Insulin as a physiological modulator of glucagon secretion

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Bansal P, Wang Q. Insulin as a physiological modulator of glucagon secretion. Am J Physiol Endocrinol Metab 295: E751–E761, 2008. First published July 22, 2008; doi:10.1152/ajpendo.90295.2008.—Glucose homeostasis is regulated primarily by the opposing actions of insulin and glucagon, hormones that are secreted by pancreatic islets from β-cells and α-cells, respectively. Insulin secretion is increased in response to elevated blood glucose to maintain normoglycemia by stimulating glucose transport in muscle and adipocytes and reducing glucose production by inhibiting gluconeogenesis in the liver. Whereas glucagon secretion is suppressed by hyperglycemia, it is stimulated during hypoglycemia, promoting hepatic glucose production and ultimately raising blood glucose levels. Diabetic hyperglycemia occurs as the result of insufficient insulin secretion from the β-cells and/or lack of insulin action due to peripheral insulin resistance. Remarkably, excessive secretion of glucagon from the α-cells is also a major contributor to the development of diabetic hyperglycemia. Insulin is a physiological suppressor of glucagon secretion; however, at the cellular and molecular levels, how intraislet insulin exerts its suppressive effect on the α-cells is not very clear. Although the inhibitory effect of insulin on glucagon gene expression is an important means to regulate glucagon secretion, recent studies suggest that the underlying mechanisms of the intraislet insulin on suppression of glucagon secretion involve the modulation of KATP channel activity and the activation of the GABA-GABA receptor system. Nevertheless, regulation of glucagon secretion is multifactorial and yet to be fully understood.

diabetes; hyperglycemia; γ-aminobutyric acid; adenosine 5′-triphosphate-sensitive K+ channel

Both the insulin-producing β-cells and the glucagon-secreting α-cells are electrically excitable; however, each cell type features a unique set of ion channels (72, 89). Unlike in β-cells, α-cells are electrically silent in the presence of insulin-stimulatory glucose concentrations (56). Regulation of glucagon secretion is mediated by electrical machinery comprised of ion channels and is modulated by glucose and paracrine factors (Fig. 1). The α-cells contain a large tetrodotoxin (TTX)-sensitive Na+ current that inactivates at intermediate voltages (56, 119) and are equipped with two types, low-voltage activated (T-type) (56, 88) and high-voltage activated (L- or N-type) (11, 56, 88, 89, 95, 104, 156), of voltage-gated Ca2+ channels. T-type Ca2+ channels are partly responsible for initiation of the depolarization cascade that is required to elicit exocytosis (56, 72). Activated by the mild depolarization caused by activation of the T-type Ca2+ channel, the TTX-sensitive Na+ channel continues the depolarization cascade (56) to trigger the influx of Ca2+ ions via either the L-type (56) or N-type Ca2+ channel (55, 59, 95, 104), although in some cases blockade of the L-type Ca2+ channel has no effect on α-cell secretion (55, 95, 104). In comparison, pancreatic β-cells also express TTX-sensitive Na+ channels (20, 37, 54, 149), but they are half-maximally inactivated at a more negative voltage (Vh = −47 mV) (56, 89). Mouse β-cells lack low-voltage-activated Ca2+ channels (89) and possess only L-, P/Q-, N-, and R-type Ca2+ channels (149),

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whereas a recent study using human \( \beta \)-cells found that they lack N- or R-type \( \text{Ca}^{2+} \) channels and express the T-type isoform (20). Rat \( \beta \)-cells express T- and L-type \( \text{Ca}^{2+} \) channels (6).

Both \( \alpha \)-cells and \( \beta \)-cells possess ATP-sensitive \( \text{K}^{+} \left( \text{K}_{\text{ATP}} \right) \) channels, which are weakly inwardly rectifying \( \text{K}^{+} \) channels that are gated by the intracellular levels of adenosine triphosphate (ATP) (121). The \( \text{K}_{\text{ATP}} \) channels set the resting membrane potential of the cell (17, 56, 72) and thus link the metabolic state of a cell to its electrophysiological activity. In the \( \beta \)-cells, a postprandially increased blood glucose concentration enhances glucose metabolism (i.e., increases the \([\text{ATP}]_i\)) (137). As a result, the \( \text{K}_{\text{ATP}} \) channels close, leading to membrane depolarization and subsequent activation of voltage-gated \( \text{Ca}^{2+} \) channels (137). The increased \( \text{Ca}^{2+} \) entry triggers insulin release (137). Both the \( \alpha \)-cell and \( \beta \)-cell \( \text{K}_{\text{ATP}} \) channels are comprised of the SUR1 (sulphonylurea receptor) (138) and Kir6.2 (pore-forming) subunits (17, 138) yet were thought to be operated differently in the \( \alpha \)-cells (61). Interestingly, it has been demonstrated by Olsen et al. (104) that the glucagon secretion pathway in isolated rat \( \alpha \)-cells mimics the stimulus-secretion mechanism in \( \beta \)-cells. In these studies the authors found that prolonged treatment of 20 mM glucose causes closure of \( \text{K}_{\text{ATP}} \) channels and stimulates glucagon exocytosis (104). This effect is glycolysis dependent and mimicked by treatment with the \( \text{K}_{\text{ATP}} \) channel blocker tolbutamide (104). Similar results were obtained by Franklin et al. (42). Although one must be cautious when interpreting results from experiments on isolated \( \alpha \)-cells because they are removed from any potential paracrine regulation from neighboring \( \beta \)-cells or somatostatin-secreting \( \delta \)-cells, these findings suggest that glucose metabolism and increased ATP production in \( \alpha \)-cells leads to \( \text{K}_{\text{ATP}} \) channel closure, \( \alpha \)-cell membrane depolarization, and glucagon secretion (104). However, a biphasic effect
of K<sub>ATP</sub> channel activity on glucagon secretion has recently been demonstrated in isolated mouse islets, because low doses of tolbutamide stimulate glucagon secretion (95) while inhibiting glucagon secretion at high concentrations (61, 95). These findings suggest that a certain degree (i.e., narrow window) of α-cell K<sub>ATP</sub> channel activity sets the α-cell membrane potential within a range that is critical for the operation of the machinery of glucagon secretion (Fig. 1). Nevertheless, the effects of modulating α-cell K<sub>ATP</sub> channels are complex and yet to be fully understood. It is clear that the α-cell must be subject to a regulatory mechanism other than the direct action of glucose (i.e., paracrine or intrasilet) to ensure that glucagon secretion is inhibited during hyperglycemia.

**Physiological Role of Glucagon**

Glucagon exerts a variety of biological actions via the activation of the glucagon receptor, which is a member of a subfamily of G protein-coupled receptors. Glucagon receptors are present in the islet β-cells and a wide array of tissues, including the liver, kidney, adipose tissue, brain, adrenal gland, duodenum, and heart (24). Glucagon has been shown to modulate heart muscle contractility (108), ghrelin secretion (5), and gastrointestinal motility (102), among other actions (Table 1). Most importantly, glucagon protects against hypoglycemia by stimulating net hepatic glucose production through promotion of glycogenolysis and glucoseogenesis and simultaneous inhibition of glycolysis and glycogenolysis (70, 146).

Generation of mouse models featuring a whole body knock-out of the glucagon receptor gene (Gcgr<sup>−/−</sup>) has advanced our understanding of glucagon signaling and its roles in the regulation of glucose homeostasis. For instance, Gcgr<sup>−/−</sup> mice display reduced fasting blood glucose levels and improved glucose tolerance compared with the wild-type mice (50, 106). Interestingly, despite lacking glucagon action, these mice still retain their counterregulatory capacity since their glucose levels could be restored to the basal levels within 60 min during hypoglycemia induced by insulin injection (50). The clear counterregulatory mechanism functioning in the lack of glucagon action has yet to be determined; however, a remarkable increase in basal cAMP signaling in Gcgr<sup>−/−</sup> liver and enhanced responsiveness to epinephrine in the Gcgr<sup>−/−</sup> hepatocytes and white adipose tissues might be, in part, responsible for this compensation (50). The presence of marked α-cell hyperplasia in Gcgr<sup>−/−</sup> islets also suggests that glucagon signaling is required for the preservation of normal islet cell growth and function (50, 131). These islets also exhibit impaired insulin secretion when treated with insulin secretagogues such as arginine and carbachol, indicating that loss of glucagon signaling may impact the insulin secretion pathway (131). Interestingly, whole body insulin sensitivity in Gcgr<sup>−/−</sup> mice was enhanced as indicated by hyperinsulinemic euglycemic clamp studies (131), which may represent a compensatory mechanism for a reduction in β-cell function. Furthermore, one such compensatory pathway could be the elevation of glucagon-like peptide 1 (GLP-1) signaling as a consequence of increased production of the hormone by the α-cells in the face of whole body glucagon resistance (31, 50). GLP-1 is an insulinotropic hormone that exerts a variety of biological actions in mammals, including simulation of insulin secretion, promotion of β-cell growth, improvement of peripheral insulin sensitivity, and moderation of body weight gain (39). It is thus not surprising that the elevated levels of GLP-1 may explain why Gcgr<sup>−/−</sup> mice are protected against streptozotocin-induced β-cell destruction and high-fat diet-induced obesity (31). However, it is not clear whether the improved action of insulin on glucose clearance in Gcgr<sup>−/−</sup> mice is a result of direct glucagon action or indirect GLP-1 action on the insulin-sensitive tissues. It is important to note that the remarkable elevation of glucagon levels in the Gcgr<sup>−/−</sup> mice might have further facilitated the GLP-1 signaling, because glucagon can, although with reduced affinity and efficacy, bind and activate the GLP-1 receptor (32).

**Hyperglucagonemia Causes Diabetic Hyperglycemia**

Hyperglucagonemia has been established as a major contributor to the development of diabetic hyperglycemia (128, 143). A recent study using mice that were administered glucagon via osmotic minipump for 28 days showed that chronic hyperglucagonemia caused elevated blood glucose levels, impaired glucose tolerance, and glomerular abnormalities (91), symptoms that are representative of early-stage type 2 diabetes. Humans with type 2 diabetes have day-long plasma glucagon concentrations that are higher than normal (116), and this hyperglucagonemia causes inappropriate elevation of hepatic glucose output due to enhanced glucoseogenesis (48) and glycogenolysis (48, 127). Interestingly, clinical studies have demonstrated that challenge with glucagon results only in a transient and small rise in the glucose and insulin levels of healthy individuals (117). However, in insulin-withdrawn diabetes patients, the glycemic response to hyperglucagonemia is five to 15 times greater than in nondiabetic controls (128). These findings suggest that the action of glucagon is diabetogenic only under the conditions of insulin deficiency (117, 128, 143). Studies in human subjects with type 1 diabetes, a disease characterized by insufficient insulin production due to autoimmun-

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**Table 1. Effects of glucagon signaling in various organs**

<table>
<thead>
<tr>
<th>Receptor Location</th>
<th>Effect</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Stimulates glycogenolysis and glucoseogenesis</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Inhibits glycogenolysis and glycogen synthesis</td>
<td>70</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>Inhibits ACTH-stimulated cortisol production</td>
<td>98</td>
</tr>
<tr>
<td>Kidney</td>
<td>Stimulates bicarbonate secretion</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>Reduces urinary acidification</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Increases glomerular mesangial cell proliferation</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Promotes natriuresis</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Stimulates K&lt;sup&gt;+&lt;/sup&gt; excretion</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Increases glomerular filtration rate</td>
<td>2</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>Stimulates brown fat thermogenesis</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Stimulates brown fat cell growth</td>
<td>14</td>
</tr>
<tr>
<td>Duodenum</td>
<td>Inhibits gastrointestinal motility</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>Promotes relaxation of Sphincter of Oddi</td>
<td>26</td>
</tr>
<tr>
<td>Stomach</td>
<td>Stimulates ghrelin secretion</td>
<td>73</td>
</tr>
<tr>
<td>Heart</td>
<td>Increases heart rate and myocardial contractility</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>and improves atrioventricular conduction</td>
<td>47</td>
</tr>
<tr>
<td>Brain</td>
<td>Suppresses ghrelin secretion</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Stimulates somatostatin release from hypothalamus</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>Stimulates neuronal ketone metabolism</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Suppresses feeding</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Stimulates sympathetic nervous activity</td>
<td>83</td>
</tr>
<tr>
<td>β-Cell</td>
<td>Promotes glucose-stimulated insulin secretion</td>
<td>66</td>
</tr>
<tr>
<td>α-Cell</td>
<td>Stimulates α-cell exocytosis</td>
<td>94</td>
</tr>
</tbody>
</table>
mune destruction of the β-cells, have demonstrated that hyper-
glucagonemia could develop even after an oral glucose chal-
lenge (1, 145). However, administration of exogenous insulin in
these patients is able to suppress excessive glucagon secre-
tion induced by hyperglycemia (136) or other stimuli such as
arginine (51). Therefore, the insulin action is essential to exert
the suppressive effect of glucose on α-cells since, in its ab-
ence, glucose is unable to suppress glucagon release in vivo
(58, 64). These studies provide an explanation underlying the
observation of unsuppressed glucagon secretion in diabetes
despite the presence of hyperglycemia, presumably due to
relatively insufficient insulin levels in the internal environment
of the islet or as a result of α-cell insulin resistance (68,
91, 141).

Intraislet Insulin Action on Glucagon Secretion

Actions of insulin are initiated by binding of the hormone to
its receptor at the cell surface in insulin-responsive tissues,
which activates a signaling complex through recruitment of
adaptor molecules, including the insulin receptor substrate
family. Upon tyrosine phosphorylation, these proteins interact
with signaling molecules through their SH2 domains, which
results in the activation of diverse signaling pathways, includ-
ing phosphatidylinositol (PI) 3-kinase/Akt signaling and
MAPK activation. These pathways act in a coordinated manner
to regulate glucose transport, protein and lipid synthesis, and
mitogenic responses (12).

Within the islets, the α-cells are major targets of insulin
action. Studies on islet microvasculature have suggested that
the α-cells lie downstream from the β-cells and are therefore
presumably bathed by high concentrations of insulin (18). It is
thought that the islet is arranged such that a core of β-cells is
surrounded by a thin layer of α- and δ-cells (7, 18, 25, 84, 105).
The directionality of microvascular perfusion within the islet
has been investigated in perfused rat (125), dog (134), rhesus
monkey (135), and human (133) pancreas by infusing insulin-
neutralizing antibodies through the arterial and venous systems
of the organ, representing anterograde and retrograde perfu-
sion, respectively. The perfusate was then analyzed for pan-
creatic hormone secretions, using radioimmunoassays to deter-
mine the directional effects of abolishing insulin signaling on
α- and δ-cell secretion. These “passive immunization” exper-
iments demonstrated a β → α → δ directionality of islet blood
flow and strongly suggested that intraislet insulin regulates
hormone secretion from α-cells.

Consistent with an important role for insulin in the α-cell,
insulin receptor and the insulin-signaling molecules are ex-
pressed highly in pancreatic α-cells (13, 71, 77, 107, 147) at
levels that are similar to those in the hepatocytes (42). The
insulin receptor plays a role in modulating α-cell function, as
siRNA-mediated “knockdown” of the insulin receptor abol-
ishes glucose-regulated glucagon secretion from the α-cells
(36). ACTION OF insulin on suppression of glucagon secre-
tion has been demonstrated directly using cultured clonal glucagon-
secreting α-cell lines, including αTC6 and In-R1-G9 cells (71,
77, 157). These studies have demonstrated that insulin-induced
suppression of glucagon secretion could be blocked by the PI
3-kinase inhibitor wortmannin (71, 157) or ablation of Akt
kinase activity using dominant negative approaches (157),
suggesting that insulin-suppressed glucagon secretion is medi-
atated by the PI 3-kinase/Akt-dependent signaling pathway.
Although results derived from clonal cell lines may not nec-
essarily be representative of actual α-cell physiology, insulin
also inhibits glucagon secretion in isolated rat islets treated
with pyruvate, an intermediate of glycolysis that selectively
stimulates glucagon secretion (69). The role of intraislet insulin
in suppressing glucagon secretion from the α-cells has been
illustrated further in studies using isolated rat and human islets
and islet hormonal RIAs (157). In these studies, challenging
islets with 20 mM glucose results in insulin secretion associ-
ated with suppressed glucagon secretion; however, blockade of
insulin signaling by the PI 3-kinase inhibitor wortmannin pre-
vents glucose from suppressing glucagon secretion, whereas
the glucose-stimulated insulin secretion is not affected, indicating
that the insulin-signaling pathway mediates the glucose-induced
glucagon secretion in the islets (157). Studies in rat pancreata
have shown that glucose-induced suppression of glucagon secretion
is blocked in the presence of insulin-neutralizing antibodies (96),
which further support the physiological role of insulin in mediating
glucose effect on the α-cells. These findings may provide a
mechanism underlying the well-characterized push-pull phenom-
enon between the secretion of insulin and glucagon in response
to glucose challenge (144) (Fig. 2). Thus, it is hypothesized that
defects in the intraislet insulin-signaling pathway may contribute
to the development of diabetic hyperglucagonemia and hypergly-
cemia, because α-cells with insulin resistance imposed by chronic
treatment with high glucose and insulin displayed reduced insulin
responsiveness, including blunted insulin-stimulated Akt phos-
phorylation and insulin-suppressed glucagon secretion in the
α-cells (157). Therefore, it is conceivable that the postprandial
hyperglucagonemia in type 2 diabetes may be due to the loss of
intraislet postprandial suppression of glucagon secretion driven
by insulin (144). This hypothesis has recently been verified by
in vivo studies using pigs treated with or without alloxan (79,
100), a chemical that selectively destroys β-cells and causes
diabetic hyperglycemia. Postprandial insulin secretion induced a
suppression of glucagon secretion via inhibition of glucagon pulse
mass (100); on the contrary, the postprandial insulin-driven sup-
pression of glucagon secretion was lost in the pigs treated with
alloxan, which caused a reduction of β-cell mass and a deficit in
postprandial insulin secretion (100). Despite this, it has been
shown that glucose can inhibit glucagon secretion at concentra-
tions below that required to elicit insulin secretion (95, 104, 123),
indicating that glucose may directly inhibit glucagon secretion in
the α-cells.

Hypoglycemia is a serious acute complication in type 1
diabetic patients undergoing intensive insulin therapy (33).
Defects in glucose counterregulation have been attributed to
tonic inhibition of α-cell activity due to intraislet hyperinsu-
linemia (9), which prevents a decrement in intraislet insulin
during hypoglycemia that would normally act as the switch for
α-cells to secrete glucagon (114). This “switch-off” hypothesis
has been supported by studies in rats rendered diabetic via
treatment with the β-cell-selective toxin streptozotocin (159)
as well as by using isolated rat and human islets (65) and a rat
model of spontaneous type 1 diabetes (161). These studies
demonstrated that cessation of exogenous insulin administra-
tion prior to induction of hypoglycemia produces a glucagon
secretory response that would otherwise be absent during
continuous insulin infusion (65, 159, 161). A clinical study in
nondiabetic humans attributed a 30% decrease in the counter-
regulatory glucagon response to hypoglycemia after somatostatin infusion was administered to abolish any decrement in intraislet insulin during hypoglycemia (57), indicating that there may be other mechanisms involved in triggering glucagon secretion in humans. However, the specificity of the switch-off hypothesis has become broad, since a recent study has suggested that a decrease in the intraislet concentration of Zn$^{2+}$ and not insulin is the switch required to trigger glucagon secretion during hypoglycemia in rats (160). The effects of Zn$^{2+}$ on $\beta$-cell function are discussed later in this review, but these results highlight the importance of $\beta$-cell secretory products on $\alpha$-cell glucagon secretion.

Intraislet Insulin Modulates $\alpha$-Cell $K_{ATP}$ Channels

Insulin’s action as a negative regulator of glucagon secretion may involve diverse machineries, including modulating the $\alpha$-cell $K_{ATP}$ channel activity. As mentioned above, glucagon secretion requires $\alpha$-cell depolarization to trigger the release of glucagon-containing granules. Conversely, hyperpolarization of the $\alpha$-cell as a consequence of $K_{ATP}$ channel activation can block glucagon secretion (61). Insulin has been shown to activate $K_{ATP}$ channels in hypothalamic neurons in rats (132) and isolated mouse $\beta$-cells (75), resulting in membrane hyperpolarization and cessation of electrical activity (75, 132). Interestingly, a recent study showed that, in isolated mouse $\beta$-cells, glucose could directly enhance $K_{ATP}$ channel conductance by upregulating $K_{ATP}$ channel plasma membrane localization (158). Furthermore, it has also been demonstrated that activation of ERK2 enhanced the activity of $K_{ATP}$ channels in HEK-293 cells transiently transfected with the $K_{ATP}$ channels through phosphorylation of the Kir6.2 subunit, leading to a decrease in ATP sensitivity (92). It is interesting to note that ERK2 is a component in the Ras-MAPK pathway that is activated by insulin (139). Since the Ras-MAPK pathway is PI 3-kinase independent, it is plausible that insulin modulates $K_{ATP}$ channel function via PI 3-kinase-dependent and/or -independent pathways.

Treatment of isolated rat $\alpha$-cells with insulin can also transiently enhance $K_{ATP}$-mediated currents (42), suggesting that insulin-mediated suppression of glucagon secretion from the $\alpha$-cells could occur via modulation of $K_{ATP}$ currents. Interestingly, this effect is not blocked by wortmannin (42), which suggests the potential involvement of nonclassical PI 3-kinases in glucagon secretory competence. Studies in transgenic mice expressing green fluorescent protein under the control of the mouse insulin promoter, which facilitates differentiation between $\beta$-cells and non-$\beta$-cells within the pancreatic islet (89), have characterized the $K_{ATP}$ channels in $\alpha$-cells to be five times more sensitive to intracellular ATP-mediated channel inhibition than $\beta$-cell $K_{ATP}$ channels, although the densities of $K_{ATP}$ channels found in both cell types are equal (89). In isolated $\alpha$-cells from mice expressing insulin promoter-green fluorescent protein, insulin markedly reduces the sensitivity of $K_{ATP}$ channels to ATP in a wortmannin-sensitive fashion (87). Interestingly, although glucose can dose-dependently modulate the intracellular [ATP] in the $\beta$-cells, glucose has no effect on (35) or increases the intracellular [ATP] less efficiently in $\alpha$-cells compared with $\beta$-cells (69). Therefore, it is conceivable that glucose-induced suppression of glucagon secretion could occur through insulin-mediated desensitization of $\alpha$-cell $K_{ATP}$

![Fig. 2. The reciprocal response of insulin (•) and glucagon (○) to changes in glucose concentration in the perfused dog pancreas (144). Figure is reproduced from the article by Dr. R. H. Unger (144) with permission from the author.](image-url)
channels to ATP inhibition, increasing channel activity and causing the \(\alpha\)-cell to become hyperpolarized. It is of great interest to further determine how insulin precisely reduces the \(\alpha\)-cell \(K_{ATP}\) channel sensitivity and its downstream cascades.

**Intraislet Insulin Promotes GABAergic Inhibition of Glucagon Secretion**

\(\gamma\)-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system and is synthesized from glutamic acid by glutamic acid decarboxylase (82). Three types of the GABA receptor (GABAR) have been identified: type A GABAR (GABA\(_A\)R), a heteropentameric ligand-gated Cl\(^-\) channel that is antagonized by the compound bicuculline (93); GABA\(_B\)R, a heterodimeric G protein-coupled receptor (19, 28); and GABA\(_C\)R, which is also a ligand-gated Cl\(^-\) channel; however, it is bicuculline insensitive, homopentameric, and active at lower concentrations of GABA than GABA\(_A\)R (28). Activation of GABA\(_A\)R results in membrane hyperpolarization as a consequence of an inward Cl\(^-\) flux (78). Although GABAergic signaling is an essential regulatory component of neural excitability accounting for 20–30% of all synaptic signaling in the mammalian brain (154), GABA also modulates the endocrine function of the pancreas (43, 74). Pancreatic \(\beta\)-cells contain high concentrations of GABA (21, 46, 140) and glutamic acid decarboxylase (45, 53). GABA is localized in both synaptic-like microvesicles (21) and the insulin-containing large dense core vesicles (22, 44) within the \(\beta\)-cells. The functional GABA\(_A\)Rs are expressed in both \(\alpha\)-cells (63, 118) and \(\beta\)-cells (38).

The GABA-GABA\(_A\)R system has been suggested to negatively regulate glucagon secretion (118, 153). GABA administered to isolated mouse and rat islets, as well as to perfused rat pancreas, can inhibit the release of glucagon (52), whereas inhibition of the GABA\(_A\)R by bicuculline (47, 118) or SR95531 (153) prevents GABA-mediated suppression of glucagon secretion from the \(\alpha\)-cells but does not affect the insulin secretion (157). However, although controversial (47), the release of GABA from the \(\beta\)-cells does not appear to be stimulated by glucose (130, 151, 155), or the increase in GABA release by high glucose may be too small to detect (118). However, the failure to detect an increase in GABA release from the \(\beta\)-cells in response to increased glucose concentrations does not exclude the possibility that there is an increase in the responsiveness of GABA\(_A\)Rs on \(\alpha\)-cells. Evidence supporting this idea is the finding that GABA\(_A\)Rs can be phosphorylated directly by Akt, the important insulin-signaling molecule, resulting in translocation of the receptors from intracellular pools to the cell surface (152). Furthermore, a recent study also suggested that, at postreceptor levels, glucose can modulate GABA\(_A\)R activity through increasing cell surface abundance of the GABA\(_A\)Rs (8). Consistent with the role of insulin in modulating GABAergic signaling in the central nervous system (148, 150, 152), insulin enhances inhibitory GABA currents in clonal glucagon-secreting \(\alpha\)-cells and isolated rat \(\alpha\)-cells upon phosphorylation of the \(\beta\)-subunit of GABA\(_A\)R and plasma membrane recruitment of the GABA\(_A\)Rs by insulin (157). The rapid regulation of neurotransmitter receptor numbers in the postsynaptic domain by direct receptor phosphorylation is an important means of modulating synaptic plasticity (30, 152). Increasing cell surface abundance of GABA\(_A\)Rs in pancreatic \(\alpha\)-cells, as a result of direct modulation by glucose or indirectly by insulin, in response to increased glucose concentrations may be a major mechanism underlying glucose-induced suppression of glucagon secretion. This conception is in accord with a recent study using a well-designed in situ patch-clamp recording technique that illustrates that GABA released from the \(\beta\)-cells could diffuse across the islet interstitium to activate GABA\(_A\)Rs in neighboring cells (153). It is interesting to note that, in vitro, high glucose concentrations stimulate glucagon secretion from isolated rat \(\alpha\)-cells via the glucose-sensing pathway as used by the \(\beta\)-cells (104), further emphasizing the crucial importance of islet paracrine signaling in the regulation of glucagon release in vivo. The intraislet insulin pathway and these modulators appear to constitute precise regulatory networks that operate to regulate glucagon release and, hence, to control glucose homeostasis. Given that in diabetic individuals, insulin deficiency is associated with elevated glucagon levels, which rapidly return to normal with insulin treatment, it is possible that defects in this pathway may contribute to diabetic hyperglucagonemia and subsequent hyperglycemia. This pathway may also partly explain that type 1 diabetic hypoglycemia that occurs in insulin-treated patients may be due to a lack of suppression by endogenous insulin that may potentially render the \(\alpha\)-cells hypersensitive to the suppression induced by exogenous insulin (9).

**Insulin Suppresses Glucagon Gene Expression**

Transcriptional regulation of the glucagon gene by insulin appears to be an important way to regulate glucagon production and secretion. The inhibitory effects of insulin on glucagon biosynthesis are mediated through a downregulation of proglucagon gene expression (29, 109, 110) that requires activation of PI 3-kinase and Akt (126). The insulin response element that mediates insulin effects on glucagon gene transcription is located in the proximal promoter region of the proglucagon gene (111), and several proteins have been identified to act in trans with this region to facilitate insulin-induced transcriptional alterations (49, 111, 126). Furthermore, it has recently been identified that FoxO1, a member of the forkhead transcription factor family, is required for the regulation of proglucagon gene expression by insulin by direct binding of FoxO1 to a forkhead consensus binding site in the preproglucagon gene promoter region (99). In vitro studies in clonal glucagon-secreting cells have demonstrated that insulin induced nuclear exclusion of FoxO1 and a subsequent reduction in proglucagon gene transcription. Experiments employing direct-site mutagenesis and RNA interference have also demonstrated that diminishment of FoxO1 binding eliminated transcriptional regulation by glucose or insulin, whereas FoxO1 silencing abolished the acute regulation by insulin, but not glucose, of glucagon secretion (99). Therefore, these studies further support that intraislet insulin is a key modulator of \(\alpha\)-cell function. However, this transcriptional regulation may be more critical for long-term maintenance of \(\alpha\)-cell glucagon content instead of for short-term responses to changes in blood glucose levels.
Regulation of Glucagon Secretion is Multifactorial

Although the intraislet insulin-mediated suppression of glucagon secretion plays an important role in modulating glucagon secretion via mechanisms involving protein-protein interactions, signal transduction, receptor translocation, and transcriptional regulation (Fig. 3), it should be noted that factors other than intraislet insulin, such as Zn$^{2+}$, may also influence glucagon secretion from α-cells. Insulin molecules are complexed with Zn$^{2+}$ ions to permit stable storage of insulin within large dense core vesicles (81). Zn$^{2+}$ is coreleased with insulin from the β-cells (113) and may be an important inhibitor of glucagon secretion (42, 69). Upon liberation, Zn$^{2+}$ is transported into the α-cells via Ca$^{2+}$ channels and Zn$^{2+}$ transporters (62). Zn$^{2+}$ has been shown to inhibit pyruvate-stimulated glucagon secretion in perfused rat pancreas (69), reduce the release of glucagon in isolated α-cells when incubated in the absence of glucose (42), and inhibit glucagon release from αTC6 cells as well as mouse islets at low glucose concentrations (62). Also, a recent study in rats has found that a decrement in intraislet Zn$^{2+}$ is required for a normal glucagon secretory response to hypoglycemia (160). Zn$^{2+}$ may inhibit glucagon secretion by binding directly to two histidine residues on the SUR1 subunit (10) of the K$_{ATP}$ channel and enhancing their activity (42, 112), as it has been reported to enhance K$_{ATP}$ channel-mediated current in clonal rat β-cells (16); however, studies in mouse α-cells have shown no effect of Zn$^{2+}$ on K$_{ATP}$ channels (62, 87). These findings potentially explain why ZnCl$_2$ has no effect on intracellular Ca$^{2+}$ oscillations or glucagon secretion in isolated mouse α-cells in the absence of glucose (115), since mouse α-cells express fewer K$_{ATP}$ channels than rat α-cells (11, 115).

Furthermore, there are a number of reported regulators/modulators that may participate in the process of glucagon secretion. Among them are sympathetic and parasympathetic nerves (4), somatostatin (27), ghrelin (122), L-glutamate (142), and GLP-1 (97). However, L-glutamate (40, 86) and GLP-1 (103) are also known to stimulate insulin secretion, which suggests that, in vivo, the effects of these molecules on the α-cells may be indirectly caused by raising intraislet insulin concentrations. For a thorough and up-to-date review of the effects these substances have on α-cells, refer to Ref. 60.

Fig. 3. Proposed mechanisms of intraislet insulin suppression of glucagon secretion. Insulin secreted from β-cells acts in a paracrine manner to activate the insulin-signaling cascade in α-cells. This has 3 main consequences: 1) activation of K$_{ATP}$ channels, 2) enhancement of type A GABA receptor (GABA$_A$R)-mediated GABA current, and 3) inhibition of proglucagon gene transcription. First, insulin decreases the α-cell K$_{ATP}$ channel sensitivity to ATP inhibition in a phosphatidylinositol 3-kinase-dependent manner, causing an increase in the efflux of K$^+$ ions from the α-cell and hyperpolarizing the α-cell membrane potential. The resultant cessation of action potentials prevents an increase in cytosolic [Ca$^{2+}$] and inhibits glucagon secretion. Second, activation of the insulin receptor (IR) promotes membrane translocation of GABA$_A$R and enhances GABA-mediated Cl$^-$ influx. The α-cell membrane potential becomes hyperpolarized, and glucagon secretion is suppressed. Finally, insulin inhibits proglucagon gene transcription, possibly representing a long-term mechanism for regulating α-cell function. Other modulators of α-cell glucagon secretion, including Zn$^{2+}$ and somatostatin, are not shown.
Conclusion

As a counterregulatory hormone for insulin, glucagon plays a critical role in maintaining glucose homeostasis. Glucagon increases hepatic glucose output by enhancing glycogenolysis and gluconeogenesis in the liver. In diabetic subjects, abnormal secretion of not only insulin but also glucagon leads to hyperglucagonemia and altered insulin-to-glucagon ratios, which predominantly contribute to diabetic hyperglycemia. Given that insulin is a physiological suppressor of glucagon secretion, understanding the precise molecular mechanism of glucagon secretion by intrasial insulin may facilitate our effort to develop strategies to combat diabetic hyperglycemia and to prevent hypoglycemia in insulin-treated patients.

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