1. General Information

In 1907, Michael Lane described his anatomical observations of the pancreatic islets of Langerhans as cells with distinctive anatomical properties versus the earlier theory as those of “exhausted acini” (58). In his characterization of stained hamster pancreatic sections, Lane described the existence of two distinct types of cells within the islet, which he referred to as the large α and smaller β cells (58). Four decades later, Sutherland and de Duve established that the α-cells of the pancreatic islet are the primary source of glucagon (95, 96). Initially labeled as a contaminant, the physiological role of glucagon was first described in 1921, when Banting and Best conducted their classical experiments of insulin’s actions. Banting and Best tested their first pancreatic extracts in depancreatized dogs and observed an initial transient rise in blood glucose followed by the insulin-induced hypoglycemia (36). Several years later, Murlin and his colleagues credited this transient rise in blood glucose to a hormone they named “Glucagon” or “Hyperglycemic-Glycogenolytic Factor” (28, 36, 71). Insights into the regulation of glucagon release came from elegant cross-circulation experiments performed by Foa and his colleagues in the 1950s, showing that hypoglycemia triggered by the injection of insulin in a donor dog induces the release of glucagon, which secreted through the donor blood via a pancreatic-femoral anastomosis causes a hyperglycemic response in a recipient dog (28). Bensley and Woerner added to these observations with their suggestion that glucagon induces liver glycogenolysis and thereby promotes a rise in blood glucose levels (4).

Glucagon is a 29-amino acid peptide derived from the tissue specific-processing of proglucagon in pancreatic α-cells through cleavage by prohormone convertase 2 (PCSK2) (Figure 1) (30). In contrast, processing of proglucagon to glucagon-like peptides (GLP-1, GLP-2), oxyntomodulin and glicentin occurs in intestinal enteroendocrine cells (Figure 1) (62). Glucagon plays a major role in antagonizing the effects of insulin and maintaining glucose homeostasis by promoting hepatic gluconeogenesis and glycogenolysis and inhibiting glycogen synthesis. Therefore, the secretion of glucagon is normally induced in states of decreasing blood glucose, such as fasting and increased energy expenditure, to sufficiently induce a rapid, yet transient, rise in blood glucose. However, aberrant secretion of glucagon is a characteristic of Types I and II Diabetes. Increased secretion of...
glucagon is observed in patients with Type II Diabetes, leading to an increase in hepatic glucose output and exacerbating the hyperglycemic state (19, 63, 79, 84, 106, 107). In contrast, failure to secrete adequate glucagon in response to hypoglycemia is a limiting factor for glucose control, contributing to the morbidity and mortality of patients with Type I Diabetes (15, 33).

2. Glucagon and the Endocrine Pancreas

The pancreatic islet comprises five major types of polypeptide-secreting cells: insulin-secreting β-cells (65-80% of total islet cells), the glucagon secreting α-cells (15-20%), somatostatin-secreting δ-cells (3-10%) and pancreatic polypeptide-secreting cells (3-5%), along with ghrelin-positive cells which are mostly observed in early development (103). The five major islet cell types are aligned on blood vessels at no particular order or structured organization within the human islet (8) (Figure 2A). In contrast, the rodent islet shows a more-defined architecture placing the β-cells in the core and the α, δ and PP-cells lying at the mantle of the islet (Figure 2B). This unique structure in the rodent islet suggests an organized system allowing paracrine interactions between the peptides released. This is supported by studies showing that arterial blood is directed from the core of the rodent islet (insulin-secreting β-cells) to the periphery (6). Therefore, during a rise in blood glucose, the pancreatic α-cells are exposed to high levels of secreted insulin leading to the inhibition of glucagon secretion and glucagon gene transcription.

3. Regulation of Glucagon Release

The secretion of glucagon by the α-cells is regulated by the effects of paracrine/endocrine factors as well as neuronal inputs. Glucagon release is inhibited after carbohydrate-rich meal and the consequent rise in blood glucose and insulin secretion. However, a meal rich in amino acids induces glucagon release. Parasympathetic (vagal) and sympathetic (Epinephrine,
Norepinephrine, Galanin, Neuropeptide Y) nerve stimulations induce the secretion of glucagon from the pancreatic α-cells.

**Glucose**
Glucagon is secreted by the pancreatic α-cells in states of decreasing blood glucose; however, whether changes in glucose concentrations alone can regulate glucagon secretion still remains unclear. High glucose concentrations inhibit glucagon release in the intact islet; however, high glucose induces glucagon release in dispersed, isolated α-cells. These data support the notion that the paracrine interactions of the islet cells, in particular the secreted insulin/GABA and somatostatin, act as primary regulators by suppressing glucagon secretion in hyperglycemia (76). However, the chronic exposure of α-cells to high glucose levels has been shown to induce α-cell dysfunction and insulin-resistance, closely mimicking the diabetic state (21, 92).

Rat α-cells express glucokinase and glucose transporter GLUT1, an isoform with a lower capacity compared to GLUT2, which is the predominant form in insulin-secreting β-cells (42). Despite differences in metabolism of glucose by the two cell types, studies have shown that they share similar inherent mechanisms of activation (76). Alpha cells have high ATP concentrations under low glucose, which rise further after stimulation with high glucose. The glucose-stimulated inhibition of glucagon secretion was associated with an inhibition of AMPK activity and activating AMP-activated protein kinase (AMPK) in turn inhibited secretion (59). Therefore, α-cells do have intrinsic mechanisms, which respond to glucose stimulation, but synergize with extrinsic paracrine signals to regulate secretion under high glucose stimulation (31).

Contrary to the regulation of glucagon release in high glucose conditions, it has been shown that extrinsic paracrine signals (insulin/GABA, somatostatin) do not play a role in regulating glucagon secretion in low glucose concentration (1-6 mM) (99). This is supported by *in vivo* findings showing that inhibiting insulin signaling in the pancreatic α-cells of mice through a loss of α-cell insulin receptors leads to an increase in glucagon secretion in both hyperinsulimic-hypoglycemic and STZ-induced hypoinsulimic-hyperglycemic state. Therefore, these data shows that insulin is necessary for inhibiting glucagon secretion in hyperglycemia; however, insulin does not play a role in regulating glucagon secretion in low-glucose conditions (52).

**Insulin and GABA**
Pancreatic α-cells are exposed to high levels of insulin secreted from the β-cells in the islet. Insulin is a potent inhibitor of glucagon secretion and glucagon gene transcription (2, 67, 78, 101, 107). Data have shown that the diminished insulin release during hyperglycemia associated with diabetes paradoxically stimulates the release of glucagon (19, 79, 106). Studies utilizing *in vitro* approaches have shown that insulin receptors are very abundant on pancreatic α-cells and activate the phosphatidyl inositol 3-kinase (PI3K)-Akt pathway leading to inhibition of glucagon gene transcription and secretion (82, 84, 105). Insulin has been shown to induce the Akt-dependent GABA<sub> receptors translocation to the plasma membrane (which can be activated by GABA (co-released with insulin) and PI3K-dependent opening of K<sub>ATP</sub> channels, culminating hyperpolarizing the plasma membrane and inhibiting glucagon secretion (29, 105). Although, the precise mechanisms responsible for changes in the α-cell function in diabetes remain unclear, a recent study by Kawamori and colleagues showed that inhibiting insulin signaling in the pancreatic α-cells of mice through a loss of α-cell insulin receptors leads to altered glucose metabolism, including mild glucose intolerance, hyperglycemia and hyperglucagonemia (52).

GABA (γ-Aminobutyric acid) is produced from the excitatory amino acid glutamate and co-released with insulin from the pancreatic β-cells by high glucose and glutamate stimulation. GABA can diffuse within the islet interstitium to activate GABA<sub> receptors present on the cell-surface of α-
cells (102). This nonpeptidal neurotransmitter has been shown to act as a suppressor of amino acid-stimulated glucagon release in the mouse and isolated α-cells via GABA_A receptors (35). Data have suggested that glucose-stimulated insulin release and the subsequent activation of the Insulin Receptor-PI3K-Akt pathway induces the activation and translocation of GABA_A receptors to the plasma membrane (105). The GABA co-released with insulin from the β-cells can activate the newly translocated cell-surface GABA_A receptors and increase Cl⁻ inhibitory currents, subsequently hyperpolarizing the plasma membrane (102). The hyperpolarization of the membrane closes the voltage-dependent Ca²⁺ channels, which lowers the free cytoplasmic Ca²⁺ levels and reduces glucagon exocytosis. (80,102).

**Glucagon and Glutamate**

Perfusion experiments in the human and rat pancreases have shown that glucagon suppresses insulin and somatostatin release (7,94). Glucagon receptor knock-out mice exhibit α-cell hyperplasia and hyperglucagonemia, which has been suggested to be due to a lack of autocrine signals of glucagon on the α-cell (36). Contrary to this theory, a recent study has shown that implanted wild-type islets in mice with liver-specific deletion of the glucagon receptor also develop α-cell hyperplasia (64). These data suggest that a circulating factor generated after the disruption of glucagon signaling in the liver can increase α-cell proliferation independent of direct pancreatic input.

Glutamate is a major excitatory neurotransmitter in the central nervous system, which has also been implicated in the regulation of glucagon release. An elegant study published by Cabrera and colleagues described the positive autocrine signal of glutamate in the human, monkey and mouse islets (9). The authors proposed a mechanistic model where glutamate co-released with glucagon potentiates glucagon secretion through acting on the inotropic glutamate receptors on the α-cell membrane and creating a positive autocrine loop (9).

**Somatostatin**

Somatostatin, secreted by the islet δ-cells, has been long accepted as a glucagon-suppressing peptide. Exogenous somatostatin inhibits glucagon release in isolated α-cells, as well as in healthy and diabetic patients (11,34). In addition, islets isolated from somatostatin-deficient mice have reduced glucose-suppression of glucagon release (41).

4. **Physiological Actions of Glucagon at Target Tissues**

Glucagon exerts its physiological action on target tissues via the G-protein coupled glucagon receptor, which is found on multiple tissues including the liver, fat, intestine, kidney and brain (50,68).

In the liver, glucagon counteracts the anabolic properties of insulin by promoting gluconeogenesis and glycogenolysis which consequently increases glucose output. The hepatocyte is exposed to high levels of glucagon released by the pancreas via the portal vein. The mechanistic actions of glucagon on the hepatocyte are mediated by glucagon’s binding and activation of the glucagon receptor on the cell membrane, which upon conformation change activates G proteins. The subsequent activation of adenylate cyclase leads to the increase in intracellular cyclic adenosine monophosphate (cAMP) levels and the activation of protein kinase A (PKA) (50). The second messenger cAMP can activate cyclic nucleotide-gated ion channels, exchange proteins activated by cAMP (EPAC) and protein kinase A (PKA). Glucagon’s activation of the liver’s glucagon receptor leading to hepatic glucose output has been shown to act through the gluconeogenic process is also promoted by the activity of additional transcription factors.
The activated CREB binds to the promoter region of the transcriptional coactivator PGC-1 gene, increasing its transcription. PGC-1 and the nuclear transcription factor hepatocyte nuclear factor-4 (HNF-4) further promote gluconeogenesis by increasing the transcription of PEPCK gene and therefore PEPCK activity (50).

In the adipocyte, glucagon activates the cAMP-PKA pathway, leading to the phosphorylation and activation of hormone-sensitive lipase and the subsequent breakdown of triglycerides (lipolysis) and release of diacylglycerol and free fatty acids into the circulation. The liver can further utilize glycerol and free fatty acids for gluconeogenesis or re-esterification of free fatty acids to form ketone bodies.

Glucagon’s physiological effects on the heart and kidney have also been described, although glucagon’s actions in these tissues are less understood mechanistically compared to those in the liver and fat. In the heart, glucagon has been described as a vasodilator, which lowers blood pressure by decreasing the vasculature resistance in the liver and spleen. Glucagon has diuretic effects on the kidney, increasing glomerular filtration and electrolyte excretion (26).

Glucagon receptors are expressed in the brain and data has suggested that circulating glucagon can pass the blood-brain barrier to modulate its effects in the central nervous system. Glucagon infused in the central nervous system has anorexigenic effects in rats, chicks and sheep. In addition, intravenous infusion of glucagon has been shown to suppress appetite in humans; however, the direct link between glucagon and central food intake regulation in humans is unclear. Although, the mechanisms of glucagon’s activity on the brain remain largely unclear, the involvement of the hypothalamic corticotropin-releasing factor (CRF) and the activation of the hypothalamic-pituitary-adrenal (HPA) axis are implicated to be involved in modulating glucagon’s suppression of food intake (27).

Glucagon relaxes the GI tract from esophagus to colon and as a result is often used to quiet the bowel before endoscopic retrograde cholangiopancreatography (ERCP) or bowel imaging studies (69). In the esophagus it is used to relax the muscle before removal of foreign objects. Glucagon also will relax the sphincter of Oddi (94). These effects are almost certainly pharmacological but are short lived and without other deleterious effects.
5. Glucagon and the Exocrine Pancreas

Crystalline glucagon was first prepared in 1953 and early reports injecting mg amounts of glucagon into rodents included a description of degranulation of acinar cells along with pancreatic atrophy (10,49,57,81). This finding was interpreted by Jarett as due to inhibition of protein synthesis due to lowered plasma amino acid levels \textit{in vivo} as glucagon did not inhibit protein synthesis measured by incorporation of 3H-leucine \textit{in vitro} (48). However, these results are difficult to interpret because the animals given megadoses of glucagon lost weight and were reported to appear ill. In a subsequent and more sophisticated study, Adler (1) infused glucagon IV into rats at 10-80 μg/kg/hr and found that the pancreas lost 80% of three digestive enzymes after 24 hours and EM showed viable cells but with few granules. However, the 50 μg/kg/hr equated to 1.2 mg glucagon in 24 hours and clearly represented a non-physiological treatment. \textit{In vitro} studies with isolated lobules showed that \textit{in-vivo} pretreatment reduced subsequent protein synthesis and intracellular transport after 30 minutes of infusion in vivo but not at 24 hours. In a more recent study, Kash et. al (57) reported that 30 μg/kg of glucagon every 8 hours reduced the trophic effect of caerulein over a 5 day period, but on its own did not affect pancreatic mass, protein or DNA.

With the knowledge of a possible relationship between the endocrine and exocrine pancreas (43), the effects of exogenous glucagon on pancreatic secretion was studied in a variety of species both with and without anesthesia. Initially most studies were carried out in unanesthetized dogs with pancreatic fistulas, the predominant animal model for GI physiology at the time, and glucagon (most often prepared by Eli Lilly) was shown to inhibit the volume, bicarbonate and protein or enzyme content of pancreatic secretion stimulated by food, acid, secretin or CCK (24,44,45,47,55,56,73,74,85). In most cases a large amount of glucagon was used (20-30 μg/kg/hr) and resulting plasma levels of glucagon were not measured or related to physiological levels. Similar inhibition of pancreatic secretion has also been seen in studies carried out in rats (1,5,83), cats (54) and humans (12,18,25,40,53). The mechanism of the inhibition remains unclear, but has been assumed by most authors to be at the level of the pancreas because the effect of exogenous secretagogues was inhibited. Possible loci include inhibition of pancreatic blood flow, the resulting hyperglycemia, lowering of plasma calcium as well as inhibition of the secretory mechanism. Glucagon could also be having an effect on the nervous system either centrally or within the pancreas.

Some of these possible inhibitory loci could be better controlled using a perfused pancreas model. Glucagon has been reported to inhibit secretion in the perfused pancreas of the cat (104) and rat (98). In the latter study, infusion of amino acids was shown to increase glucagon and inhibit pancreatic secretion and this effect could be blocked by infusing an antibody to glucagon (98). Other studies, however, gave different results. In a study in the perfused dog pancreas glucagon had no effect (72), but in a study in perfused rat pancreas, glucagon increased the basal flow and protein output. However, when glucagon was combined with secretin, it decreased the volume and protein output (91). Other \textit{in vitro} studies have been carried out using pancreatic segments or lobules. In studies of rat pancreas lobules, glucagon increased amylase secretion and potentiated effects of acetylcholine, CCK or electric field stimulation to activate nerves (86,87). By contrast, glucagon was reported to increase amylase release from mouse segments (66) or have no effect on \textit{in vitro} release of amylase by mouse (16) or rat pancreatic fragments (1). While all of the positive in vitro studies imply a direct effect on the pancreas, there is no clear overall pattern.

With the development of isolated pancreatic acini and isolated acinar cells, the effects of glucagon were studied and compared to the structurally
related peptides, secretin and VIP. Natural purified glucagon was shown to stimulate amylase release and increase cyclic AMP at high concentrations (1 to 100 µM) in isolated acini from guinea pig, rat and mouse acini (77,89,90). The effect was similar to that of secretin, but observed at much higher concentrations. The material did not interact with VIP receptors on acini and did not elute with purified synthetic glucagon, so its nature is unknown. Most importantly, synthetic glucagon had no effect on amylase secretion (3,77). Glucagon receptor mRNA has been identified in pancreas and in isolated islets by several techniques (22,39,70). However, none of these studies specifically evaluated exocrine pancreas or showed receptor mRNA in acini or ducts. In summary, the studies of glucagon on isolated acini and the current state of receptor knowledge do not support a direct effect of glucagon on acinar cells. Moreover, some of the stimulatory effects of glucagon on isolated perfused pancreas or pancreatic fragments could have been due to a contaminant in natural glucagon.

Because of the effect of glucagon to inhibit secretion in animals and humans, it was studied as a possible therapeutic agent in pancreatitis. In animal models, glucagon protected against hemorrhagic pancreatitis in mice, but only when given before the inducing choline-deficient ethionine-supplemented (CDE) diet (65). In pigs with retrograde injection of bile salts, glucagon infusion had a protective effect when started 18 hours later (100). Although early studies in humans reported some positive results (53), controlled trials failed to show a significant improvement (17,23,75).

In summary, endogenous glucagon from the endocrine α-cells may have an action on the exocrine pancreas, but its mechanism of action and physiological importance is not clear. Supraphysiological administration can affect the exocrine pancreas, but its physiological significance is unclear. Glucagon affects insulin secretion and body metabolism and these could also secondarily affect the exocrine pancreas. Whether increased glucagon levels in diabetes could contribute to the reduced exocrine function reported in diabetics is currently unclear and worthy of further attention.

6. Tools for the Study of Glucagon

Detection and Measurement
Monoclonal and polyclonal antibodies against human, rat and mouse glucagon are commercially available for the detection of glucagon and proglucagon by immunoblotting, immunohistochemistry, immunofluorescence, and immunoprecipitation (Sigma, Abcam, Santa Cruz, Cell Signaling). RIAs and ELISAs are also commercially available for the measurement of glucagon in human, mouse and rat serum, plasma as well as tissue extracts and culture media (Millipore, Alpco, R&D Systems). A disadvantage in using a RIA or ELISA to measure glucagon levels in the serum or plasma of mice is the large volume of sample required to detect baseline levels (50-100 µl) in a concentration range of 20-400 pg/mL. Further modifications of these assays maybe required, but are not always sufficient, to accommodate smaller volumes. A new ELISA recently developed by Mercodia (Uppsala, Sweden) requires smaller volume (25 µl) and offers higher specificity of detection (5-414 pg/mL).

Cell Lines
A glucagon-secreting cell, alpha TC-1, is the only readily available cell line for in vitro approaches to study the regulation of glucagon synthesis and secretion and can be obtained from the American Type Culture Collection (ATCC; Manassas, VA). This cells line was extracted from adenomas developed in transgenic mice expressing the SV40 large T-antigen under the rat preproglucagon promoter and further differentiated in two clones (clones 6 and 9) which do not express insulin/proinsulin mRNA, somatostatin or pancreatic polypeptide (37).
InR1G9 is another glucagon-secreting cell line often referred to in the literature. The InR1G9 cell line is derived from hamster glucagonoma (20,21,78).

**Animals**

Loss of α-cells have been observed in transgenic animal models with transcription factor mis-expression resulting in either a loss of α-cells or diverting α-cell-fate into a different endocrine cell lineage (13,14,38,60,92). The loss of α-cell transcription factors Arx, Pax6 and Foxa2 results in a dramatic complete loss of α-cells and circulating glucagon levels (38,60,92). In contrast, ectopic expression of Pax4 drives endocrine precursor cells and mature α-cells to adapt a β-cell fate (14). Thorel and colleagues were able to ablate 98% of α-cells using an inducible system of dipheria toxin-mediated cell deletion in adult mice (97). Animal models designed to block glucagon’s actions by a genetic or pharmacological inhibition of glucagon receptor and inhibiting active glucagon synthesis using prohormone convertase 2 knock-out mice, have been described (30,32,61,64).

**6. References**

27. Filippi BM, Abraham MA, Yue JT, and Lam TK. Insulin and glucagon signaling in the central nervous system. *Reviews in Endocrine & Metabolic Disorders*, 2013. PMID: 23959343


58. Lane MA. The cytological characters of the areas of Langerhans. *Am J Anat* 409-422, 1907.


