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Review

New insights into the role of connexins in pancreatic islet function and diabetes

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ABSTRACT

Multi-cellular systems require complex signaling mechanisms for proper tissue function, to mediate signaling between cells in close proximity and at distances. This holds true for the islets of Langerhans, which are multicellular micro-organs located in the pancreas responsible for glycemic control, through secretion of insulin and other hormones. Coupling of electrical and metabolic signaling between islet β-cells is required for proper insulin secretion and effective glycemic control. β-cell specific coupling is established through gap junctions composed of connexin36, which results in coordinated insulin release across the islet. Islet connexins have been implicated in both Type-1 and Type-2 diabetes; however a clear link remains to be determined. The goal of this review is to discuss recent discoveries regarding the role of connexins in regulating insulin secretion, the regulation of connexins within the islet, and recent studies which support a role for connexins in diabetes. Further studies which investigate the regulation of connexins in the islet and their role in diabetes may lead to novel diabetes therapies which regulate islet function and β-cell survival through modulation of gap junction coupling.

1. Introduction

The pancreatic islets of Langerhans are responsible for blood glucose homeostasis through the regulated secretion of insulin and other hormones. In β-cells within the islet, membrane depolarization and calcium influx drives the coordinated release of insulin. Gap junctions are intercellular channels, composed of two connexon hemi-channels each made up of six connexin subunits, which readily transfer ions, metabolites and other small molecules between cells. For a more in depth review on connexins in general and their role in the endocrine system, we refer readers to the review article by Bosco et al. [1]. In the islet, gap junctions composed of connexin36 (Cx36, also known as Gjd2) provide electrical and metabolic coupling between β-cells which regulates electrical activity and insulin secretion. Under high glucose, gap junctions facilitate the coordination of electrical activity across the islet which leads to synchronized release of insulin from individual β-cells. Under low glucose, gap junctions repress electrical activity which contributes to the inhibition in the release of insulin. While the role of Cx36 gap junctions is becoming increasingly established in the islet, a link between gap junction coupling and diabetes is also beginning to emerge. Recent studies in mouse models have shown a correlation between decreased gap junction coupling, disruptions to glucose homeostasis and altered islet function; similar to that observed in models of diabetes. This suggests that gap junction coupling may be one underlying factor, of many, which contributes to disease development. As such, this review will discuss the specific role of Cx36 gap junctions in islet function and insulin secretion, the known pathways for regulation of Cx36, and current evidence for the role of Cx36 in Type 1 and Type 2 diabetes. We also note that discussion of a number of roles Cx36 plays in the islet can also be found in reviews by Meda and colleagues [2–5] or by Perez-Armendariz [6]. Current evidence suggests that regulation of insulin secretion from the islet can be modulated through gap junction coupling. Understanding the regulation of Cx36 gap junctions and the role they play in diabetes has the potential to uncover new therapies which restore more physiological blood glucose control and which could potentially deter the onset of disease.

2. The role of connexins in the islet

2.1. Connexins and gap junctions

In the human genome, 22 connexin species have been identified, while only 19 have been identified in the mouse [7,8].

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Connexin species are identified by the molecular weight of the protein, where for example Cx36 has a molecular weight of 36 kDa [9]. Most connexins share a very similar gene structure and therefore a similar protein structure, where all connexins are composed of 4 membrane spanning domains connected by 2 extracellular and 1 intracellular loop [8]. The N-terminal, 2 extracellular loops and 4 transmembrane regions are highly conserved between different connexins, while the intracellular loop and C-terminal are highly variable [10]. Both the N- and C-terminal are located in the cytoplasm, positioning them for post-translational modifications by intracellular kinases and phosphatases [11], and for interactions with connexin-associated proteins [12].

Connexin proteins oligomerize around a central hydrophilic space into a 6-protein hemi-channel called a connexon during trafficking from the ER to the Golgi complex [13]. Connexons may be homomeric, composed of only one type of connexin protein, or heteromeric, composed of different species of connexins [14]. Connexons are then trafficked to the plasma membrane via Golgi-derived vesicles mediated through microtubules or the actin cytoskeleton [15]. Connexons are inserted into a lipid raft in the membrane [13], where they can rapidly diffuse and aggregate at tight junctions or other non-junctional areas of the membrane. At tight junctions, adhesion molecules such as zonula occludens-1 on the surface of neighboring cells have decreased the gap between cells to ~2 nm [16,17]. At this point, connexon hemi-channels may remain uncoupled [18], or they may interact with a connexon from a neighboring cell via extracellular loops and form an intercellular channel. In many cell types uncoupled hemi-channels can play a role in cell signaling. However, no known role for uncoupled Cx36 hemi-channels has been established in the islet to date and recent studies suggest that β-cells do not have functional uncoupled hemi-channels [19].

Connexons which have formed intercellular channels aggregate in the membrane and form plaques, termed gap junctions [20]. Gap junctions provide electrical and metabolic coupling between cells [21]. Electrochemical gradients drive the rapid diffusion of ions, metabolites, nucleotides, small peptides and other small molecules [16,22], where the selectivity and conductance of gap junctions to these molecules is largely determined by the species of connexin that comprises the hemi-channels [23]. Although gap junctions are made up of many intercellular channels, the individual connexin channels are only open about 10% of the time [24]. Gating of connexin channels can be controlled through environmental factors, such as electric current and voltage across the channel, cytosolic pH, or changes in intracellular free-calcium activity ($[Ca^{2+}]_i$) [25,26]; as well as through post-translational phosphorylation [11]. Connexin channels are also very short lived, with a half-life of ~4 h. Gap junctions are internalized from the center of a plaque where the hemi-channel and connexin protein are degraded, while new hemi-channels are inserted at the periphery of the plaque [13,14]. While gap junctions play a role in cell signaling through electrical and metabolic coupling, recent evidence suggests that connexin hemi-channels may also effect changes in gene expression and cell proliferation through interactions with other proteins or through insertion into non-junctional regions of the plasma membrane [27].

2.2. Connexin36 gap junctions

Insulin-secreting β-cells within the pancreatic islet are exclusively coupled by Cx36 gap junctions in mice, and strongly coupled by Cx36 gap junctions in humans [28–30]. To date, Cx36 has been found to be highly expressed in the brain [31], the adrenal medulla [32], the retina [33], in the murine carotid body, ileum, and colon [34], as well as the pancreatic islet [30]. While Cx36 is expressed by insulin producing β-cells in the islet, it has also been reported to be expressed in non β-cells [30]. Data in studies examining electrical activity within the islet suggest that coupling may occur between α-cells and β-cells based on synchronous oscillations being observed [35]. However, direct evidence of functional gap junctions has only been found between β-cells [36]. Also recent studies from our lab have demonstrated that no functional gap junctions exist between α- and β-cells [submitted]. Cx36 gap junction coupling has been found to be very heterogeneous in mouse islets [28,36]. Cx36 gap junctions studied in β-cells are distinct from gap junctions composed of other connexin species in that they preferentially exchange cationic molecules [37], are minimally voltage sensitive with a half-activation voltage of only ±85 mV [38], and have a small unitary conductance (~6 pS) [36]. Compared to gap junctions formed by other species of connexins, Cx36 gap junction only have an open probability of ~0.8% [39].

3. Connexin36 regulation of insulin secretion

3.1. Role of connexin36 under high glucose

In β-cells, a series of metabolic and electrical events regulates insulin secretion in response to elevations in blood glucose. Glucose uptake and metabolism leads to a generation of ATP, closure of ATP-sensitive potassium channels (K_{ATP}), subsequent membrane depolarization, activation of voltage gated calcium channels and an elevation of $[Ca^{2+}]_i$, as outlined in Fig. 1. This $[Ca^{2+}]_i$ elevation triggers insulin granule exocytosis and release from individual β-cells. The dynamics of insulin release are tightly coupled with electrical activity and therefore calcium signaling across the islet. Glucose driven oscillations in electrical activity modulated by K_{ATP} channel gating leads to oscillations in $[Ca^{2+}]_i$ [40]. Glucose stimulated insulin secretion is biphasic, with a burst release in the 1st phase and a pulsatile release in the 2nd phase [41]. While the mechanism underlying biphasic release of insulin are under debate, one proposed mechanism is that different phases originate from two separate pools of insulin granules; one population which is docked to the plasma membrane and is readily available for insulin release, and one population of reserve granules which must be trafficked to the cell membrane for insulin release. The initial pulse of glucose stimulated $[Ca^{2+}]_i$ stimulates release of insulin from docked granules, resulting in the initial increase of insulin characterized as 1st phase release. The subsequent oscillations in $[Ca^{2+}]_i$ trigger exocytosis of insulin granules in a pulsatile manner, characterized as the 2nd phase insulin release [41]. Another theory is that the biphasic nature arises from two subpopulations of β-cells which exclusively respond to during either the first or second of insulin secretion [42]. However, the action of Cx36 gap junctions in coordinating β-cell $[Ca^{2+}]_i$ and insulin responses across the islet argues against this.

Under stimulatory levels of glucose, electrical coupling provided by Cx36 gap junctions allows for transfer of a depolarizing current, synchronization of K_{ATP} channel-regulated membrane depolarization, and the coordination $[Ca^{2+}]_i$ oscillations across the islet [28,29,43], as depicted in Fig. 1. As such insulin secretion is released in a coordinated and oscillatory fashion. This has been demonstrated in isolated islets from mice with knockout of the Cx36 gene, *Gjd2*. In one study, islets from Cx36 deficient mice showed a loss of glucose-stimulated $[Ca^{2+}]_i$ oscillations and a loss of pulsatile insulin secretion [29]. Further studies in Cx36 deficient isolated islets have shown the loss of $[Ca^{2+}]_i$ oscillations arises from a loss of synchronization between β-cells, where individual β-cells still show irregular, heterogeneous oscillations that lack any synchronization [28]. This is similar to the irregular and heterogeneous oscillations observed in isolated β-cells, dissociated from the islet [44] and is consistent with Cx36 being the sole connexin that forms gap