (proglucagon), which converted into pancreatic 29-amino acid glucagon and a 10 K Da protein that did not exhibit glucagon-like immunoreactivity [26]. Analysis of proteins from the gut revealed two peptides of 8 K Da and 12 K Da, respectively, both reacting with a non-specific glucagon antiserum [24]. In the late 1970s, peptides reacting with a non-specific glucagon antiserum were also found in the rat brain [27,28]. Soon after glicentin was isolated from the porcine gut, and the amino acid sequence revealed that within this peptide the entire 29-amino acid sequence of glucagon was embedded between a 33 amino acid N-terminal and a 8 amino acid C-terminal addition of amino acids [29].

In the early 1980s proglucagon amino acid sequences from cDNAs and gene sequences were isolated from the anglerfish, rat, hamster, cattle and humans [30] and revealed how the mammalian preproglucagons all contained not only the sequence of glucagon and glicentin, but also two additional glucagon-like peptides, namely, glucagon-like peptide 1 (GLP-1) and glucagon-like peptide 2 (GLP-2) separated from glucagon by two small intervening peptides I and II, respectively (Fig. 1). From RNA analyses it became evident that the glucagon gene is transcribed in the pancreatic alpha cells, in a discrete set of neurons within the nucleus of the solitary tract and in specific enteroendocrine cells of the gut (L cells) [31–35]. While the proglucagon mRNA transcripts are the same in these tissues, specific posttranslational processing results in secretion of peptides structurally related, but with very different effects [36]. The 160 amino acid proglucagon molecule is post-translationally processed by the processing enzymes prohormone convertase 1 (PC1) (also known as neuroendocrine convertase 1) and prohormone convertase 2 (PC2) (also known as neuroendocrine convertase 2). Thus, the final products of proglucagon in a given cell / tissue is dependent on the presence, relative activity and specificity of these processing enzymes. The processing enzymes have generally been considered tissue-specific with PC2 being expressed in pancreatic alpha cells liberating glucagon and three other peptides (glicentin-related pancreatic polypeptide, intervening peptide 1 and a major proglucagon fragment [37,38]) and PC1 being expressed in enteroendocrine L cells and in neurons in the nucleus of the solitary tract, liberating glicentin, oxyntomodulin, intervening peptide 2, GLP-1 and GLP-2 [38–40] (Fig. 1). The structural resemblance between these peptides is noticeable: The major proglucagon fragment (secreted from pancreatic alpha cells) span the amino acid sequence of both GLP-1, intervening peptide 2 and GLP-2 while glicentin

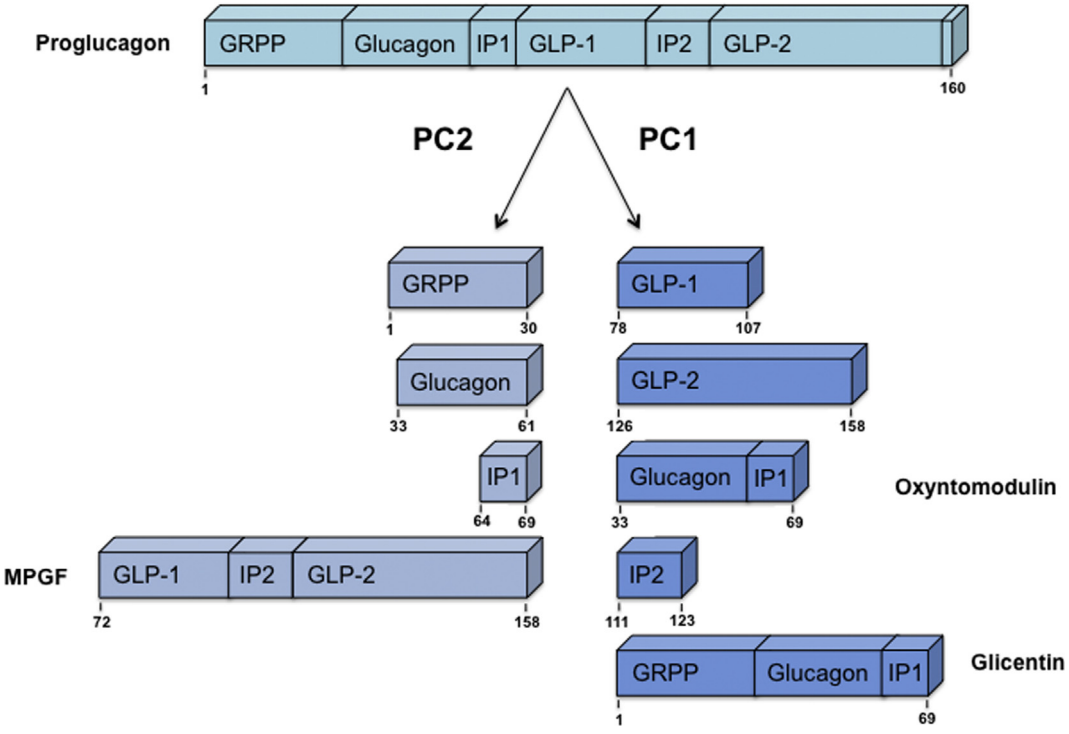

Fig. 1 – Differential processing of proglucagon by prohormone convertase 1 (PC1) and prohormone convertase 2 (PC2), respectively. GRPP, glicentin-related pancreatic polypeptide; GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2; IP1, intervening peptide 1; IP2, intervening peptide 2; MPGF, major proglucagon fragment. The numbers indicate amino acid positions in the 160-amino acid proglucagon sequence.

and oxyntomodulin (secreted from enteroendocrine L cells) both include the full sequence of 29-amino acid glucagon. Although the general idea is that the processing enzymes are tissue specific, it has been shown that under certain circumstances activity of PC1 may also be present in pancreatic alpha cells [41–43] and it has been speculated that GLP-1 secreted from alpha cells could play an important role in regulating glucose metabolism [44]. In 2009 it was speculated how PC2 expression in enteroendocrine L cells could result in co-secretion of common L cell products and true 29-amino acid 'pancreatic-type' glucagon from the gut [45] and a few years later it was shown that PC2 may also be present in enteroendocrine cells in the gut [46] - including in cells staining positive for GLP-1 [47,48].

4. Extrapancreatic glucagon

As alluded to above the first notion of possible extrapancreatic glucagon secretion was suggested already in 1948 when it was shown that a "glycogenolytic substance" could be extracted from the gastric mucosa of dogs and rabbits [6]. Since then the question of the existence of extrapancreatic 'true' glucagon has been sought answered by several investigations in totally pancreatectomised animals and humans. Overall, these studies have reported considerable variation between species and provided opposing conclusions regarding the presence or absence of extrapancreatic glucagon. Studies in totally pancreatectomised dogs reported findings of "glucagon immunoreactivity" in plasma [49–52] and it was suggested that the gastric mucosa [50] as well as the ileum could be responsible for this glucagon-like immunoreactivity [51]. A study in ducks reported no trace of glucagon immunoreactivity following total pancreatectomy [53], and the diverging results from studies in totally pancreatectomised humans have contributed to the uncertainty of the existence of extrapancreatic glucagon [54–66]. One of the most important reasons for the controversies regarding the existence of extrapancreatic glucagon is that it has proved difficult to accurately measure fully processed 29-amino acid glucagon [67]. Since the glucagon RIA was introduced, immunochemical approaches have remained the most common, and most accurate, methods for the quantification of plasma glucagon levels, including RIAs and enzyme-linked immunosorbent assays (ELISAs). However, the sensitivity and specificity for these glucagon assays are challenged by the biology of the 'glucagons'. As described above the enteroglucagons, glicentin and oxyntomodulin, both contains the entire 29-amino acid glucagon sequence (corresponding to amino acids 33–61 of proglucagon (Fig. 1), and, thus, cross-reactivity of side-viewing antibodies is possible with antisera directed at residues within the core 29-amino acid glucagon sequence. Antibodies specific to the free C-terminus of glucagon should circumvent this problem, and ought not to react with glicentin and oxyntomodulin. However, glucagon has been shown to constitute a substrate for enzymes with various endopeptidase and exopeptidase activity (such as dipeptidyl peptidase 4), and thus, several truncated forms of glucagon (e.g. glucagon 3–29, 18–29 and 19–29) exist [68]. Also, 'glucagon' exists in an N-terminally extended form (glucagon 1–61), which poses an additional problem for assays based on C-terminally directed anti-glucagon antibodies [69]. Lastly, glucagon circulates in the low picomolar range, which demands a very high degree of sensitivity to detect small, but, nevertheless, potentially clinical relevant changes in glucagon levels. In line with these challenges, an assessment in 2014 of commercially available glucagon assays demonstrated poor specificity and suboptimal sensitivity for many assays [70]. Thus, interpretation of much of the existing literature reporting measurements of glucagon, and extrapancreatic glucagon in particular, remain challenging.

To improve specificity of glucagon measurements a 'sandwich' assay approach employing a combination of N and C-terminal anti-glucagon antibodies thus targeting each end of the glucagon peptide has been developed. This assay improves sensitivity and specificity compared to conventional RIA methods, and only show low cross-reactivity against other proglucagon fragments [71]. Furthermore, technical progress within liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography tandem-mass spectrometry (LC-MS/MS) has made the quantification of low-abundant peptides possible. These methods have a high specificity and in addition they make it possible to distinguish modified peptides, including oxidized peptides, from intact peptides [72]. We recently applied these novel analytical methods in a study examining totally pancreatectomised patients undergoing an oral glucose tolerance test and a subsequent isoglycaemic intravenous glucose infusion [1]. From sandwich ELISA measurements we could show an increase in circulating glucagon levels following oral ingestion of glucose, whereas intravenous glucose infusion suppressed glucagon concentrations in these patients (Fig. 2) [1]. The robust elevations in plasma glucagon concentrations in these patients following oral glucose ingestion were substantial and approximating concentrations seen in healthy subjects during hypoglycaemic conditions [73]. Subsequent LC-MS/MS analysis of plasma in the fasting state and 30 min following oral glucose ingestion confirmed that 29-amino acid glucagon was circulating and that the concentration increased following oral glucose ingestion in these totally pancreatectomised patients (Fig. 2). Importantly, pancreatic polypeptide and C-peptide were undetectable underscoring the completeness of pancreatectomy, and thus eliminating the possibility that the observed glucagon responses could originate from remnant pancreatic tissue. The finding of extrapancreatic glucagon secretion measured with a sandwich ELISA method was confirmed in a study of totally pancreatectomised patients during a mixed meal test [74].

Taken together, the progress in analytical methods and recent studies in totally pancreatectomised patients have provided compelling evidence of an extrapancreatic source of 29-amino acid glucagon hitherto considered a pancreas-specific hormone.

5. Secretion of extrapancreatic glucagon

Although the exact source of extrapancreatic glucagon secretion remains to be elucidated, the fact that oral nutrient administration elicits glucagon secretion in totally pancreate-

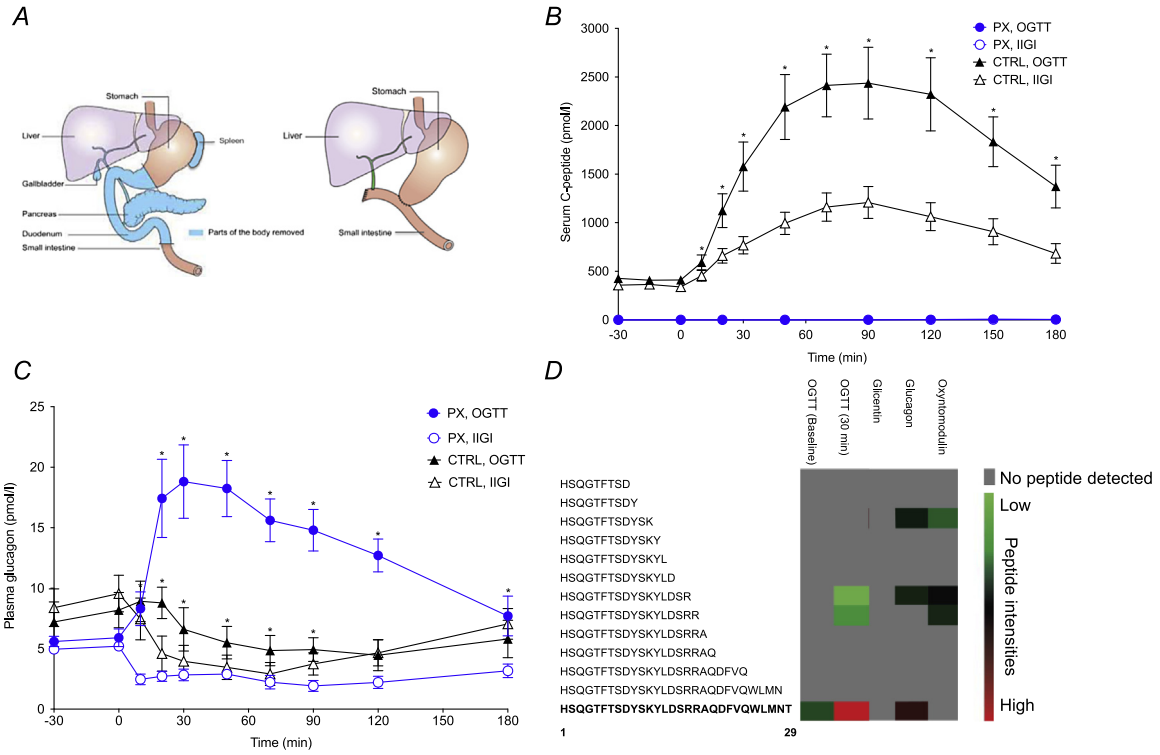

Fig. 2 – Illustration of the anatomy before (left) and after (right) total pancreatectomy (*A*). Serum C-peptide concentrations (*B*) and plasma glucagon concentrations (*C*) during 75-g oral glucose tolerance test (OGTT) and isoglycaemic intravenous glucose infusion (IIGI) in totally pancreatectomised patients (PX). Tandem mass spectrometry-based proteomics of plasma from fasting (baseline) and from time point 30-min during the OGTT (*D*). Oxyntomodulin, glucagon and glicentin samples were used as control measures. The 29-amino acid sequence of glucagon is depicted in bold font. Data are mean ± SEM. Asterisks (*) indicate significant differences. Adapted from Lund et al. [1].

ctomised patients while intravenous glucose administration does not, draws the attention to the gut as the potential site of origin. In the gut, the enteroendocrine L cells, the only known proglucagon-expressing cells in the gut, are obvious candidates. Interestingly, in humans, L cells are found throughout the intestinal tract with density increasing from the duodenum to the rectum [75]. Following total pancreatectomy in humans the anatomy of the gut is changed involving removal of the distal part of the ventricle (including the pyloric sphincter) and the duodenum (Fig. 2). Thus, when these patients ingest a meal or an oral glucose load, nutrients are delivered directly from the stomach to the jejunum where L cells under normal circumstances are abundant. This likely results in a rapid and large stimulation of the L cells explaining the significantly increased responses of 'normal' L cell products (GLP-1 and oxyntomodulin) as observed in totally pancreatectomised patients following nutrient intake [1]. However, as alluded to above, for the L cells to secrete 29-amino acid glucagon an atypical posttranslational processing of proglucagon should take place in these cells. One possibility could be that the cleavage sites of PC1 on the proglucagon molecule are less specific than previously believed, and thus that atypical cleavage of proglucagon by PC1 results in secretion of normal alpha cell products including 29-amino acid glucagon. The secretion from this atypical posttranslational modification could under normal circumstances be limited. But when the anatomy is changed (as seen following total pancreatectomy or following the bariatric surgical procedure Roux-en-Y gastric bypass (RYGB)) and nutrients suddenly are bypassed the duodenum and delivered more rapidly to distal parts of the gut (where the density of L cells is greater), a substantial secretion of proglucagon-derived peptides, including 29-amino acid glucagon may occur. Another possibility is that PC2 is co-expressed in the L cells, which likely would result in formation of not only 'traditional' L cell proglucagon products, but also in traditional alpha cell products like 29-amino acid glucagon. In line with the latter hypothesis, a study from 2008 found that PC2 is present in the gut of rodents, especially in the upper two parts of the small intestine, the duodenum and the jejunum [76], and in 2011 a study of jejunal biopsies from patients with type 2 diabetes and non-diabetic control subjects described PC2 mRNA and immunohistochemical PC2-positive enteroendocrine cells [77]. This finding of PC2-positive enteroendocrine cells was subsequently confirmed in studies from 2015 comparing regional expression of the prohormone convertases in lean and morbidly obese humans [46] as well as in patients following RYGB surgery [47] and most recently in a study in patients with type 2 diabetes and non-diabetic individuals [48]. Interestingly, the observation of co-localisation of PC2 and GLP-1 in the small intestinal epithelium may suggest that some proglucagon-producing cells perhaps harbour processing capability to produce 29-amino acid glucagon [77]. In contrast, a recent study from Jorsal and colleagues found no co-

staining of glucagon and PC2 in small intestinal mucosa biopsies (Jorsal et al., unpublished); a finding favouring the hypothesis of posttranslational processing of proglucagon to 29-amino acid glucagon in the gut by other processing enzymes (e.g. unspecific processing by PC1).

While the regulation of pancreatic glucagon secretion has been carefully described [78] less is known about the regulation of extrapancreatic glucagon secretion. As described in detail above, the studies reporting on extrapancreatic glucagon secretion should be seen in the light of the applied glucagon assays and at the time of most of these studies, the sensitivity and specificity of the glucagon assays could (and should) be questioned. In the 1970s and 1980s studies of totally pancreatectomised dogs showed how oral administration of glucose and arginine elicited significant responses of gut glucagon-like immunoreactivity [50,79,80], as did hypoglycaemia (a potent stimulus of pancreatic glucagon secretion) in conscious depancreatised dogs but not when the dogs were put under full anaesthesia, suggesting an involvement of the nervous system in the glucagon-like immunoreactivity response [81]. In studies of the isolated perfused dog stomach, arginine infusions (often used as a stimulus to evaluate maximal pancreatic glucagon secretion) resulted in increments in glucagon-like immunoreactivity [82]. Also, infusion of the gut hormone glucose-dependent insulinotropic polypeptide (GIP) was shown to increase glucagon levels in the gastric vein after intragastric instillation of a meal in anesthetised dogs [83]. In depancreatised dogs, infusions of the gut hormones secretin and cholecystokinin were shown not to result in a glucagon-like immunoreactive response, but during co-administration with arginine, cholecystokinin potentiated the arginine-induced glucagon-like immunoreactive response [84].

Human studies providing insight into the regulation of extrapancreatic glucagon are scarce. In 1976, a study in totally pancreatectomised humans showed that intravenous infusion of arginine did not elicit responses of glucagon-like immunoreactivity [56]; a finding confirmed the same year in a case report showing no changes in circulating glucagon-like immunoreactivity following intravenous arginine infusion in a totally pancreatectomised patient [55]. In 1981 a study examining the antidiabetic actions of somatostatin in totally pancreatectomised humans found that oral glucose elicited a significant response of glucagon-like immunoreactivity and that this response was blunted when somatostatin was infused simultaneously [80]. In 1983 a study examining totally pancreatectomised humans found glucagon-like immunoreactivity responses following a meal rich in fat and carbohydrates and concluded that the human intestine is capable of generating all the molecular forms of glucagon normally present in plasma [66]. In the light of today's knowledge about the origin and secretion of the multiple gut peptides derived from proglucagon and the questionable specificity of the employed assays, the above studies likely report concentrations of not only 29-amino acid glucagon, but a range of peptides with glucagon-like immunoreactivity. However, more recent studies applying sensitive and specific analyses (mass spectrometry-based proteomics and mass spectrometry-validated sandwich ELISA) in totally pancreatectomised humans, have shown how oral ingestion of glucose represents a strong stimulus for the secretion of extrapancreatic 29-amino acid glucagon whereas intravenous glucose administration seems to suppress circulating concentrations [1]. Furthermore, it was shown that ingestion of a mixed meal in totally pancreatectomised patients elicits robust elevations in plasma glucagon concentrations [74], a response that could be completely eliminated by single-dosing of the short-acting GLP-1 receptor agonist lixisenatide (known to possess glucagonostatic properties) [74]. Interestingly, this was accompanied by a ~50% reduction in postprandial glucose excursions (despite the fact that no bolus insulin was administered with the meal test) [74]. The mechanism(s) behind this suppressive effect of GLP-1 on extrapancreatic glucagon secretion is not clear. It may be a direct effect on the extrapancreatic glucagon-secreting cells or a paracrine effect involving GLP-1-mediated stimulation of somatostatin-secreting enteroendocrine cells [85], corresponding to the glucagon-lowering effect of GLP-1 mediated through somatostatin in the pancreas [86]. Also, lixisenatide-induced deceleration of upper gastrointestinal motility including gastric emptying may be involved. Two case-reports published in 2017 showed how octreotide (a somatostatin analogue) and 3 days of subcutaneous somatostatin treatment, respectively, significantly suppressed both fasting and postprandial glucagon levels in totally pancreatectomised patients and found that this correlated with improvement of postprandial hyperglycaemia [87,88] and an increase of nocturnal hypoglycaemic events [88]. This may indicate that extrapancreatic glucagon is affecting glucose levels both in the fasting state and postprandially. The effect of intravenous infusion of arginine was recently evaluated by Juel et al. in totally pancreatectomised humans (applying mass spectrometry-validated sandwich ELISA) and no glucagonotropic effect was seen [89].

In light of recent investigations of extrapancreatic glucagon secretion with novel and specific analytical modalities, it seems likely that enteroendocrine cells constitute the origin of extrapancreatic glucagon secretion. It appears that the stimulus for secretion is closer linked to that of other enteroendocrine cells, as for instance normal L cell products (luminal stimulation with nutrients), than to the secretion for the pancreatic alpha cells. Nevertheless, regulation of extrapancreatic glucagon is currently poorly understood and investigations using the novel and more specific analytical modalities are warranted. Results from studies in totally pancreatectomised patients looking at extrapancreatic glucagon secretion during hypoglycaemia and during treatment with drugs known to affect glucagon levels (e.g. sodium-glucose co-transporter 2 inhibitors and dipeptidyl peptidase 4 inhibitors) are awaited.

6. Physiological role of extrapancreatic glucagon?

The physiological implication of extrapancreatic glucagon secretion is unclear. It has been speculated that gut-derived glucagon secretion could play an unrecognised pathophysiological role in postprandial hyperglycaemia considered a core trait in diabetes [1]. Thus, patients with diabetes show increased glucagon secretion following ingestion of carbohydrates (likely depending on secretion of extrapancreatic glu-

cagon from the gut) whereas intravenous administration of glucose suppresses glucagon concentrations in these patients [90–93]. As some studies suggest that postprandial hyperglucagonaemia could be responsible for as much as 50% of the pathological increment in plasma glucose excursions following oral glucose ingestion in patients with diabetes [94,95], extrapancreatic glucagon secretion after carbohydrate ingestion may have important pathophysiological implications by contributing to hyperglycaemia.

In theory the secretion of extrapancreatic glucagon could also constitute a physiological protective mechanism to counteract the potent glucose-lowering mechanisms exerted by the incretin hormones (e.g. their insulinotropic effect) and the suppression of endogenous glucose production exerted by ingested carbohydrates. Such a mechanism may be particular important in patients who have undergone RYGB surgery (or total pancreatectomy surgery) where nutrients are directly delivered to the distal part of the small intestine where the density of L cells is very high resulting in a GLP-1-mediated 'overshoot' of insulin relative to the amount of carbohydrate ingested, increasing the risk of postprandial hypoglycaemia [96]. In these patients, surgery-induced metabolic benefits are typically accompanied by postprandial hyperglucagonaemia [97,98]. Extrapancreatic glucagon secretion from the gut could explain the paradoxical postprandial hyperglucagonaemia observed after RYGB surgery as a recent study examining mucosal biopsies pre- and postoperatively from these patients found extractable glucagon in biopsies harvested postoperatively (Jorsal et al., unpublished). Nevertheless, accelerated uptake of amino acids [99] and amino acid-induced alpha cell secretion after RYGB may also contribute to the postprandial hyperglucagonaemia associated with RYGB.

In addition to the potential contribution of extrapancreatic glucagon to protect from postprandial hypoglycaemia, the secretion of glucagon from the gut could also stimulate glucagon-mediated satiety and work in concert with other gut hormones such as GLP-1 and peptide YY to induce satiety [100].

7. Conclusions

Since the first notion of a hyperglycaemic factor in the gastrointestinal tract in dogs and rabbits almost 70 years ago the possible existence of extrapancreatic glucagon secretion has been sought elucidated. The research in this field has advanced with the discovery and characterisation of the multiple glucagon-like peptides secreted from the gut alongside a recognition of limitations of many glucagon assays regarding the specific measurement of 29-amino acid glucagon. With recent development in analytical techniques, reliable detection of 29-amino acid glucagon in human plasma has been made possible, and, in the last few years accumulating evidence points to an extrapancreatic source of glucagon secretion in humans – most likely the gut. The secretion of extrapancreatic glucagon seems to be regulated differently from pancreatic glucagon secretion and to resemble that of other enteroendocrine cells where gastrointestinal luminal stimulation of nutrients act as mediators of secretion. However, studies on the anatomical localisation and physiological regulation of extrapancreatic glucagon secretion are needed to provide a clearer picture. Furthermore the physiological and/or pathophysiological effects of extrapancreatic glucagon secretion in health and disease warrant further elucidation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.diabres.2018.06.013.

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