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Review

Alpha cell dysfunction in type 1 diabetes

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ABSTRACT

Type 1 diabetes is characterized by selective loss of beta cells and insulin secretion, which significantly impact glucose homeostasis. However, this progressive disease is also associated with dysfunction of the alpha cell component of the islet, which can exacerbate hyperglycemia due to paradoxical hyperglucagonemia or lead to severe hypoglycemia as a result of failed counterregulation. In this review, the physiology of alpha cell secretion and the potential mechanisms underlying alpha cell dysfunction in type 1 diabetes will be explored. Because type 1 diabetes is a progressive disease, a synthesized timeline of aberrant alpha cell function will be presented as an attempt to delineate the natural history of type 1 diabetes with respect to the alpha cell.

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by selective destruction of the pancreatic beta cells [2,50]. Beta cell loss can occur over extended periods of time (months to years), but due to the remarkable compensatory capacity of the beta cells, overt diabetes symptoms such as hyperglycemia and resulting polydipsia and polyuria might not be present until ~80% of beta cells have been lost [2,50]. The Islets of Langerhans are comprised of multiple cell types that are intimately linked through an incompletely understood intercellular communication network, such that isolation of one islet cell type renders those cells incapable of a normal secretory response to their normal stimuli. Thus it is not surprising that the loss of beta cells during the progression of T1D leads to a progressive dysfunction of other islet cell types, particularly the alpha cells. Hyper- or hyposecretion of glucagon from the alpha cells can lead to significant and severe disruptions in glucose homeostasis, thus exacerbating hyperglycemia or preventing a normal counterregulatory response to hypoglycemia. The purpose of this review is to highlight the potential mechanisms underlying alpha cell dysfunction in T1D and to provide a synthesized timeline of the progression of the dysregulation of glucagon release in patients with T1D.

2. Regulation of glucagon secretion (Fig. 1)

Although the regulation of insulin release from the beta cells by glucose has been well characterized and described, low glucose-stimulated glucagon secretion from the alpha cell is not fully understood. Investigation of the mechanisms driving glucagon inhibition by glucose or stimulation by low glucose levels has been hampered by multiple species-specific differences in islet architecture and cell signaling. For example, although most studies of alpha cell function utilize mouse or rat models, rodent islets are characterized by the localization of beta cells predominantly in the center of the islet, surrounded by alpha, delta, and other cell types, which comprise the outer layer of the islets [40,53]. This is in sharp contrast to human islets, in which alpha, beta, and delta cells are randomly distributed throughout the islet [8,53]. Furthermore, while rodent islets are composed predominantly of beta cells (60–80% of total cell numbers, compared to 15–20% alpha cell content), the composition of the human islet is more heterogeneous, with fewer beta cells (50–60%) and higher numbers of alpha cells (30–45%) [5,6,8]. These differences could have important implications for the role of paracrine signaling and intercellular communication on alpha cell regulation and glucagon secretion. In addition, important species-specific variations in protein expression could drive differences in the regulation of glucagon secretion between mice, rats, and humans. While mouse alpha cells produce L-, N-, T-, and R-type calcium channels, rats alpha cells express L- and N-type calcium channels [14,16,29]. However, the expression of N-type calcium channels in mouse alpha cells is relatively low, and studies evaluating their activity using ω-connotoxin are hampered by the finding that ω-connotoxin exerts a non-specific effect in alpha cells [43]. Human alpha cells produce L-, T-, and P/Q-type calcium channels [41]. These differences could significantly impact how electrical coupling of glucose concentration to glucagon secretion is controlled between rodent models and humans. Thus, although human islets and purified alpha cells can be difficult to obtain, it is essential to confirm the findings of rodent alpha cell studies using human cells in order to fully understand human alpha cell physiology. However, comparative studies have highlighted fundamental similarities in the regulation of glucagon secretion with respect to both

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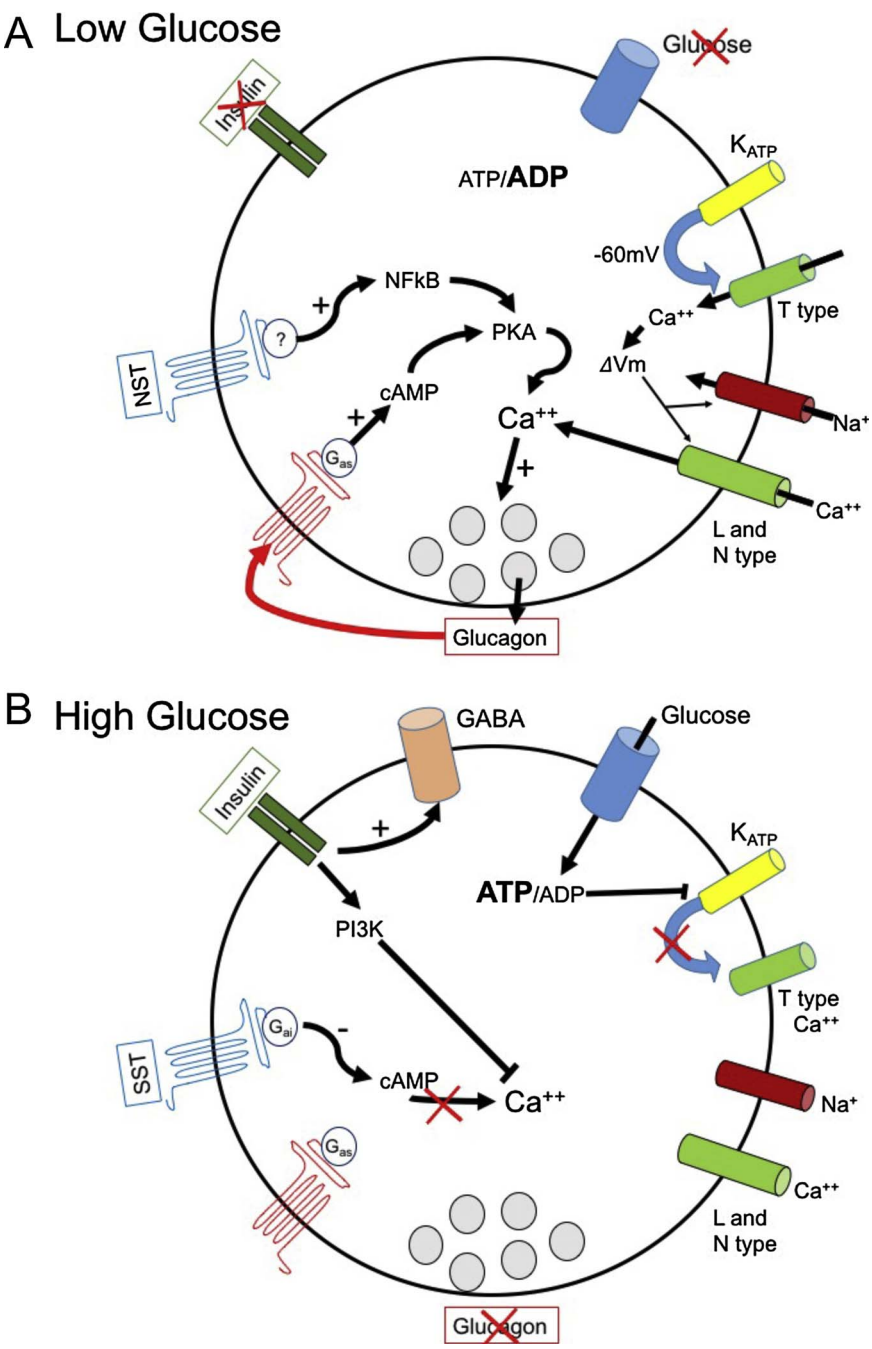

Fig. 1. Regulation of glucagon secretion. A) In low glucose, a decrease in the ATP/ADP ratio causes ATP-sensitive postassium channels (K_{ATP}) to generate a membrane potential of ~60 mV, leading to opening of low voltage (T type) calcium channels. This causes a downstream opening of sodium and high voltage (L and N type) calcium channels, intracellular accumulation of calcium, which drives fusion of glucagon-containing vesicles with the membrane and release of glucagon from the alpha cell. In addition, glucagon secretion is stimulated by neuronostatin (NST) from the delta cell and by glucagon itself. The inhibitory effect of insulin is negligible due to reduced release of insulin from the beta under low glucose conditions. B) In high glucose conditions, the ATP/ADP ratio increases, leading to closure of K_{ATP} channels and membrane depolarization to an extent that downstream ion channels involved in action potentials are deactivated. In addition, insulin exerts potent inhibitory actions through PI3 K and the translocation of GABA receptors to the membrane. Somatostatin (SST) from the delta cells inhibits cAMP production, thus reducing downstream calcium flux.

electrical activity and endocrine and paracrine signaling.

2.1. Electrical regulation of glucagon release

Like beta cells, alpha cells produce multiple ion channels that are capable of generating action potentials depending on extracellular glucose concentrations [16]. Many of these channels are similar or the same as proteins expressed by the beta cells, particularly the ATP-sensitive potassium channels, which couple extracellular glucose concentrations to calcium-stimulated hormone secretion in both beta and alpha cells. Several models of stimulus-secretion electrical coupling in alpha cells have been proposed and reviewed extensively elsewhere [40,44]. Briefly, the "regenerative model," [14,29,40] posits that at low glucose levels, the intracellular ATP/ADP ratio is low, causing the ATP-sensitive potassium channels to generate a membrane potential of approximately −60 mV. This results in the opening of low voltage-activated calcium channels (i.e. T-type), leading to membrane depolarization to the extent that sodium and high voltage-activated calcium channels (i.e. N-type) channels are activated, and subsequently the formation of regenerative action potentials and calcium-induced glucagon secretion (Fig. 1A). However, Rorsman and colleagues have shown that exposure to a suppressive concentration of glucose (6 mM) altered the electrical activity of alpha cells such that action potentials occurred at a higher frequency and lower amplitude [64]. This led to a reduction of P/Q-type calcium channel activity, which subsequently inhibited glucagon secretion [64]. Thus, stimulus-secretion electrical coupling may not be due to electrical silencing, but rather the result of electrical tuning. A second model suggests that exposure to high glucose inhibits secretion of glucagon via an ATP-sensitive potassium channel-independent mechanism [26,60]. In this model, a depolarizing calcium store-operated current would be suppressed. Regardless, the direct stimulus-secretion coupling of extracellular glucose concentration to

glucagon secretion remains controversial. Several studies using human and mouse alpha cells and islets indicate that low extracellular glucose concentrations directly stimulate, and high glucose inhibits, glucagon release [1,29], and ATP-sensitive potassium channels play an essential role in this coupling. Rorsman and colleagues have shown that the activity of ATP-sensitive potassium channels in alpha cells is low under hypoglycemic conditions and completely inhibited in high glucose [64]. Closure of these channels leads to membrane depolarization, inactivation of sodium channels, and ultimately reduced calcium-mediated exocytosis of glucagon [64]. In humans, a single nucleotide polymorphism in the KCNJ11 subunit of ATP-sensitive potassium channels has been linked to inadequate suppression of glucagon secretion in response to high blood glucose levels [54]. However, paracrine factors, particularly other islet hormones such as insulin and somatostatin, also appear to be critical regulators of glucagon release from alpha cells.

2.2. Endocrine and paracrine regulation of glucagon release

In human islets, the random distribution pattern of islet cell types promotes intracellular communication and the opportunity for paracrine and autocrine regulation of hormone secretion [8]. Alpha cells express numerous hormone receptors, the activation of which initiates inhibitory or stimulatory effects on alpha cell secretory activity. Insulin appears to be the major inhibitory factor regulating glucagon release through multiple intracellular mechanisms [40]. First, insulin, acting via its tyrosine kinase receptor, has been shown to inhibit glucagon release from alpha cells by activating phosphatidylinositol 3 kinase (PI3 K) [20]. Insulin also stimulates the translocation of GABA receptors to the alpha cell membrane, sensitizing the alpha cell to GABA produced by the beta cells [61]. Furthermore, insulin hyperpolarizes the alpha cell membrane by increasing the activity of ATP-sensitive potassium channels, resulting in reduced glucagon release [25]. Somatostatin, produced by the delta cells of the islet, also exerts inhibitory actions on the alpha cell via interaction with its G protein coupled receptors, SSTR1-5, which are coupled to $G_{\alpha i}$. The predominant somatostatin receptor expressed by the alpha cells is SSTR2 [19,23,52]. Engagement of this receptor by somatostatin inhibits adenylyl cyclase and cAMP production, leading to a downstream reduction in calcium flux (Fig. 1B). Additionally, somatostatin can affect alpha cell electrical activity by activating potassium channels and inducing membrane hyperpolarization [15].

Recently an additional, biologically active peptide hormone encoded by the somatostatin preprohormone was identified [48]. This hormone, neuronostatin, appears to have an opposite, complementary effect on the alpha cell to that of somatostatin (Fig. 2). Treatment of isolated rat islets or immortalized mouse alpha cells with neuronostatin enhanced both proglucagon mRNA expression and glucagon secretion in response to low glucose [11,46]. Using a unique Deductive Ligand-Receptor Matching Strategy [63], the neuronostatin receptor was identified as the orphan G protein coupled receptor, GPR107, which is expressed by alpha cells, but not beta cells [11]. The activation of GPR107 on alpha cell membranes stimulates glucagon production and release via the phosphorylation of protein kinase A (PKA) (Fig. 1A). Interestingly, neuronostatin does not alter cAMP levels in alpha cells, but rather activates PKA through a cAMP-independent, nuclear factor kB (NFkB)-dependent mechanism [11]. Elevated cAMP has been shown in multiple studies to enhance glucagon release; however, the studies describing neuronostatin signaling suggest that molecules downstream of cAMP, such as PKA or NFkB, instead of or in addition to cAMP, could serve as molecular hinge points responsible for regulating ultimate vesicular fusion of glucagon release from alpha cells.

Like somatostatin, neuronostatin is produced by the delta cell of the islet [48], and thus could act as a paracrine regulator of glucagon secretion. However, somatostatin and neuronostatin are produced by other gastrointestinal tissues as well, including the D cells of the stomach and intestine [48]. Thus, plasma levels of neuronostatin, which are elevated in the fasted state [11], could reflect either islet or intestinal production. Although the physiology of neuronostatin has not been fully elucidated, the peptide has been proposed as a novel counterregulatory hormone that primarily functions as a positive regulator of glucagon production and release [47]. The production of neuronostatin in digestive organs could also indicate that neuronostatin potentially functions similarly to that of glucagon like peptide 1 (GLP-1), which primes beta cells to release insulin in response to a meal. Likewise, neuronostatin could act as a priming factor for the alpha cell, which could enhance the alpha cell response to low glucose. However, this hypothesis remains untested.

In addition to neuronostatin, a well-known positive regulator of glucagon secretion is glucagon itself, which acts in an autocrine fashion to enhance glucagon release by stimulating intracellular cAMP levels [28] through an interaction with a GPCR coupled to Gas. Furthermore, alpha cells have been shown to secrete non-classical islet peptides, including glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [35,59]. These incretins can exert autocrine activity in the alpha cell to reduce (GLP-1) or enhance (GIP) glucagon secretion through an interaction with their respective GPCRs [10,18,49]. Although several hormone-stimulated intracellular signaling molecules, particularly cAMP, have been identified as important regulators of glucagon secretion, these intracellular signaling events are clearly coupled to the electrical activity of the alpha cell membrane. Thus, in an intact physiological system, both paracrine factors and membrane potential differences play essential roles in alpha cell secretory activity (Fig. 1).

3. The alpha cell in type 1 diabetes

In the setting of type 1 diabetes, the islet undergoes multiple cellular and subsequently endocrine changes that ultimately lead to metabolic homeostatic failure and hyperglycemia. T1D is characterized by a selective loss of beta cell mass and insulin secretion, which play an important role in the eventual resultant hyperglycemia. However, multiple lines of evidence suggest an alternative view, championed by Unger and colleagues [55], that glucagon hypersecretion is the major culprit in T1D-associated hyperglycemia. Indeed, disruption of glucagon receptor signaling via global knockout prevented the metabolic derangements associated with T1D in streptozotocin-treated mice [24]. Similarly, treatment with glucagon receptor antagonists appears to improve glycemic control in both rodents and humans with diabetes [3,21,22,30,33,36,56], suggesting that glucagon receptor blockade could be an effective treatment for patients with diabetes and further substantiating the hypothesis that diabetes-associated hyperglycemia is primarily due to alpha cell dysfunction. It is important to remember that T1D is a progressive disease, and that study of alpha cell function during the different stages of T1D has been hampered by the inability to detect T1D early in development (i.e. before significant beta cell loss and overt hyperglycemia) and by a paucity of pancreas tissue from patients with T1D available for research. Thus the existing data on alpha cell function across the natural history of T1D in humans is limited to “snapshots” of distinct points in time, and animal studies are limited by species-specific differences, as highlighted above. Nonetheless these data contain valuable information particularly when viewed in light of complementary studies conducted at other time points.

3.1. Pathology of T1D progression

As opposed to the beta cell component of the endocrine pancreas, which declines in number and total mass as T1D progresses, alpha cells have been shown in humans and in multiple rodent models, including non-obese diabetic (NOD) mice and streptozotocin (STZ)-treated mice and rats, to be hypertrophic and hyperplastic [13,37,39]. In a comprehensive study of changes in alpha cell mass across T1D progression

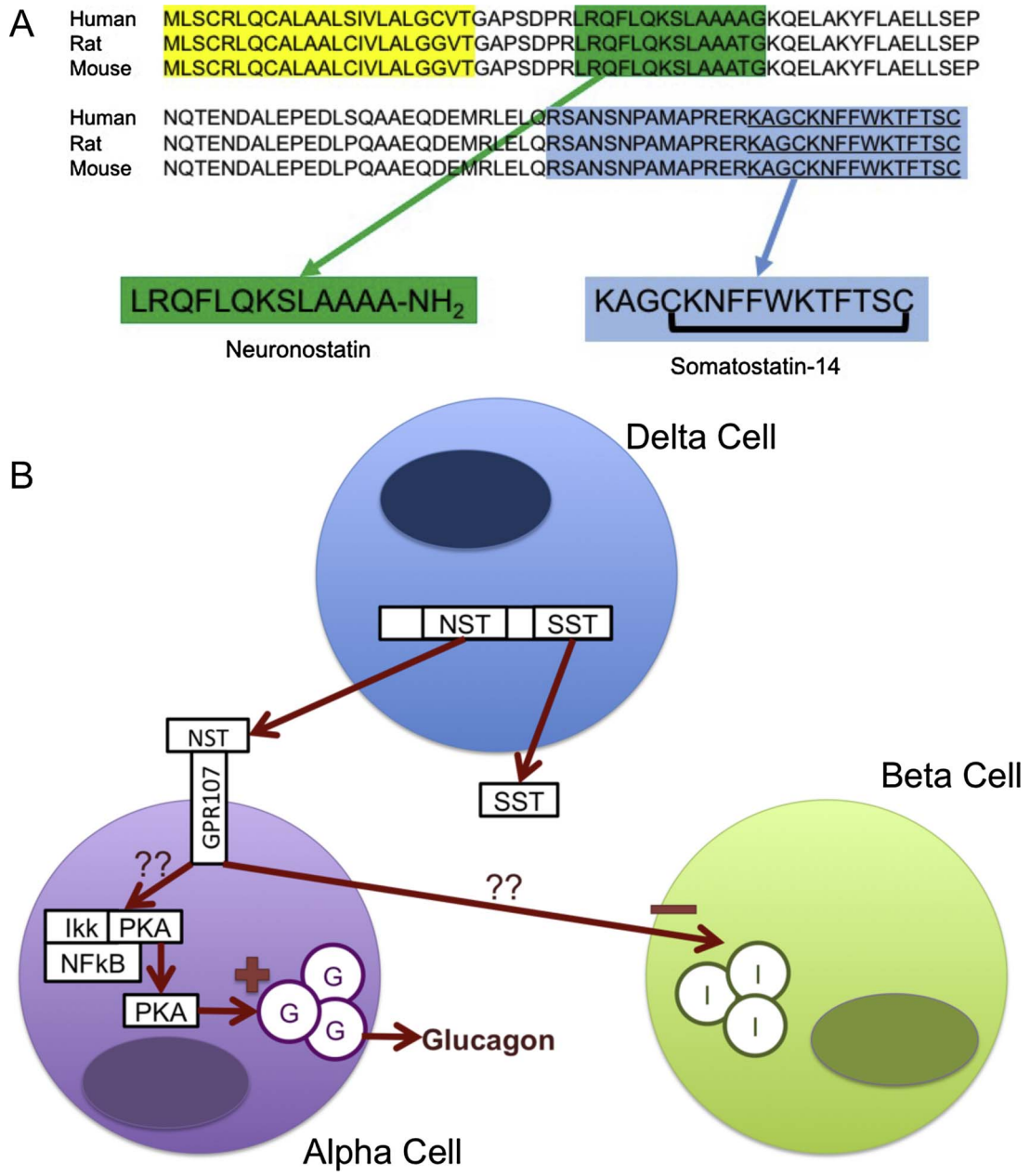

Fig. 2. Summary of neuronostatin production and action. A. Neuronostatin (NST) is derived from the somatostatin (SST) preprohormone, and is largely conserved across multiple species, including human and rodents. Unlike SST, which is cyclized by disulfide bonds (denoted by black bar), NST is C-terminally amidated. B. NST is produced by delta cells and acts through the GPCR, GPR107, which is produced by alpha cells, but not beta cells, to stimulate protein kinase A (PKA) activity. NST stimulates PKA activity by enhancing the release of PKA from the NFkB complex (independent of cAMP) through the action of an unknown G protein. This leads to enhanced release of glucagon (G) from the alpha cell, as well as increased glucagon mRNA expression. NST also inhibits insulin (I) release from beta cells through a direct action on the alpha cells via an unknown mechanism.

in NOD mice, alpha cell mass was increased compared to wildtypes at four weeks of age, prior to full diabetes onset; however, no changes in alpha cell numbers or mass were detected during T1D progression from 4 to 24 weeks of age [39]. However, alpha cell hypertrophy was detected in STZ-treated mice following development of overt diabetes. These studies have been contested in several reports in which a decline [38] or no change in alpha cell mass [9] was observed. These discrepancies could be explained at least in part by timing of tissue collection, since any changes in alpha cell mass appear to occur early in disease progression, prior to overt hyperglycemia [39].

Alpha and beta cells of the islet share a common embryonic origin, and several reports have demonstrated the ability of beta cells to transdifferentiate into alpha cells [51]. Likewise, multiple lines of evidence suggest that alpha cells can transdifferentiate into beta cells [7,32,34,62], indicating remarkable plasticity of the pancreatic islet cells. Thus, an increase or decrease in alpha cell numbers could be explained in part by transdifferentiation of beta cells to alpha cells or vice versa, respectively, although this remains controversial. A simpler explanation of alpha cell hypertrophy or hyperplasia could lie in the loss of insulin-mediated inhibition of alpha cell secretory activity, eventually leading to aberrant alpha cell expansion and ultimately hyperglucagonemia and hyperglycemia.

3.2. Natural history of alpha cell dysfunction in type 1 diabetes (Fig. 3)

Regardless of the mechanisms underlying the pathological changes in alpha cell numbers or mass associated with T1D, the alpha cell undergoes significant functional changes that dramatically affect glucose

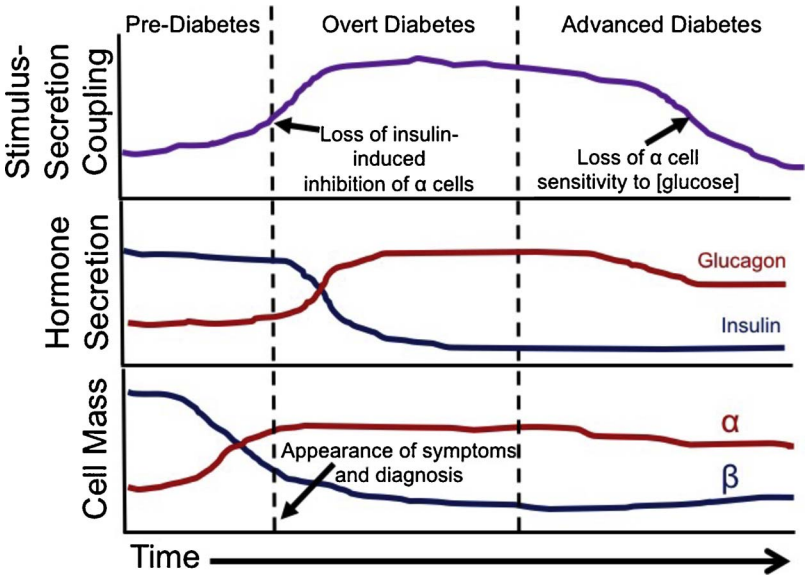

Fig. 3. Natural History of Alpha Cell Dysfunction in Type 1 Diabetes. Changes in alpha cell mass, glucagon secretion, and stimulus-secretion coupling in the alpha cell potentially underlie alpha cell dysfunction in patients with type 1 diabetes. Pre-diabetes represents the period (months to years) prior to diagnosis of type 1 diabetes. Overt diabetes is the period from diagnosis to approximately 10 years following diagnosis. Advanced diabetes indicates established disease, 10 years or more from diagnosis of type 1 diabetes.

homeostasis. Since T1D is a progressive disease, the functional implications of alpha cell dysfunction depend on the stage of T1D of the individual patient (Fig. 3). During the pre-diabetic state, prior to the appearance of hyperglycemia-related symptoms (i.e. polydipsia and polyuria), the beta cells are selectively destroyed via autoimmune mechanisms, and the alpha cell component begins to expand. However, because beta cells are able to compensate for cell loss by increasing insulin secretion, insulin levels and blood glucose levels, and consequently glucagon release remain relatively normal. The stimulus-secretion coupling of the alpha cells, or the ability of the alpha cells to respond appropriately to extracellular glucose concentrations and paracrine signals, should remain intact during this period. Following loss of ~80% of the beta cell mass, insulin secretion fails, leading to deficient glucose disposal by insulin sensing tissues such as skeletal muscle, and ultimately hyperglycemia. During this phase, the patient becomes overtly diabetic and develops symptoms of diabetes. The expanded alpha cell mass, uninhibited by insulin, begins to secrete aberrantly high levels of glucagon, leading to a significantly reduced insulin to glucagon ratio, and enhanced glucagon-stimulated catabolism, driving hepatic glucose production. Stimulus-secretion coupling of the alpha cell is impaired, due at least in part to the loss of insulin signaling, which causes changes in the electrical activity of the alpha cell membrane. Even if treatment is successfully initiated and glycemia is well-managed, this phase is still often marked by hyperglucagonemia and subsequent hyperglycemia, particularly following a meal. As time from diagnosis increases, particularly after 10 or more years from diagnosis (advanced diabetes), alpha cell mass likely is not further affected, but stimulus-secretion coupling is reduced leading to a loss of adequate counterregulation, and potentially life-threatening hypoglycemia. Thus, the natural history of alpha cell dysfunction is associated with two distinct phenomenon, hyperglucagonemia and impaired counterregulation, with separate etiologies and clinical implications.

3.2.1. *Hyperglucagonemia and exacerbation of hyperglycemia*

As in patients with type 2 diabetes (T2D), patients with T1D tend to exhibit hyperglucagonemia in spite of high blood glucose levels. Unger and colleagues propose that this paradoxical increase in glucagon release is the driving factor underlying hyperglycemia in T1D and T2D [55]. Patients with T2D exhibit high basal levels of plasma glucagon as well as postprandial hyperglucagonemia, which significantly contributes to high basal and postprandial blood glucose levels [27]. In patients with T2D, hyperglucagonemia is thought to be due to resistance of the alpha cell to the suppressive effects of insulin and hyperglycemia, as well as to the dysregulation of incretins (e.g. glucose-dependent insulinotropic polypeptide, GIP, and glucagon-like peptide 1, GLP-1), which can alter glucagon release [27]. Hyperglucagonemia associated with T1D has a complex etiology that includes (as mentioned above) potentially functional and physical alpha cell hypertrophy and hyperplasia as well as disinhibition of alpha cells due to the loss of the influence of insulin. These processes could be influenced by the relative resistance of alpha cells to glucose toxicity, lipotoxicity, and inflammatory cytokines [57,58]. While beta cell viability and secretory function are negatively impacted by high glucose, lipids, and inflammatory cytokines, such as IL1beta, alpha cell mass and glucagon secretion are enhanced in the presence of these factors [57,58]. In addition, the concentration response curve of the effect of glucose on alpha cells appears to be bell-shaped [17,45]. The functional implications of this bell-shaped concentration response curve are that while low glucose stimulates glucagon release, and moderately high glucose levels inhibit glucagon secretion, very high glucose concentrations can increase glucagon release from alpha cells. Thus, extreme hyperglycemia, such as that observed in some patients with T1D at the time of diagnosis, and in non-controlled patients with T1D, or initially high plasma glucose levels that occur postprandially, could lead to aberrant glucagon secretion. This could initiate a vicious cycle, in which very high glucose increases glucagon release, which in turn acts in an autocrine fashion to stimulate further glucagon release and subsequently hepatic glucose production. The cellular mechanisms mediating the bell-shaped concentration response curve of alpha cells to glucose are unclear, but could include changes in ion channel expression or function or the capacity of alpha cell specific glucose transporters. Further research is necessary to determine the underlying causes of hyperglucagonemia in patients with T1D and to identify potential therapeutic targets to treat hyperglucagonemia, particularly postpradial hyperglucagonemia, which is a common and serious problem in patients with T1D.

3.2.2. *Failure of counterregulation in response to hypoglycemia*

Patients with diabetes who have experienced multiple hypoglycemic events or who have an increased disease duration, particularly those who are greater than ten years from diagnosis, are susceptible to a condition known as “hypoglycemia unawareness.” Hypoglycemia unawareness is associated with an inability to recognize the normal signs of hypoglycemia, such as sweating and shakiness, which can delay self-treatment and lead to dangerously low plasma glucose levels [4,42]. Patients with hypoglycemia unawareness therefore are at an increased

risk of developing severe hypoglycemia, in which they are unable to self-treat, which can lead to seizures, coma, and death. Hypoglycemia unawareness is often associated with blunted hormonal responses to hypoglycemia. In particular, glucagon secretion is impaired, leading to decreased hepatic glucose production in spite of dangerously low blood glucose levels [4,12]. The failure of counterregulation in patients with advanced diabetes has been shown to be due to insufficient glucagon secretion, but not glucagon production; adequate glucagon peptide is synthesized but it is not released, as the stimulus-secretion coupling of extracellular glucose concentration to glucagon secretion is impaired (Fig. 3) [4,12,31]. Numerous potential mechanisms could underlie this failure of counterregulation in response to hypoglycemia, including aberrant glucose transport or ion channel activity, as well as a loss of paracrine signaling or an inability to respond to paracrine signals. For example, reduced release of neuronostatin, which increases glucagon secretion in isolated islets and immortalized alpha cells, could lead to impairment of glucagon secretion [11,46,47]. Alternatively, decreased expression of GPCRs in alpha cells, such as the glucagon receptor and the neuronostatin receptor, GPR107, could desensitize the alpha cells to these positive secretory signals. Given that up to 40% of patients with diabetes are impacted by some level of hypoglycemia unawareness and associated insufficient counterregulation, investigation of these potential mechanisms and the development of therapeutic strategies to restore glucagon secretion in these individuals are essential.

4. Conclusions

Alpha cell dysfunction in T1D is a complex phenomenon that changes over the course of disease progression and can significantly impact glucose homeostasis, even in patients practicing intensive insulin therapy. The physiological consequences of alpha cell dysfunction include a propensity for both hyperglycemia due to paradoxical hyperglucagonemia, particularly postpradial hyperglucagonemia, and insufficient glucagon release resulting in hypoglycemia and hypoglycemia unawareness. Emerging therapies designed for optimal glucose regulation, such as beta cell replacement and closed-loop artificial pancreas systems, as well as glucagon receptor antagonists, are promising technologies that could eventually mitigate the hyperglycemia and hypoglycemia associated with alpha cell dysfunction and T1D. However, new therapeutic strategies must be developed to bridge the gap to treat or, hopefully prevent aberrant glucagon secretion in patients with T1D.

Disclosures

Dr. Yosten has nothing to disclose.

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