Is zinc an intra-islet regulator of glucagon secretion?

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Abstract More than a decade ago, zinc was suggested to have a role as an intra-islet regulator of glucagon secretion. Several lines of experimental evidence have since provided support for this hypothesis, though contradictory observations have also been reported. Meanwhile, Slc30A/ZnT8, a zinc transporter expressed in insulin and glucagon secretory granules, was identified. Furthermore, genome wide association analyses revealed it to be a candidate causative gene for type 2 diabetes mellitus. Recent progress in gene manipulation in animals yielded considerable information on the role of zinc in islet cells. In this mini-review, data pertaining the roles played by zinc in islet hormone secretion are summarized and discussed.

Keywords Glucagon · Insulin · Zinc · Slc30A8/ZnT8 · α -cells

Mechanisms of glucagon secretion

Glucagon, which is secreted mainly from pancreatic α -cells when blood glucose levels drop, stimulates release of glucose from the liver (for a comprehensive review, see [1]). The molecular mechanisms underlying glucagon secretion are not as yet fully understood, however. Two

mechanisms of glucagon secretion have been postulated, one paracrine and the other intrinsic [1, 2].

Metabolic activation of α -cells triggers glucagon secretion

Paracrine regulation of glucagon secretion was proposed several decades ago. Since glucose stimulates the secretion of insulin and somatostatin, while inhibiting glucagon release, α -cell secretion was speculated to be suppressed by β -cell or δ -cell secretory products. Several lines of evidence support the existence of such paracrine inhibitory mechanisms [2]. The evidence includes the observation that metabolic activation of α -cells triggers glucagon secretion. This is reasonable since secretion is an energy-requiring process. The hypothesis is based on the observation that pyruvate stimulates glucagon secretion [3], which was reported long ago [4, 5] but has not since been widely recognized. Similar observations were made when the dicarboxylate transporter was expressed mainly in rat islet α -cells [6]. When α -cells were engineered to be activated by dicarboxylates or tricarboxylates, such as α -ketoglutarate, malate and fumarate, glucagon was secreted in response to these compounds. These data indicated that metabolic activation of α -cells triggers glucagon secretion from islets.

These earlier studies also suggested that simultaneous activation of β -cells suppresses glucagon secretion [3, 6]. Pyruvate does not stimulate insulin secretion, since mature β -cells do not possess pyruvate transporters on their cell surface. Adenovirus-mediated expression of monocarboxylate transporter (MCT-1) by the insulin promoter in rat islets endowed β -cells with the capacity to secrete insulin in response to pyruvate [7]. This was subsequently confirmed using a murine model in which MCT-1 is expressed

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in β -cells [8]. When β -cells are engineered to be activated by pyruvate, pyruvate no longer exerts a stimulatory effect on α -cells in islets of Langerhans [3]. Therefore, α -cells are capable of secreting glucagon only under circumstances in which β -cells are quiescent.

Intrinsic mechanisms underlying suppression of glucagon secretion via glucose-derived signals

There are also data favoring intrinsic mechanisms of hormone secretion, i.e. indicating that paracrine inhibition of glucagon secretion does not necessarily occur. One of the arguments against paracrine mechanisms is that glucagon secretion is clearly suppressed when the glucose concentration is raised from 0 ~ 1 mM to 2.5 ~ 7 mM, while insulin secretion is essentially unchanged from basal levels at these glucose concentrations but is stimulated at glucose concentrations higher than 5 ~ 7 mM [2]. To answer this argument, proponents of the paracrine view of glucagon regulation have suggested somatostatin involvement. Somatostatin could be important for suppression of glucagon secretion at 2.5 ~ 7 mM glucose, since its secretion is triggered at these lower glucose concentrations [2]. However, another finding favoring intrinsic mechanisms as modulators of glucagon secretion is that electrophysiological or Ca^{2+} recording in single α -cells clearly demonstrated suppressed α -cell electric activity or intracellular Ca^{2+} concentrations at 2.5 to 7 mM glucose as compared to that at 0 or 1 mM glucose [9–11]. A major weakness of the intrinsic hypothesis is that none of the postulated molecular mechanisms convincingly explain this observation. There are two major proposed mechanisms: one is based on special orchestration of ion channels in α -cells and the other involves regulation of intracellular Ca^{2+} via store-operated Ca^{2+} entry. An increase in glucose concentrations from 0 ~ 1 mM to 2.5 ~ 7 mM would activate the endoplasmic reticulum Ca^{2+} storage triggered by increased ATP synthesis from glucose and subsequent shut off of store-operated Ca^{2+} entry at the plasma membrane, leading to suppressed glucagon secretion (see review [2]).

These data on the control of glucagon secretion by glucose can be summarized overall, in our view, by assuming that on balance glucagon secretion is suppressed by both intrinsic mechanisms and by paracrine mechanisms through δ -cells at 1 to 5 ~ 7 mM glucose, and that glucagon secretion is activated by intrinsic mechanisms but suppressed by paracrine mechanisms through β -cells and δ -cells at glucose concentrations higher than 5 ~ 7 mM (Fig. 1). Note that intrinsic mechanisms operate in opposite directions at low and high glucose concentrations. At 1 to 5 ~ 7 mM glucose, intrinsic mechanisms operate to achieve the suppression of glucagon secretion, and at glucose concentrations higher than 5 ~ 7 mM, glucose

metabolically activates α -cells and operates to promote stimulation [3, 12, 13]. Failure of glucagon suppression by glucose is indeed a hall mark of both type 1 and type 2 diabetes, probably secondary to β -cell dysfunction [1].

Roles of zinc in the regulation of glucagon secretion

The importance of zinc (Zn^{2+}) in diabetes has been long discussed, since this metal ion affects both insulin secretion and insulin action (see review [14]). Zn^{2+} is stored in insulin secretory granules and is secreted together with insulin. Genome wide association studies have recently revealed, *Slc30A/ZnT8* to be a candidate causative gene for type 2 diabetes mellitus, thereby accelerating research on the association between zinc and diabetes. Moreover, *Slc30A/ZnT8* strongly correlates with proglucagon expression in human islets [15]. Conversely, rare variants in the *SLC30A8* gene have been associated with lowering of blood glucose and protection from T2D [16].

Evidence favoring or opposing roles of zinc in glucagon suppression

The idea that glucagon secretion is inhibited by Zn^{2+} released from β -cells was initially proposed as being analogous to mossy fiber synaptic modulation [3]. Pancreatic perfusion experiments showed that pyruvate-stimulated glucagon secretion was suppressed by 30 μM ZnCl_2 . The effects of Zn^{2+} were subsequently confirmed by static secretion and electrophysiological experiments in purified rat α -cells [12]. Thereafter, several laboratories tested Zn^{2+} effects on glucagon secretion, yielding conflicting and thus confusing results.

At least three laboratories confirmed the effects of Zn^{2+} . Wheeler's group reported glucagon secretion at 1 mM glucose to be suppressed by 20 μM Zn^{2+} in isolated islets from mice with a CD1 background [17]. Piston's group also found glucagon secretion at 1 mM glucose to be suppressed by 30 μM Zn^{2+} in both isolated islets and purified α -cells from B6 mice [13]. In addition, Robertson and colleagues showed glucagon secretion to be enhanced when perfusion of the rat pancreas with 30 μM Zn^{2+} was terminated as compared to when pre-perfusion without Zn^{2+} was stopped, highlighting Zn^{2+} suppression of glucagon secretion [18].

At least three research groups have reported that Zn^{2+} does not suppress glucagon secretion. Rutter's group demonstrated 30 μM ZnCl_2 to exert no effect on glucagon secretion in static secretion experiments using isolated islets from CD1 mice [9]. Rorsman and colleagues showed 30 μM Zn^{2+} to increase glucagon secretion from isolated

Fig. 1 A proposed regulatory mechanism of glucagon secretion by glucose. Glucagon secretion by glucose may be regulated by at least three mechanisms. The intrinsic mechanism exerts suppression and stimulation of glucagon secretion at low and high glucose concentrations, respectively. The β -cell and δ -cell secretory activities may cause suppression of glucagon secretion at glucose concentrations higher than 5 ~ 7 mM (β -cells) as well as at hypoglycemia (δ -cells). Combined effects of these mechanisms result in fine tuning of glucagon secretion

Ishihara & Wollheim Figure 1

human islets [11]. In addition, Gilon's group presented evidence that there are no changes in intracellular Ca^{2+} levels in response to the addition of 30 μM Zn^{2+} to purified mouse α -cells [10].

The reasons for these discrepancies are unclear: differences in experimental settings and species differences in islet sources may partially explain these differences.

If Zn^{2+} is a mediator suppressing glucagon secretion, what is the mechanism by which it exerts this effect? One putative Zn^{2+} target is the sulfonylurea receptor-1 (ABCC8). Zn^{2+} binding to histidine residues of the sulfonylurea receptor-1 reportedly activates ATP-dependent potassium channels [19], and may affect Ca^{2+} entry into cells as well as exocytosis. Piston's group offered the alternative hypothesis that Zn^{2+} plays a role in the steps after Ca^{2+} entry based on the failure to detect any changes in intracellular Ca^{2+} when Zn^{2+} was added [13]. More recently, Cav2.3, an R-type Ca^{2+} channel subunit, was proposed to be a target of Zn^{2+} acting to suppress glucagon secretion [20]. When a Zn^{2+} chelating agent was administered to wild-type mice, serum glucagon levels rose, while Cav2.3-deficient mice exhibited higher glucagon levels and no glucagon responses to Zn^{2+} chelation. More detailed analyses are clearly needed for the role of Zn^{2+} in glucagon suppression to be established.

Reports favoring or opposing roles of insulin in glucagon suppression

Historically, insulin was the earliest candidate factor suppressing glucagon secretion (for review see [1]). Many of the aforementioned studies tested effects on α -cell

responses of insulin in addition to Zn^{2+} . Effects of insulin in the perfused pancreas, isolated islets and purified α -cells from rats were demonstrated electro-physiologically or by direct measurement of secreted glucagon [12]. The groups of Rutter [9], Piston [13], Wheeler [21] and Rorsman [11] demonstrated exogenous insulin (17–100 nM) to inhibit glucagon secretion in isolated islets from mice or humans. In contrast, according to one report, no effect of insulin (100 nM) was observed when intracellular Ca^{2+} was measured in purified α -cells or glucagon secretion was measured in isolated and perfused mouse islets [10].

Although analyses of exogenous insulin in glucagon suppression experiments yielded discrepancies, an α -cell specific knockout model of the insulin receptor appeared to establish the roles of insulin in the suppressive effects of glucose on glucagon secretion [22]. It is, however, possible that zinc is required for the suppressive action of insulin on glucagon secretion, as zinc-free insulin failed to mimic the suppressive effect on glucagon release in β -cell-depleted rats [18].

Lessons from murine models with ZnT8 genetic modulations

Genome wide association studies have identified ZnT8, a major vesicular Zn^{2+} transporter responsive to the accumulation of Zn^{2+} in insulin secretory granules, as a candidate gene for type 2 diabetes susceptibility. This finding has accelerated research into the action of Zn^{2+} in pancreatic islet biology. Several murine models with a modified ZnT8 locus have been generated [23–27]. Very

Fig. 2 Possible effects of insulin and Zn^{2+} secreted from ZnT8-deficient β -cells. Reduced Zn^{2+} secretion from β -cells may attenuate putative suppressive effects of Zn^{2+} on glucagon secretion. At the same time, reduced Zn^{2+} secretion from β -cells attenuates suppressive effects of Zn^{2+} on insulin secretion itself, resulting in net increase of insulin secretion, augmenting suppressive effects of insulin on glucagon secretion. Thus, combined effects on glucagon secretion of reduced Zn^{2+} secretion could be neutral

recently, an elegant study was conducted using β -cell ZnT8 overexpressing mice and highly specific β -cell ZnT8 knockout mice [28]. Interestingly, ZnT8 deletion reduced Zn^{2+} secretion from β -cells and increased insulin secretion, while overexpression of the transporter increased Zn^{2+} output and reduced insulin secretion. Therefore, Zn^{2+} secreted from β -cells appears to be a negative autocrine regulator of insulin secretion [27, 28]. When glucagon secretion was studied in several of these ZnT8 knockout mice, either global or β -cell specific, none of the studies showed any changes in glucagon secretion [23–27]. The authors concluded that Zn^{2+} has no role as a glucagon suppressing factor. However, another view of these data is plausible. As illustrated in Fig. 2, when Zn^{2+} secretion from β -cells is reduced, insulin secretion rises, such that the suppressive effect of insulin is increased. At the same time, reduced Zn^{2+} secretion from β -cells may attenuate the suppressive effect of Zn^{2+} on α -cells, augmenting glucagon secretion. Therefore, reduced Zn^{2+} secretion from β -cells exerts both positive and negative effects on α -cells. This could explain why no changes in glucagon secretion were observed. Interestingly, Rutter and colleagues reported [29] glucagon secretion at 1 mM glucose from isolated islets to be approximately 1.8-fold higher in α -cell specific ZnT8 knockout mouse islets than in wild-type islets, suggesting that Zn^{2+} secreted from α -cells is a negative autocrine regulator of glucagon secretion.

Conclusion and perspectives

In the last decade, methodological advances in cellular physiology and genetic manipulation have enhanced our understanding of the role of Zn^{2+} in islet cell function.

However, precise mechanistic investigations are still necessary before we can conclude that Zn^{2+} plays a physiologically relevant role in the regulation of glucagon secretion. The identification of a direct target(s) of Zn^{2+} in α -cells is particularly important, and it is anticipated that α -cell specific modulation of such a molecule(s) will answer this interesting question.

Compliance with ethical standards

Conflict of interest Authors have nothing to declare related to this review.

Ethical standards This article does not contain any studies with human or animal subjects performed by any of the authors.

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