Molecular Mechanism Underlying the Intra-Islet Regulation of Glucagon Secretion

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1. Introduction

Glucagon secreted from pancreatic α-cells plays central roles for counteracting hypoglycemia by modulating hepatic glucose metabolism (Gromada et al., 2007). In addition, glucagon also contributes to the maintenance of glucose homeostasis together with insulin from β-cells. During hyperglycemia such as post-prandial state, insulin secretion from β-cells is stimulated while glucagon secretion from α-cells is suppressed, leading to a lowering of blood glucose levels due to enhanced hepatic- and adipose glucose uptake and suppressed hepatic glucose output. In contrast, in hypoglycemia such as starvation, glucagon secretion is promoted while insulin secretion is reduced, causing elevated blood glucose levels via the effects of glucagon, including enhanced hepatic glucose output and breakdown of lipids and proteins to provide glucose that is critical to the central nervous system. Thus, both glucagon and insulin are pivotal in systemic energy homeostasis, and the balance between these two hormones determines the metabolic state of various organs in response to changes in energy status.

In both type 1 and type 2 diabetes, both of which exhibit a global increase in incidence, an imbalance between the two hormones appears to significantly impact glucose homeostasis (Unger, 1978). Insufficient insulin secretion and systemic insulin resistance both contribute to hyperglycemia due to quantitative and qualitative insulin shortage. In addition, abnormal elevations in circulating glucagon, due to lack of normal suppression mechanisms, worsens the hyperglycemia via enhanced hepatic glucose output. On the other hand, in patients undergoing treatment for diabetes, an increased incidence of hypoglycemia likely occurs due to a poor glucagon response. Whether this poor glucagon response is a consequence of impaired effects of insulin due to repeated treatment with exogenous insulin or other factors is not fully understood (Gerich et al., 1973). Therefore, diabetes can be recognized as “state where adequate hormones cannot work appropriately” when intra-islet hormone balance is focused on. These observations have prompted consideration of glucagon in the overall therapeutic approach to treat patients with both type 1 and type 2 diabetes. Furthermore, novel therapeutic approaches targeting Glucagon-like peptide (GLP)-1 action in α-cells...
(GLP-1 analogues and DPP-4 inhibitors) are also being considered given the potential for GLP-1 to have direct suppressive effects on α-cells, thus these enabled comprehensive control of islet hormone balance including improvement of both insulin and glucagon secretion. Therefore, it becomes more important to understand the underlying molecular mechanisms for the regulation of glucagon secretion to apply new therapeutic approaches to diabetes targeting α-cell dysfunction.

2. Functions of glucagon

2.1 Functions of glucagon
Glucagon is a 29 amino acid peptide hormone, secreted by pancreatic α-cells mainly in hypoglycemic state, and exerts multiple biological effects on a wide range of organs (Kawamori et al., 2010). Glucagon has important functions in vivo for sustaining appropriate blood glucose level. In physiological states, glucagon is released into the bloodstream in response to hypoglycemia to oppose the action of insulin in peripheral tissues, and works as a counter-regulatory hormone to restore normoglycemia. Secreted glucagon works predominantly on the liver, and promotes hepatic gluconeogenesis, glycogenolysis, and simultaneously inhibits glycolysis and glycogenesis (Exton et al., 1966; Unger and Orci, 1977), thus contributing to restoring glucose homeostasis by counteracting the action of insulin. In contrast, insulin suppresses hepatic glucose output while enhancing hepatic glucose uptake and glycogenesis, indicating that a balance between these two hormones at the hepatocyte determines hepatic glucose metabolism, thus systemic glycemic homeostasis. In addition to countering hypoglycemia and opposing the effects of insulin in the liver, glucagon has impacts the function of several metabolic organs together favoring the maintenance of glucose homeostasis. For example, in the adipose tissue, glucagon enhances lipid decomposition, while, in contrast, the lack of detectable glucagon receptors in skeletal muscle indicates glucagon has little effect in regulating systemic glucose metabolism by acting on skeletal muscle (Christophe, 1996). Glucagon can also stimulate insulin secretion from pancreatic β-cells (Scheen et al., 1996) and indirectly impact hepatic glucose output. Taken together, these actions indicate an important role for glucagon in maintaining glucose homeostasis.

2.2 Molecular mechanism underlying glucagon action
The glucagon receptor is a G-protein (Gs/Gq) coupled type receptor (Jelinek et al., 1993) and is widely expressed in insulin target organs, such as liver, adipose, β-cells and brain, with the exception of skeletal muscle (Burcelin et al., 1995). Following binding and conformational changes of the receptor the activation of Gs leads to recruitment of adenylate cyclase to the cellular membrane, causing an increase in intracellular cyclic adenosine monophosphate (cAMP) levels and subsequent activation of protein kinase A (PKA) (Weinstein et al., 2001). On the other hand, activation of Gq induces activation of phospholipase C, upregulation of inositol 1,4,5-triphosphosphate, and the subsequent release of intracellular calcium (Ca²⁺) (Wakelam et al., 1986). The action of glucagon is relatively complex and involves the coordinate regulation of transcription factors and signal transduction networks which converge to regulate amino acid, lipid and carbohydrate metabolism. For example, in the liver, elevated PKA activity activates various downstream
targets leading to the suppression of glycolysis and glycogenesis, and the enhancement of gluconeogenesis and glycogenolysis (Jiang and Zhang, 2003). In islet cells, the elevation of cAMP by glucagon has been reported to stimulate insulin and glucagon secretion from β- and α-cells respectively (Huypens et al., 2000; Ma et al., 2005) by PKA dependent and independent mechanisms. Upregulation of cAMP activates cAMP-regulated guanine nucleotide exchange factors (cAMPGEFs / Epac), which modulates intracellular Ca$^{2+}$-ion mobilization, enhancing exocytosis (Holz et al., 2006; Ma et al., 2005).

2.3 Anatomical characteristics of pancreatic islets and α-cells
Pancreatic islets possess unique anatomical characteristics and are composed of five different endocrine cell types distributed as islands randomly within the exocrine pancreas. Among these five endocrine cells in islets, the α-cells account for approximately 20% of islet cells.

In adult rodents, β-cells are restricted mostly to the islet core, while α-cells, somatostatin-secreting δ-cells, pancreatic polypeptide-secreting PP-cells, and ghrelin-secreting ε-cells, are scattered along the periphery of the islet and surrounding β-cells. It is likely that this distribution and arrangement of different islet cell types is teleologically important for physiological regulation between the cells since the blood flows from the center of the islets toward periphery; i.e. β-cells to non-β-cells in the islet microcirculation system (Bonner-Weir and Orci, 1982; Stagner and Samols, 1986), suggesting that secreted insulin regulates hormone secretion from other islet cell types. This architecture is typically preserved in rodent islets, while in humans, non-β-cells are often observed both at the periphery and also seemingly in clusters within the center of islets (Cabrera et al., 2006). This implies several possibilities; 1) rodent cellular hierarchy in the islets does not apply to human islets, or 2) human islets consist of several clover-leaf like ‘rosettes’, with each rosette resembling the basic islet architecture observed in rodent islets (Bonner-Weir and O’Brien, 2008) suggesting
the arrangement and interaction of the different cell types in human islets is similar to that in rodents. Recent studies report that in large human islets blood vessels penetrate and branch inside islets, and α-cells located within the core of islets are placed along these vessels and surrounded by β-cells (Bosco et al.). Thus, according to this report, in human islets, α-cells which appear to be placed in the islet core are still ‘peripheral’ in the islets since blood vessels are usually considered to be placed outside the islets. Given the direction of intraislet microcirculation described above, intraislet auto-/paracrine effects between islet cells especially from β- to non-β-cells can be applied to human islets.

2.4 Excessive glucagon secretion in diabetes

Glucagon plays critical roles in glucose homeostasis largely by regulating hepatic glucose metabolism. However, circulating glucagon levels are often elevated in both type 1 and type 2 diabetes, thus are suggested to contribute to the development of insulin resistance (e.g. hepatic insulin resistance) and exacerbation of diabetes (Ahren and Larsson, 2001; Dinneen et al., 1995; Larsson and Ahren, 2000; Unger, 1978). In addition, the absence of postprandial glucagon suppression in diabetes patients also contributes to postprandial hyperglycemia (Mitrakou et al., 1992; Raskin and Unger, 1978; Sherwin et al., 1976). Another potential contributor to the excess glucagon levels is a relative increase in α-cells compared to β-cells in pancreatic islets in both type 1 (Orci et al., 1976) and type 2 diabetes (Rahier et al., 1983; Yoon et al., 2003). Moreover, in type 1 diabetic islets, an increase in α-cell area and number, and dysregulated cell-type distribution in islets is due to specific β-cell destruction. Although the precise mechanism(s) of relative hyperglucagonemia in the diabetic state is still obscure, β-cell dysfunction is a possible candidate since β-cell secretory products, including insulin, are known to suppress glucagon secretion (see section 4.1.). Thus altered (impaired) β-cell function in diabetes can potentially induce inappropriately elevated glucagon in hyperglycemic states by impairing the intraislet influence of β-cells on glucagon regulation (Meier et al., 2006a).

2.5 Defective glucagon response to hypoglycemia in diabetes

Diabetes patients (both type 1 and type 2) frequently develop defective counter-regulatory responses to hypoglycemia that is associated with reduced or absent glucagon secretory responses. A defective glucagon secretory response to hypoglycemia in hyperinsulinemic states frequently exacerbates a hypoglycemic attack, and limits intensive glucose control by insulin therapy (Amiel et al., 1988; Gerich et al., 1973). Moreover, hypoglycemia associated autonomic failure is induced especially in patients with frequent exposure to hypoglycemia leading to a worsening phenotype (Cryer, 1994). This defective response to hypoglycemia includes sympathoadrenal and neurohormonal responses against hypoglycemia such as epinephrine, cortisol and growth hormone that act to decrease blood glucose further, finally leading to sudden states of hypoglycemia and hypoglycemia unawareness (Amiel et al., 1988; Gerich et al., 1973). How diabetes induces these defective responses to hypoglycemia is still under investigation and suggested theories include alteration in brain glucose transport and metabolism by frequent exposure to hypoglycemia (Criego et al., 2005) and/or defective intraislet β-cell effects on α-cell function, such as the “switch-off” of insulin (Hope et al., 2004; Zhou et al., 2004) or Zinc iron (Zhou et al., 2007) (see section 4.).
3. Regulation of glucagon secretion

3.1 Factors involved in glucagon secretion
The secretion of glucagon from α-cells is stimulated in response to hypoglycemia, and suppressed by hyperglycemia in vivo. However, the regulation of glucagon secretion is not simply determined only by glucose concentration, but is complex and finely controlled by additional contribution of neural, hormonal, and intra-islet interactions (Gromada et al., 2007). While it is still not conclusive whether α-cells can directly sense glucose concentration outside the cells and subsequently respond in glucagon secretion (section 3.2.), additional mechanisms which contribute to the secretion of glucagon have recently been revealed. For example, the central nervous system is reported to sense glucose concentration largely through the hypothalamus, and to modulate secretion of islet hormones via the autonomic nervous system (section 3.3.). In addition, circulating autonomic neurotransmitters such as γ-amino-butyric acid (GABA), epinephrine and norepinephrine can stimulate glucagon secretion from α-cells. As described above, various regulatory mechanisms for the glucagon secretion than glucose were uncovered. Among them, it is recently revealed that intra-islet regulation by neighboring β-cells plays critical roles in the physiology of glucagon secretion from α-cells (see section 4).

3.2 Regulation of glucagon secretion by glucose and other nutrients
The secretion of glucagon from α-cells is elevated in response to hypoglycemia and suppressed by hyperglycemia in vivo. While some studies suggest a direct suppressive effect of glucose on α-cell secretory function (Ravier and Rutter, 2005; Vieira et al., 2007), the paradoxical stimulation of glucagon secretion by high glucose in isolated islets and α-cell lines (Franklin et al., 2005; Olsen et al., 2005; Salehi et al., 2006) suggests that additional mechanisms contribute to the secretion of glucagon in response to glucose. Also, it is still not conclusive whether α-cells can directly sense glucose concentration outside the cells then respond in glucagon secretion or not.

Amino acids such as L-arginine are potent stimulators of glucagon secretion (Gerich et al., 1974). This is physiologically relevant to prevent hypoglycemia after protein intake since amino acids also stimulate insulin secretion. L-glutamate is produced, secreted by various cell types including neural cells, and acts as a neurotransmitter. In islet α-cells, glutamate is contained in glucagon secretory vesicles (Yamada et al., 2001). Interestingly, a recent study shows that glutamate secreted by α-cells functions as an autocrine positive feedback signal for glucagon secretion (Cabrera et al., 2008), as α-cells express glutamate transporters and receptors (Hayashi et al., 2001). Low glucose stimulates glutamate release from α-cells, which in turn acts on α-cells in an autocrine manner leading to membrane depolarization and glucagon secretion (Cabrera et al., 2008).

3.3 Involvement of nervous system and neurotransmitters
While glycemia might modulate glucagon secretion directly, several reports indicate the involvement of the central and/or autonomic nervous systems in the regulation of glucagon secretion (Ahren, 2000; Bloom et al., 1978; Evans et al., 2004; Marty et al., 2005). Hypoglycemia is a critical condition for body especially since glucose is an essential fuel for the central nervous system. Thus in response to hypoglycemia, the nervous response immediately triggers various counterregulatory mechanisms to protect the brain from energy deprivation, including the stimulation of glucagon secretion.
The dense innervations of the islets suggest that both α- and β-cells are regulated by the nervous system (Ahren, 2000). The autonomic nervous system (ANS) transmits stimuli to promote glucagon secretion especially under hypoglycemia when blood glucose must be increased to supply fuel for the body. The ANS can modulate all islet cells and regulate glucagon secretion directly via the parasympathetic pathway or indirectly by pathways that can modulate islet paracrine factors (see section 4.) (Ahren, 2000). In addition, circulating autonomic neurotransmitters epinephrine and norepinephrine have been reported to stimulate glucagon secretion from α-cells through adrenergic receptors (Schuit and Pipeleers, 1986; Vieira et al., 2004). Glucagon secretion is also modulated by other neurotransmitters including GABA (see section 4.2.) and glutamate (see section 3.2.). The precise mechanism by which the central nervous system (CNS) senses blood glucose and affects glucagon secretion is not fully understood, although several possibilities have been suggested. Glucose sensing in the CNS is suggested to be an interaction between neurons and glial cells. For example, neurons in the ventro-medial hypothalamus (VMH) have been reported to play a role in sensing hypoglycemia in the brain and triggering the responses of counter-regulatory hormones to impact hypoglycemia (Borg et al., 1995), through AMPK (McCrnimmon et al., 2004), K+ATP channels (Evans et al., 2004), and corticopitrohin releasing factor receptors (Cheng et al., 2007) in rat models. Moreover, it has also been reported that GLUT2 in cerebral astrocytes acts as a central glucose sensor in the modulation of glucagon secretion in mice (Marty et al., 2005).

4. Intra-islet regulation of glucagon secretion

In addition to glucose, various regulatory mechanisms for glucagon secretion have been detected. Among these mechanisms is the emerging concept that intra-islet regulation by secretory products from neighboring β-cells plays a critical role in determining α-cell function. This concept is supported, at least in the rodent, by the direction of the intraislet microcirculation which occurs from the core to the periphery and implicates α-cells as potential direct targets of β-cell secretory products such as insulin, (Asplin et al., 1981; Kawamori et al., 2009; Maruyama et al., 1984; Weir et al., 1976), GABA (Rorsman et al., 1989; Xu et al., 2006) and Zinc ions (Ishihara et al., 2003). In addition, another islet hormone somatostatin is reported to modulate glucagon secretion. Interestingly, glucagon itself is reported to regulate glucagon secretion. GLP-1 can suppress glucagon secretion directly and possibly indirectly by enhancing insulin secretion.

4.1 Insulin

Insulin, the major secretory product of β-cells, has been proposed as one of the intra-islet paracrine factors that can modulate the secretion of glucagon from neighboring α-cells (Asplin et al., 1981; Kawamori et al., 2009; Maruyama et al., 1984; Weir et al., 1976). Furthermore, proteins in the insulin signaling pathway are abundantly expressed in α-cells supporting an important role for insulin signaling in α-cells(Bhathena et al., 1982; Franklin et al., 2005; Patel et al., 1982).

4.1.1 Modulation of glucagon secretion by insulin

In clinical studies in human type 1 diabetes patients whose β-cell function is considered to be extinct (Asplin et al., 1981; Gerich et al., 1975), along with basic studies in insulinopenic animal models (Maruyama et al., 1984; Stagner and Samols, 1986; Weir et al., 1976), indicate
Schematic image for the β-cell-mediated suppression of glucagon secretion from α-cells via a paracrine mechanism. The β-cell secretes insulin, γ-amino-butyric acid (GABA), and zinc irons (Zn) which suppress glucagon secretion. High glucose/hyperglycaemia suppresses glucagon secretion through the nervous system and by stimulation of β-cell secretion. Somatostatin also suppresses glucagon secretion. GLP-1 suppresses glucagon secretion through β-cell mediated and direct pathways.

that insulin suppresses glucagon secretion in vivo. In insulinopenic animal models, exogenous insulin suppressed glucagon secretion (Greenbaum et al., 1991; Stagner and Samols, 1986; Weir et al., 1976). Conversely, suppression of insulin action by infusion of an anti-insulin antibody increased glucagon release (Maruyama et al., 1984). These studies clearly indicate the suppressive effect of insulin on glucagon secretion. Thus, it is conceivable that chronic and post-prandial hyperglucagonemia seen in diabetes patients (see section 2.4) is due to a lack of the direct suppression of insulin on glucagon secretion induced either by an absolute lack of insulin and/or α-cell insulin resistance (Meier et al., 2006a; Raju and Cryer, 2005).

In addition, insulin is reported to stimulate glucagon secretion through a “switch-off” mechanism (Hope et al., 2004; Zhou et al., 2004). During hypoglycemia, a decrease in intraislet insulin may act as a trigger for glucagon secretion as α-cells can sense the decrease in ambient insulin. This concept is proposed by studies wherein cessation of insulin administration in vivo pancreas perfusion experiments in insulinopenic diabetic rats induces glucagon secretion in response to hypoglycemia (Hope et al., 2004; Zhou et al., 2004). It is also possible that the defective secretory response of glucagon to hypoglycemia in diabetes patients occurs secondary to a defect in insulin sensing in α-cells (see section 2.5). Thus, insulin is a center player not only in the suppression of glucagon secretion but also the stimulation of glucagon secretion.
4.1.2 Molecular mechanisms underlying the modulation of glucagon secretion by insulin signaling

These *in vivo* reports suggest a direct effect of insulin in modulating glucagon secretion. On the other hand, recent *in vitro* studies in α-cell lines using gene knock-down techniques indicate a role for the insulin receptor and its signaling pathway in suppressing glucagon secretion by high glucose (Ravier and Rutter, 2005), as well as in stimulating glucagon secretion by low glucose concentration (Diao et al., 2005).

The direct inhibitory effects of insulin to suppress glucagon secretion has been reported to occur either by 1) reducing the sensitivity of K⁺ATP channels (Franklin et al., 2005) which regulate glucagon secretion machinery via phosphatidyl inositol 3-kinase (PI3K) (Leung et al., 2006), or by 2) modulating Akt, a critical downstream effector of PI3K, leading to recruitment of the GABA-A receptor to the cellular membrane to allow its ligand, GABA, to inhibit glucagon secretion (see section 4.2) (Rorsman et al., 1989; Xu et al., 2006).

4.1.3 The α-cell specific insulin receptor knockout mouse model

While numerous reports indicate a pivotal role for insulin in the regulation of glucagon secretion, direct molecular evidence for the importance of insulin signaling in α-cells *in vivo* has been lacking until recently. The significance of systemic insulin signaling in glucose homeostasis is well known as insulin resistance is induced in insulin target organs including the liver, the skeletal muscle and the adipose tissues under diabetic state, and impacts on glycemic metabolism in these organs. Eventually, the genetic evidence of the *in vivo* significance of insulin signaling in α-cells in the regulation of glucagon secretion was provided by investigation of the α-cell specific insulin receptor knockout (αIRKO) mice (Kawamori et al., 2009).

The αIRKO mice exhibited glucose intolerance, hyperglycemia and hyperglucagonemia in the fed state together with enhanced glucagon secretion in response to L-arginine. These results indicate that disruption of insulin receptor in α-cells enhanced glucagon secretion by diminishing the glucagonostatic effect of insulin, and provided direct *in vivo* evidence for the suppression of glucagon secretion by insulin from β-cells through intra-islet paracrine manner. Interestingly, the mutant mice also displayed blunted glucagon response to hypoglycemia indicating a defective glucagon response through insulin “switch-off” mechanism (Hope et al., 2004; Zhou et al., 2004) by disruption of insulin signaling in α-cells. The results using αIRKO mice clearly demonstrate a critical role for insulin in the regulation of α-cell function in both normo- and hypoglycemic states *in vivo*.

4.1.4 Model for the intraislet regulation of glucagon secretion from α-cells by insulin

From these findings, a possible model for the intraislet regulation of glucagon secretion by insulin can be proposed. In states of hyperglycemia, the greater insulin secretion from β-cells is stimulated and would activate insulin signaling in α-cells via paracrine manner, and represses glucagon secretion. On the other hand, in hypoglycemic state, the consequent levels of low insulin would allow the α-cells to sense the reduction in ambient insulin leading to a lack of activation of insulin signaling that in turn leads to the stimulation of glucagon secretion. This would occur in addition to possible direct stimulation by low glucose itself. Indeed, a recent clinical study reported that this proposed mechanism is actually feasible in humans (Cooperberg and Cryer, 2010). In this report, patients with type 1 diabetes were subjected to normo- and hypoglycemic clamps and the effects of insulin
analogue glulisine were evaluated. Continuous glulisine infusion suppressed glucagon secretion both under normo- and hypoglycemic states, while discontinuation of glulisine infusion stimulated glucagon secretion in hypoglycemic state. From these studies, it is proposed that insulin overrides the effects of glucose and suppresses glucagon secretion in the hyperglycemic state, and decreasing insulin levels triggers glucagon response to hypoglycemia and precedes the direct effect of low glucose.

In high glucose state, stimulated insulin secretion from β-cells acts on insulin receptor on the surface of α-cells then suppresses glucagon secretion by paracrine manner. In low glucose state, decreased insulin secretion from β-cells is recognized by α-cells as a reduction of insulin signaling in α-cells through insulin receptor, then α-cells increase glucagon secretion in response.

4.2 GABA

γ-aminobutyric acid (GABA) is produced from the excitatory amino acid glutamate by glutamic acid decarboxylase (GAD) and works as an important inhibitory neurotransmitter in neural synapses, mainly in the central nervous system (Kittler and Moss, 2003). In neurons, GABA is released by the presynaptic terminal into synaptic junctions and binds to GABA receptors on the postsynaptic membrane, inhibiting cellular electrical firing through modulation of ion channels and consequent membrane hyperpolarization (Kittler and Moss, 2003). Islets are also innervated by GABA-ergic neurons (Sorenson et al., 1991), suggesting that GABA is a potential inhibitor of α-cell function.

In addition, GABA has also been reported to be secreted from β-cells and suppress glucagon secretion from α-cells in an intraislet paracrine manner (Rorsman et al., 1989; Wendt et al., 2004; Xu et al., 2006). High glucose or glutamate levels stimulate secretion of GABA from β-cells and the secreted GABA then binds to its receptor expressed on α-cells, inhibiting glucagon secretion through cellular membrane hyperpolarization. Importantly, the GABA-A receptor is recruited to the cellular membrane by insulin-Akt signaling (Xu et al., 2006), and its activation suppresses glucagon secretion through desensitization of K^ATP channels. These observations suggest a cooperative role between insulin and GABA in the inhibition of glucagon secretion.
4.3 Zinc
Zinc ions (Zn\(^{2+}\)), co-released with insulin by β-cells in response to high glucose levels, have been reported to activate K\(^{+}\)\_ATP channels on α-cells, desensitize the channels and suppress glucagon secretion (Ishihara et al., 2003). Zn\(^{2+}\) is also reported to stimulate glucagon secretion from α-cells when its concentration falls as part of a “switch-off” mechanism (Zhou et al., 2007). However, another study reports a lack of inhibitory effect of exogenous Zn\(^{2+}\) on glucagon secretion (Ravier and Rutter, 2005), indicating that the effects of Zn\(^{2+}\) on glucagon secretion are complex and require further investigation.

4.4 Somatostatin
Somatostatin, an inhibitory hormone, secreted by neuronal and pancreatic δ-cells in islets inhibits both insulin and glucagon in a paracrine manner in the islet (Barden et al., 1977; Gerich et al., 1974; Starke et al., 1987). Somatostatin is considered to exert its suppressive effect on glucagon secretion largely through interstitial communication between α- and δ-cells (Stagner and Samols, 1986). Following binding to its receptors on α-cells somatostatin inhibits glucagon secretion by inducing plasma membrane hyperpolarization (Yoshimoto et al., 1999), suppression of cAMP elevation (Schuit et al., 1989) and direct inhibition of the exocytotic machinery via a G-protein-dependent mechanism (Gromada et al., 2001). Somatostatin secretion from islet δ-cells is stimulated by glucose (Gerber et al., 1981; Honey et al., 1980), consistent with the report that the suppressive effect of high glucose on glucagon secretion may be mediated by glucose-induced secretion of somatostatin (Hauge-Evans et al., 2009). Interestingly, global somatostatin knockout mice exhibit enhanced insulin and glucagon secretion \textit{in vivo} and \textit{ex vivo}. In addition the ability of exogenous glucose to suppress glucagon secretion is lost in islets isolated from somatostatin knockout mice (Hauge-Evans et al., 2009) and highlights the intra-islet interactions between somatostatin, glucagon, and insulin. These observations from a global knockout of somatostatin should be interpreted with caution since extra-pancreatic neuronal effects cannot be ruled out. It should also be noted that somatostatin involvement in glucagon suppression during hyperglycemia might be less important than the effects of β-cell secretion \textit{in vivo} according to the direction of intraislet microcirculation, β-α-δ (Gerich, 1990; Stagner and Samols, 1986). Interestingly, somatostatin is also reported to be involved in GLP-1 mediated suppression of glucagon secretion (see section 4.6). Further investigation is thus necessary to clarify the intra-islet relationship of islet hormones.

4.5 Glucagon
Interestingly, glucagon which is secreted by α-cells is reported to stimulate glucagon secretion (Ma et al., 2005). Upregulation of cAMP by glucagon signaling is suggested to stimulate glucagon exocytosis via a mechanism that is similar to the stimulatory effects of glucagon on insulin and somatostatin secretion (Huypens et al., 2000; Stagner et al., 1989).

4.6 Glucagon like-peptide-1 (GLP-1)
The incretin hormone, glucagon-like peptide-1 (GLP-1), is secreted by intestinal L-cells in response to food intake and is a strong stimulator of insulin secretion and also regulates β-cell mass through modulation of cellular proliferation and death (Drucker, 2006). Therefore, GLP-1 contributes to glucose homeostasis acutely by enhancing β-cell secretory function and chronically by maintaining β-cell mass. In addition to these effects on β-cells, GLP-1 is
reported to suppress glucagon secretion by directly acting on α-cells or indirectly by stimulating insulin secretion or modulating other non-β-cell hormones (e.g. somatostatin) which can in turn suppress glucagon secretion. However, the defects in GLP-1 secretion and action in type 2 diabetes likely impact the pathophysiology of the disease via abnormal regulation of both insulin and glucagon secretion (Holst et al., 2009).

Paradoxically, another incretin hormone, glucose-dependent insulinotropic polypeptide (GIP), can stimulate glucagon secretion despite stimulating insulin secretion from β-cells in a manner similar to GLP-1 (de Heer et al., 2008; Meier et al., 2003; Pederson and Brown, 1978). On the other hand, GLP-2, although derived from the same proglucagon gene as GLP-1, in intestinal L-cells, has not been reported to affect the secretory properties of β-cells but stimulates glucagon secretion in human subjects (Meier et al., 2006b), by activation of GLP-2 receptors on α-cells (de Heer et al., 2007).

4.6.1 Indirect suppression of glucagon secretion by GLP-1
GLP-1 is reported to suppress glucagon secretion directly and/or indirectly through other cell-types; β- and δ-cells. In this point, many studies were conducted and displayed pros and cons to both theories. However, considering these reports comprehensively, it is less possible that only one mechanism is working in the suppressive effect of GLP-1 on glucagon, and it is conceivable that these direct and indirect manners are both regulating glucagon secretion with interacting each other.

There are conflicting reports concerning the expression of GLP-1 receptors in α-cells (Heller et al., 1997; Moens et al., 1996). Previous studies investigating GLP-1 receptor expression in α-cells by RNA expression and immunohistochemical analyses indicate that GLP-1 receptors are not expressed in α-cells or if present are expressed at low levels (Tornoehave et al., 2008), or by only a few α-cells (Heller et al., 1997). A recent study using in situ hybridization and immunofluorescence microscopy in mouse, rat, and human pancreas identified the islet cell types that express GLP-1 receptors (Tornoehave et al., 2008) and concluded that GLP-1 receptors are not expressed in α-cells. Thus, it is unlikely that GLP-1 can exert its direct effects on α-cells to impact glucagon secretion. On the other hand, GLP-1 is a strong secretagogue for insulin from β-cells, and considering the central role for insulin in the regulation of glucagon secretion, it is reasonable to suggest that GLP-1 suppresses glucagon secretion by secreted insulin. GLP-1 is also reported to stimulate somatostatin secretion from δ-cells in response to high glucose (Orskov et al., 1988), and it is possible that the secreted somatostatin suppresses glucagon secretion (de Heer and Holst, 2007; Hauge-Evans et al., 2009). This suggestion is supported by the observation that expression of a highly specific somatostatin receptor subtype 2 (SSTR2) antagonist completely abolished the GLP-1 effect on glucagon secretion in isolated perfused rat pancreas (de Heer et al., 2008). However, considering that the direction of intra-islet microcirculation occurs from the core of islets to the mantle; from β-α-δ at least in rodents (Stagner and Samols, 1986), additional studies are necessary to explore these possibilities.

4.6.2 Direct suppression of glucagon secretion by GLP-1
In contrast, reports that GLP-1 (Creutzfeldt et al., 1996) and DPP-4 inhibitor (Foley et al., 2008) treatment suppressed excessive glucagon secretion in type 1 diabetes patients even in the absence of secretory products from β-cells, suggest a potential direct effect of GLP-1 on glucagon suppression. A recent study by De Marinis et al reported that the expression of
GLP-1 receptors in α-cells is less than 0.2 % of its expression in β-cells, and consequently GLP-1 can induce a small elevation in cAMP activating PKA followed by selective inhibition of N-type Ca\(^{2+}\) ion channels, thus suppressing glucagon exocytosis (De Marinis et al.). In contrast, receptors for epinephrine or GIP are expressed abundantly in α-cells, and these molecules stimulate electrical activity significantly leading to an increase in Ca\(^{2+}\) in α-cells, causing glucagon exocytosis to accelerate through activation of L-type Ca\(^{2+}\) ion channels (De Marinis et al.). Studies using isolated islets indicated that GLP-1 effect on glucagon suppression is independent of insulin and intra-islet paracrine effect.

### 4.6.3 Model for the GLP-1 mediated suppression of glucagon secretion

Considering these reports together, it is possible that GLP-1 suppresses glucagon secretion directly, but in postprandial state, GLP-1 enhances insulin secretion from β-cells together with another incretin GIP, and subsequently exerts suppressive effects on glucagon secretion. Further urgent investigations are necessary to understand the effects of GLP-1 on α-cell function. However, reports of GLP-1 induced suppression of glucagon secretion, in addition to its beneficial role on β-cells including augmentation of glucose-stimulated insulin secretion, promotion of β-cell proliferation, and protection of β-cells from various cytotoxicities, emphasizes the potential of GLP-1 therapy for the treatment of diabetes.

GLP-1 directly suppresses glucagon secretion from α-cells through slight increase of cAMP followed by inhibition of N-type Ca\(^{2+}\) channels (De Marinis, 2010) (57). GLP-1 also potentiates insulin secretion from β-cells then suppresses glucagon secretion through insulin effects on α-cells. Glucose stimulates insulin secretion from β-cells and suppresses glucagon from α-cells through insulin effects, while glucose can stimulate glucagon secretion from α-cells.
5. Conclusion and future perspectives

While glucagon was believed to elevate or decline simply in response to blood glucose levels, emerging work reveals a complex but sophisticated regulatory mechanism for the modulation of glucagon output from the α-cells with effects from pancreatic and endocrine hormones including insulin, somatostatin, epinephrine and incretins, nutrients and central and autonomic nervous pathways. The concept of intra-islet regulation of glucagon secretion that is mediated by insulin in a paracrine manner is now recognized as an important pathway that determines α-cell functions. Thus, disorder in intra-islet regulation of glucagon secretion is deeply involved in pathophysiology of diabetes. Considering that the diabetic state is characterized by systemic insulin resistance, that includes non-classical targets such as β-cells (Gunton et al., 2005; Kulkarni et al., 1999), it would be important to explore whether insulin resistance at the level of the α-cell underlies some of the early defects that lead to enhanced glucagon output and a consequent defect in glucose homeostasis.

Recently, new therapeutic approaches targeting excessive glucagon by suppression of glucagon secretion or inhibition of glucagon receptors and their function were tried in the treatment of diabetes, but simple inhibition of glucagon effect does not result in improvement of glucose homeostasis because of hypoglycemia by lack of glucagon effect. In future therapy in diabetes, we need to aim glucagon to work appropriately and rest properly, then improve its effects on other organs and hormonal balance between glucagon and insulin. Further studies are necessary to explore whether cells in the central and/or autonomic nervous systems can be targeted to modulate glucagon secretion for therapeutic purposes.

6. Acknowledgments

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7. References


Over the last few decades the prevalence of diabetes has dramatically grown in most regions of the world. In 2010, 285 million people were diagnosed with diabetes and it is estimated that the number will increase to 438 million in 2030. Hypoglycemia is a disorder where the glucose serum concentration is usually low. The organism usually keeps the serum glucose concentration in a range of 70 to 110 mL/dL of blood. In hypoglycemia the glucose concentration normally remains lower than 50 mL/dL of blood. Hopefully, this book will be of help to many scientists, doctors, pharmacists, chemicals, and other experts in a variety of disciplines, both academic and industrial. In addition to supporting researcher and development, this book should be suitable for teaching.

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