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Glucagon receptor signaling in metabolic diseases

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ABSTRACT

Glucagon is a peptide hormone secreted from the pancreatic alpha cells in response to hypoglycemia but in some patients with type 2 diabetes a paradoxical hypersecretion results from the intake of glucose. In rodent, antagonizing the actions of glucagon have been shown to be effective for lowering blood glucose levels and this has recently have been solidified in patients with type 2 diabetes. Although the reported increases of liver enzymes, hyperglucagonemia, and alpha cell hyperplasia resulting from glucagon receptor antagonism may potentially limit the clinical applicability of glucagon receptor antagonists, they may serve as an instrumental toolbox for delineating the physiology of glucagon. Agonizing glucagon receptor signaling may be relevant, in particular when combined with glucagon-like peptide-1 receptor analogues in the perspective of body weight lowering therapy. Here, we will focus on new conceptual aspects of glucagon biology and how this may led to new diagnostics and treatment of metabolic diseases.

1. Introduction

Glucagon is traditionally believed to be an alpha-cell derived hormone that increases blood glucose levels through increased hepatic glucose production [1]. The antagonism or agonism of the glucagon receptor has long been regarded as potential therapeutic strategies. Whereas agonism of the glucagon receptor (GCGr) is commonly used for treatment of hypoglycemia in patients with type 1 diabetes [2,3] and potentially as a weight-lowering drug for obese individuals [4], antagonizing the GCGr on the other hand has been suggested a novel drug candidate for treatment of type 2 diabetes [5].

The diabetogenic role of glucagon is widely accepted [6], as demonstrated in the early 1980s using glucagon receptor antagonists in rodents [7,8] and recently also confirmed in patients with type 2 diabetes [9]. However, a number of preclinical studies now suggest that the secretion of glucagon and alpha cell mass is controlled by amino acids whereas glucagon may contribute to hepatic amino acid turnover through ureagenesis and gluconeogenesis and thereby constituting a new endocrine feedback system [10–13].

Here, we reflect on new aspects of glucagon biology and their role and potential applicability for understanding and treating metabolic diseases. Firstly, let us look on how and why it is important to measure glucagon in clinical conditions.

2. Clinical rationale for measurement of glucagon

Measurement of glucagon may be clinically relevant for the diagnosis of glucagon-producing tumors (part of the group of pancreatic neuroendocrine tumors) [14,15]. Although the incidence of glucagonomas are extremely low (~1:1.000.000) the pathophysiology of such tumors may help us understand how *differential processing of proglucagon* results in heterogeneous clinical phenotypes: Hypoglycemia due to increased plasma levels of active GLP-1, and hyperglycemia due to increased plasma concentrations of glucagon [16]. That said, it may be of interest to profile the secretion of glucagon during daily life and upon metabolic challenges [17] and the majority of the current literature is actually based on the associations of glucagon with development of diabetes.

From the development of the first glucagon assay in 1959 [18], the majority of research investigating hypersecretion of glucagon has applied C-terminal glucagon assays, whereas a few have used, what turned out to be unreliable, sandwich and or side-viewing glucagon assays [19], which led to falsely increased secretory rates of glucagon due to cross-reactivity with other proglucagon derived peptides (Fig. 1A), namely oxyntomodulin and glicentin. Truly C-terminal assays (C-terminal wrapping assays) may be specific and provide its user with an accurate profile of glucagon secretion [20], but on the other hand these are unable to discriminate between potential N-terminally elongated and/or truncated glucagon forms that may exist in dysmetabolic conditions [16] and their importance has yet to be decided. In addition, the C-terminal assays may, dependent of the quality of the antibody used, also cross-react with proglucagon molecules. It therefore remain important to assess the specificity of antibody based measurement methods, as described previously using mass-spectrometry based

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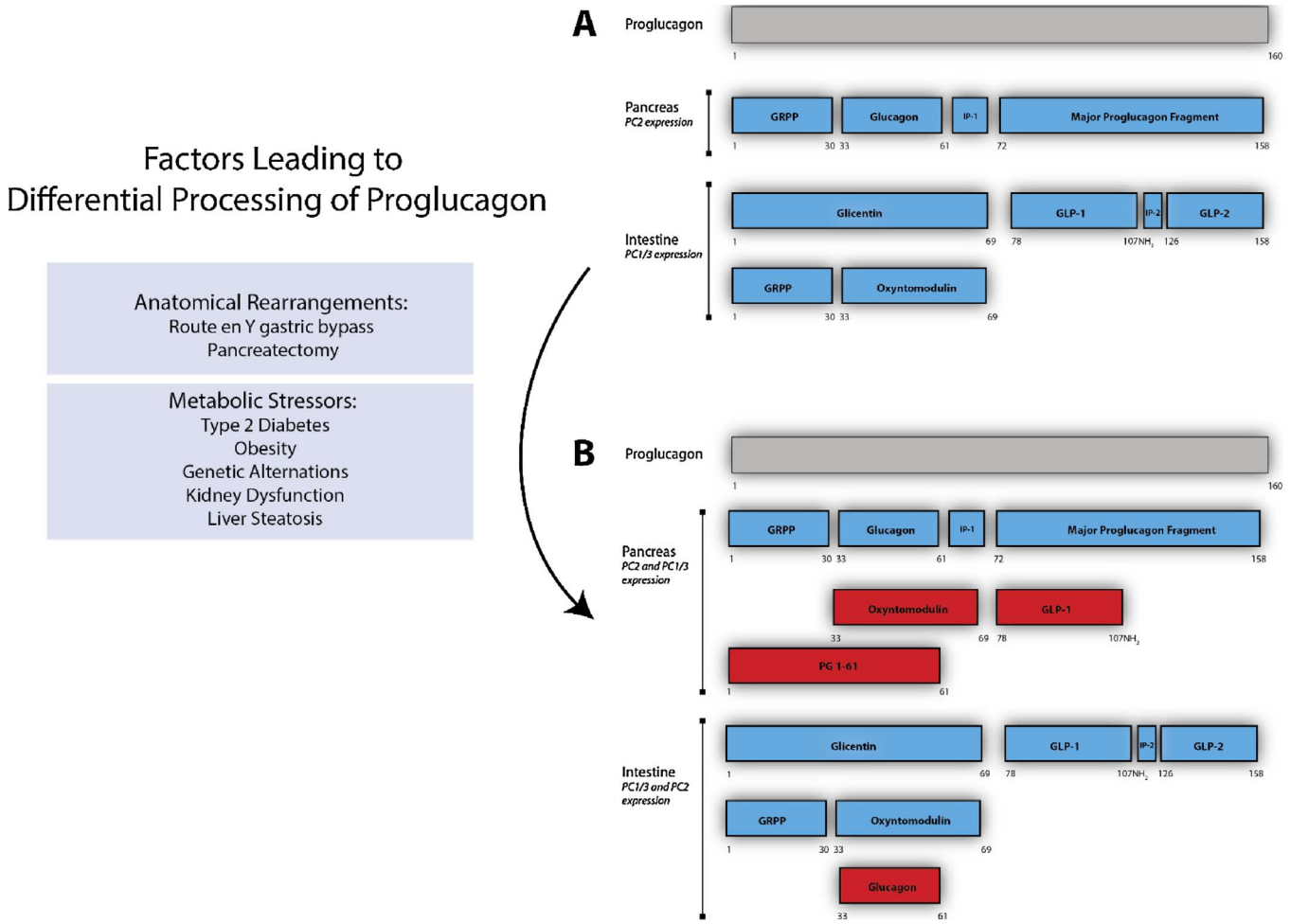

Fig. 1. Factors Leading to Differential Processing of Proglucagon.
A: The classical processing paradigm of proglucagon. Expression of prohormone convertase 2 (PC2) in the pancreatic alpha cells lead to the formation of glucagon and GLP-1 1–36NH_2 (not depicted) whereas expression of prohormone convertase 1/3 (PC1/3) in the intestinal L-cells leads to glicentin, oxyntomodulin, glicentin-related polypeptide (GRPP), glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2). Intervening peptide 1 (IP1) and intervening peptide 2 (IP2). Factors such as anatomical rearrangements and metabolic stressors may induce a differential processing pattern of proglucagon resulting in B: Expression of PC1/3 in the pancreatic alphacells leading to formation of oxyntomodulin and GLP-1, furthermore PG-1-61. Whereas in the intestinal L-cells expression of PC2 may led to the formation of glucagon.

methods and assay validation guidelines.

Whereas accurate measurement of glucagon in humans has been possible since the early 1950s proper measurements in rodents, have not. That said, plasma concentrations of glucagon have been reported in rodent studies [21] using C-terminal specific methods but what has been lacking is the secretory dynamics of glucagon to e.g. a glucagonotropic compound such as arginine. We (led by professor Holst, University of Copenhagen) recently contributed to the development of a sensitive low-volume (10 µL) sandwich ELISA, commercialized by Mercodia, which we validated in both rats and mice. Using this method we uncovered the secretory dynamics of glucagon in rodents and also demonstrated the extremely short half-life of glucagon [22]. This method furthermore, allow the clinician to discriminate between a 'true glucagonoma', which excretes excess number of glucagon molecules, compared to the tumors (primarily expressing prohormone convertase 1/3) with up to 1nmol/L (reference interval ~1–5 pmol/l at fasting) of active GLP-1 that results clinically in severe hypoglycemia [16]. As such this may guide us for stratified medicine in individuals with intractable glucagonomas: A patients with a primarily glucagon-secreting tumor may be treated with a glucagon receptor antagonist (GRA), and a patient with a GLP-1-and oxyntomodulin-producing tumor with exendin 9–39 (a GLP-1 receptor antagonist) as an addition to the standard care of somatostatin analogues.Therefore, accurate measurement of glucagon in a suitable assay is prerequisite for understanding the biology of glucagon in a variety of disease processes.

3. The molecular heterogeneity of hyperglucagonemia

To what extent the reported hypersecretion of glucagon in a variety of clinical conditions actually represents 'true' pancreatic glucagon is not well characterized [23]. The molecular heterogeneity of glucagon has primarily been investigated in subjects with glucagon producing tumors using radioimmunoassay and size-exclusion chromatography [24,25], but also normal subjects were investigated with this technique [26]. In these studies glucagon, beside of the canonical proglucagon products: oxyntomodulin, glicentin, GLP-1, GLP-2, have been reported however also N-terminal elongated glucagon-like molecules have been shown to exist. A study by Kuku et al. showed that in subjects with chronic renal failure, a glucagon-like molecule (high molecular weight ~9000 Da) exists [27]. In addition, a plasma component with a similar molecular weight was observed in uremic pigs and identified by chromatography as PG 1-61 [28]. Recently, we have described the identification of 'glucagon variants', using state-of the art mass-spectrometry based plasma proteomics, to which PG 1-61 was the major component beside native glucagon in a wide range of clinical conditions [29]. Interestingly, PG 1-61 seems mainly to be formed and detected in our circulation when the body is challenged metabolically such as due to hyperglycemia (Type 2 Diabetes) (Fig. 2B). Although, PG 1-61 may