

Sklopitev med stimulacijo in sekrecijo in medcelične interakcije celic alfa v zdravju in pri sladkorni bolezni tipa 2

Alpha Cell Stimulus–Secretion Coupling and Intercellular Interactions in Health and Type 2 Diabetes

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Izvleček

Dolgo je veljalo, da je temelj patogeneze sladkorne bolezni tipa 2 motnja delovanja celic beta, danes pa se zdi bolj realističen dvohormonski model bolezni. Celice alfa imajo pomembno vlogo pri homeostazi glukoze zaradi komunikacije z drugimi celicami v Langerhansovih otočkih in tkivih, kot so jetra, z delovanjem glukagona. Čeprav natančno izoblikovanega modela njihove fiziologije še nimamo, novi pristopi omogočajo boljši vpogled v poti izločanja glukagona in v procese, ki ga uravnavajo, kar omogoča boljši vpogled v delovanje celic alfa v zdravju in bolezni z možnimi posledicami za zdravljenje sladkorne bolezni tipa 2.

Abstract

Beta cell dysfunction has long been thought to be at the root of type 2 diabetes pathogenesis; however, a bi-hormonal model of the disease appears to be more realistic. Alpha cells play a significant role in glucose homeostasis due to their communication with other cells within the islets of Langerhans and tissues through glucagon action. Despite the lack of a concise model for their physiology, new approaches provide more insight into the pathways of glucagon secretion and processes that regulate it. This increased understanding of alpha cell function in healthy and diseased states has potential implications for managing type 2 diabetes.

INTRODUCTION

The existing understanding of type 2 diabetes mellitus (T2DM) places a strong emphasis on insulin malfunction. However, mounting evidence shows that diabetes mellitus is a bi-hormonal illness, and glucagon may play a critical role in maintaining glucose homeostasis (1,2). Disinhibited alpha cells produce excessive amounts of glucagon in the absence of insulin compared with the normal paracrine inhibition of glucagon secretion by glucose-responsive beta cells. This directly causes diabetic hyperglycemia by increasing the rate of hepatic glycogenolysis and gluconeogenesis and decreasing the rate of hepatic glucose uptake and glycogen synthesis (3). In light of the emerging glucagon-centered concept of diabetes, this review briefly examines the role of alpha cells in glucose homeostasis.

ARCHITECTURE OF THE ISLET OF LANGERHANS

Most pancreatic endocrine cells are arranged into microorgans called islets of Langerhans (4), which secrete at least five hormones: glucagon, insulin, somatostatin, pancreatic polypeptide, and ghrelin from alpha, beta, delta, gamma, and epsilon cells (5). The distribution of cell types within islets follows

distinct architectural patterns (4). The mantle-core pattern, found in mice, has the core populated almost exclusively by beta cells and the mantle by non-beta cells (6). Figure 1 shows a mouse islet with immunolabeled alpha cells in the islet mantle. In conjunction with specific microcirculation, this organization affects alpha cell physiology (7). Approximately two-thirds of mouse islets perfuse beta cells in the core first and then alpha cells in the mantle with blood containing insulin, facilitating beta cell paracrine action on alpha cells and suppressing glucagon secretion during a high glucose load (1). The paracrine action appears reciprocal, since glucagon potentiates cyclic adenosine monophosphate (cAMP)-mediated insulin secretion and glucagon blocking reduces insulin release, leading to glucose intolerance in vivo (8,9). Alpha cell glucagon secretion is further controlled by intrinsic and neuronal mechanisms (10,11).

Another important aspect of islet architecture is methodological because endocrine cell function studies are often limited to islet surface layers. The two most common methods, confocal measurement of intracellular calcium ion dynamics and electrophysiological assessment of ion channels and membrane potential in isolated islets, are limited to the superficial one to two cell layers (12,13). The mantle-

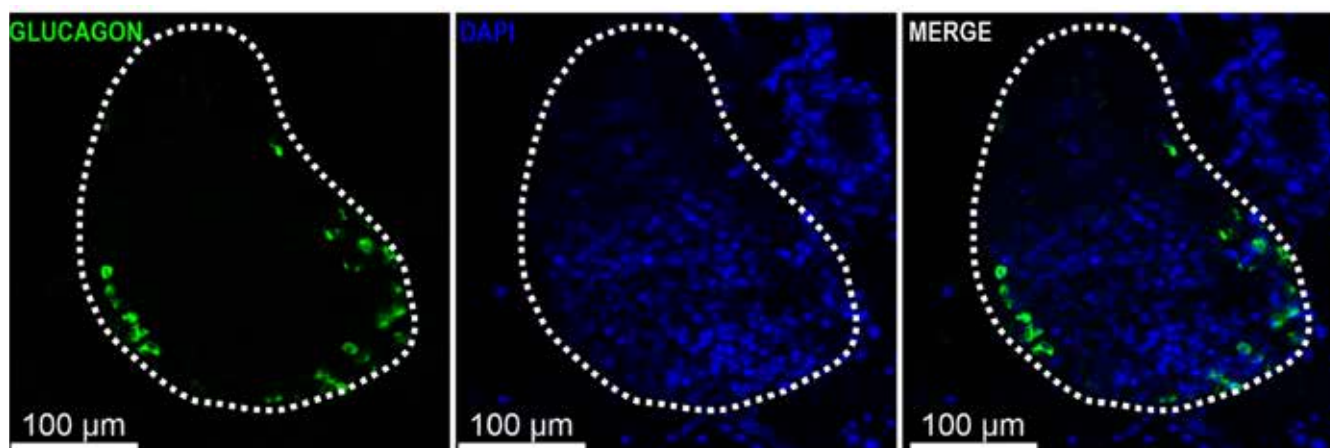


Figure 1. Morphological identification of alpha cells in mouse pancreas tissue slices. Immunolabeling of alpha cells for glucagon (with Ab against glucagon, green, left panel), labeled nuclei (blue, middle panel), and merged channels (right panel). The blue nuclei outside of the islet (broken white line) belong to acinar and ductal cells.

core pattern in mice limits the study to superficial alpha cells, rendering alpha-beta cell interaction somewhat inaccessible, and the enzymatic surface layer damage during islet isolation further complicates the investigation (14). However, the alternate acute tissue slice method, in which the islet core is removed and exposed, has proved successful in examining all mouse islet cell types (12,13).

Human islets have a similar but somewhat more complex microarchitecture (4). They consist of a layer of beta cells flanked by two surrounding layers of alpha cells that fold into U- or O-shapes within an islet (15). Despite unclear perfusion patterns, paracrine alpha-beta interactions are suggested to have a similar function in glucose homeostasis in humans as in rodents (4).

PHYSIOLOGY AND FUNCTIONAL IDENTIFICATION OF ALPHA CELLS

The stimulus-secretion coupling (SSC) pathway for insulin secretion is widely known. An increase in blood glucose concentration promotes glucose uptake by beta cells and its metabolism to adenosine triphosphate (ATP) via glycolysis and oxidative phosphorylation. The nascent ATP causes the closing of the ATP-dependent potassium channels (K_{ATP}), resulting in plasma membrane depolarization. This opens voltage-dependent calcium channels (VDCC), resulting in an influx of calcium ions that trigger the exocytosis of insulin-containing granules (16).

The mechanisms controlling glucagon release in alpha cells are less clear, despite their importance in glycemic control, mainly due to the small number of alpha cells in animal model islets and the lack of suitable methods to identify them in the past (17,18). The fundamental question is whether glucose controls alpha cell glucagon release directly or through beta and delta cell paracrine mechanisms. K_{ATP} and some types of VDCC channels that mediate electrical activity are similar in alpha and beta cells (19). However, important differences may explain how glucose affects the two cell types differently. First, K_{ATP} channel activity in alpha cells is optimal for glucagon secretion at lower glucose concentrations and is maximally inhibited by roughly 7mM glucose, whereas beta cell insulin secretion is highest at high

glucose concentrations (20). Second, although glucose-induced insulin secretion in mouse beta cells highly depends on L-type VDCCs (21), glucagon secretion from alpha cells appears to be more dependent on non-L-type VDCCs, most likely P/Q-type VDCCs, which surprisingly account for only a small fraction of total Ca^{2+} entry (19,22). Third, voltage-dependent Na^+ channels (VDSCs) are more critical for the electrical activity of alpha cells than for beta cells (23). Their involvement in action potential generation in alpha cells may explain why K_{ATP} channel closure by increased glucose concentrations does not facilitate glucagon secretion like insulin secretion from beta cells. When glucose concentration increases and K_{ATP} channels close, a sustained depolarization ensues, leading to the inactivation of VDSCs. This, in turn, disrupts the SSC pathway and causes a decrease in glucagon secretion (19). Finally, glucose appears to decouple $[Ca^{2+}]_c$ activity from glucagon secretion in alpha cells because a reduction in whole-cell $[Ca^{2+}]_c$ does not suppress glucagon secretion, even if $[Ca^{2+}]_c$ activity is required for it (24,25). This suggests that $[Ca^{2+}]_c$ permits rather than regulates glucagon secretion.

In the so-called electrophysiological model (26), glucose directly influences glucagon secretion. It adapts the SSC pathway of beta cells and accounts for most differences described earlier. It proposes that low ATP concentrations, as present at low glucose levels, stimulate the production of continuous action potentials in alpha cells via the VDSCs and VDCCs. This allows the influx of Ca^{2+} via the P/Q VDCCs to trigger glucagon granule fusion. At higher glucose concentrations, stronger inhibition of K_{ATP} channels results in sufficient membrane depolarization to inhibit VDSCs, thereby reducing the ability of P/Q-type VDCCs to open and trigger glucagon secretion. This model incorporates many physiological observations associated with glucagon secretion; however, competing models with varying degrees of validity have been proposed (20).

Although glucose directly affects glucagon release, paracrine mechanisms also regulate it. Zn^{2+} ions, gamma aminobutyric acid (GABA), insulin, and somatostatin have all been proposed to impact glucagon secretion; however, the evidence for all but somatostatin is inconsistent. Somatostatin appears to inhibit glucagon

secretion tonically, an effect that is not seen in somatostatin knockout mice. In fact, glucagon release in the absence of somatostatin and the presence of 30mM glucose is comparable to that when no glucose is present in control mice, mainly due to the influence of somatostatin (27).

In addition, perfusion experiments in the whole pancreas and batches of isolated islets showed that beta and delta cells secreted insulin and somatostatin synchronously, while glucagon secretion from alpha cells was antisynchronous (28,29). No definitive conclusions can be made about the role of intrinsic mechanisms and paracrine factors in glucagon secretion. However, it appears that intrinsic mechanisms regulate secretion under low-glucose conditions when the somatostatin concentration is low, whereas somatostatin is important at higher glucose levels. However, solid empirical evidence for this is still lacking (20). Besides intrinsic control and paracrine factors, hormones and neurotransmitters, such as acetylcholine, epinephrine, norepinephrine, glutamate, and glucose-dependent insulinotropic polypeptide, to name a few, also stimulate glucagon secretion, with epinephrine and norepinephrine being among the most potent ones (20). Several methods have been developed for identifying alpha cells within an islet, ranging from the use of transgenic mice with fluorescent protein expression (18), immunostaining (30,31), cell sorting (32), and electrophysiological methods (33), but each has certain drawbacks. Often, these methods are used in conjunction with collagenase digestion and subsequent culture of isolated islets or dispersed cells. Digestion exposes islets to injuries, resulting in functional changes (14,34), and the dispersal to individual cells deprives them of their in vivo environment and connections, resulting in changes in their function (18). Furthermore, methods such as patch clamp are limited to studying one or two cells at once, while immunostaining deforms the tissue, leading to the loss of information. Functional identification, which uses a stimulation protocol to distinguish one cell type from others based on their biological processes, largely circumvents these drawbacks. The most commonly used agents for the functional identification of alpha cells are some of the aforementioned secretagogues to which alpha cells

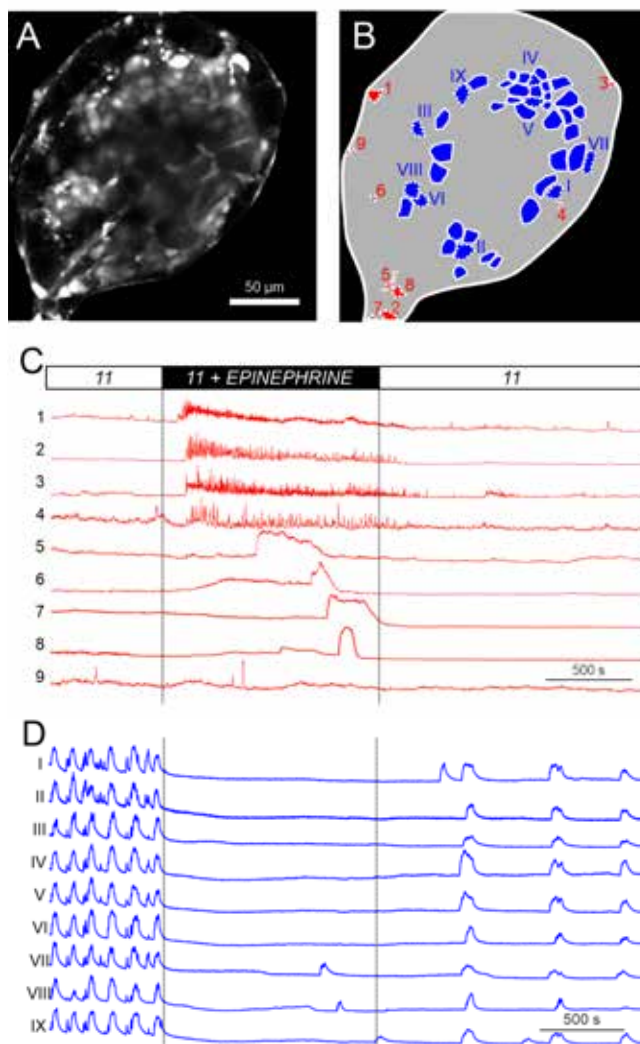


Figure 2. Functional identification of alpha cells in mouse pancreas tissue slices. (A) Cells from an islet of Langerhans in a mouse pancreas tissue slice. The cells were loaded with a calcium indicator dye. (B) A mask of alpha cells (in red) and beta cells (in blue) from panel A. Beta cells, whose activity is depicted in panel D, are indicated with dashed lines in panel B. (C and D) Functional discrimination of alpha cells and beta cells: while stimulated with 11 mM glucose, alpha cells are mostly inactive (C), while beta cells display their typical fast oscillation pattern (D). Addition of 1 μ M epinephrine activates alpha cells with a delay (C) and inhibits beta cells immediately and almost completely (D). No synchronization exists between calcium traces of neighboring or distant alpha cells, whereas beta cells are globally synchronized by means of propagating calcium waves (C and D). Note that the activity of beta cells is less following the period of complete inhibition than it was earlier.

respond, such as epinephrine, glutamate, and glucose (22,30,31,35–37). Three calcium activity patterns separate pancreatic islet cells stimulated by glucose: (1) alpha cells are active at 0.5 and 3 mM glucose but not at 11 mM glucose; (2) delta cells are active at 3 and 11 mM glucose but not at 0.5 mM glucose; and (3) beta cells are active at 11 mM but not at 0.5 and 3 mM glucose (22,30,35). However, because these protocols can be extremely time-consuming, the alpha cells in an islet are better identified by their response to glutamate, epinephrine, or both. Both are known to elicit $[Ca^{2+}]$ activity in alpha cells (35,36), while epinephrine also inhibits activity in beta (35,36) and delta cells (38). However, the concentration of epinephrine sufficient to inhibit $[Ca^{2+}]$ activity in beta cells seems to depend on the glucose concentration (11). The observed difference in responses between alpha and beta cells can be used to functionally discriminate the two major cell types, as shown in Figure 2.

ROLE OF ALPHA CELLS IN TYPE 2 DIABETES MELLITUS

T2DM is a complex multifactorial disease characterized by insulin resistance, beta cell secretory insufficiency, resultant hyperglycemia, and poor glucose tolerance (39,40). However, the complementary abnormalities of glucagon secretion, such as a diminished alpha cell secretory response to hypoglycemia and an inappropriately high glucagon secretion during hyperglycemia, also contribute to the development of T2DM (20).

Some of the factors contributing to the pathology of T2DM are directly related to alpha cell dysfunction. The glucose sensitivity in alpha cells of patients with T2DM appears to be impaired, as glucose concentrations that have a glucagonostatic effect in healthy individuals do not suppress glucagon secretion or even have a glucagonotropic effect (41,42). Furthermore, the V-shaped glucose response, generally seen only in isolated cells, in which the glucagon release is the highest at extreme glucose concentrations (0 or 10–20mM) and the lowest at about 7mM glucose, can be seen in intact islets of diabetic donors (20,43). The alpha cells

from diabetic donors also appear to lack P/Q-type Ca^{2+} channel activity at low glucose concentrations compared with healthy donors, decreasing exocytosis. Moreover, an increase in glucose concentration slightly promotes Ca^{2+} channel activity and exocytosis in cells from T2DM donors, suggesting that the typical suppression of P/Q-type Ca^{2+} activity by glucose is disrupted under these conditions (44). However, the peak total Ca^{2+} current is similar in cells from nondiabetic and diabetic donors (43). Hyperglycemia also causes alpha cells to lose their identity and transdifferentiate into beta cells in response to increased insulin demand, which is supported by an increase in bi-hormonal insulin/glucagon-positive cells that may be responsible for excessive glucagon secretion (45). How intrinsic alpha cell alterations promote T2DM development is unclear.

In addition, paracrine, endocrine, and neuronal changes may contribute to alpha cell dysfunction in T2DM. At first glance, beta cell mass reduction in T2DM can decrease circulating insulin and paracrine suppression of glucagon release by alpha cells. However, this does not seem to be the case (20,46). A similar explanation is even less likely for somatostatin-secreting delta cells, as their numbers do not decrease in T2DM (20). The inability of alpha cells to respond to glucose perturbations is likely due to impaired insulin and somatostatin inhibition. Somatostatin, and insulin, to some extent, stop electrical activity and exocytosis in intact islets from nondiabetic donors. This phenomenon is lost in islets from T2DM donors (43). It is therefore not surprising that the antisynchronous pulsatility of glucagon and insulin (28,29) is lost in prediabetes and T2DM (47,48). Additionally, the surface expression of somatostatin receptor type 2 (SSTR2) decreases in alpha cells from patients with diabetes (43), and neuropathy may influence neuronal input and glucagon response with progression (49). Although more exploration is needed, the alpha cell resistance to external inhibition may be involved in T2DM pathogenesis.

Regardless of the underlying etiology of alpha cell dysfunction, the resulting hyperglucagonemia stimulates hepatic glucose production, thus contributing to hyperglycemia in patients with T2DM (3,50). Besides glucagon produced by alpha cells, the gut is assumed

to be the main extrapancreatic source of postprandial hyperglucagonemia. This is demonstrated by the fact that oral, but not intravenous, glucose administration increases glucagon levels in patients with T2DM (51). In sum, a variety of factors affect the secretion of glucagon. Therefore, it is difficult to determine whether the impaired hormone secretion is due to an alteration in alpha cell function and/or extrinsic factors. For this reason, the most likely etiology of impaired glucagon secretion in T2D is multifactorial (20).

CONCLUSIONS

Although the contribution of alpha cells to the development of T2DM appears to be significant, an all-encompassing model of glucagon release regulation in response to glucose is still lacking. It appears that both intrinsic and extrinsic components, particularly

somatostatin, play a role in regulation, and calcium appears to play a permissive role in glucagon secretion. The identification and study of alpha cells and their significance in T2DM has been difficult in the past, but novel approaches allow for further insight into the pathways involved in alpha cell glucagon secretion, as well as the interactions of these pathways with the environment surrounding them. The acute pancreas tissue slices, in conjunction with confocal microscopy and epinephrine as an identification method, can be a viable approach for alpha cell research in their native environment, with the advantage of enabling simultaneous exploration of beta and delta cells. Extending basic calcium dynamics investigations with concurrent secretory studies can provide further information about the role of calcium in glucagon secretion, and the use of diet-induced T2DM mouse models can allow us to also assess the pathophysiological changes during T2DM development.

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