Chapter 3
Endocrine Pancreas

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Introduction

The endocrine pancreas is composed of the islets of Langerhans, which comprise approximately two million clusters of cells dispersed within the acinar tissue of the exocrine pancreas. Whereas the exocrine pancreas is responsible for secreting digestive enzymes for nutrient absorption, the endocrine pancreas regulates nutrient homeostasis and metabolism, including uptake, storage, and release of metabolic fuels. In adults, the islets constitute between 1 and 2% of pancreatic mass. At least four cell types have been identified in the islets: α-cells, β-cells, δ-cells, and pancreatic polypeptide (PP) cells. β-Cells constitute the majority of islet cells and are concentrated in the anterior head, body, and tail of the pancreas. In contrast, the posterior portion of the head, which is derived from the primordial ventral bud (versus the dorsal bud for the remainder of the pancreas), consists of mostly PP cells (Table 3.1). Recently, another subgroup of endocrine cells (epsilon cells) producing hormone ghrelin was discovered in pancreas of mice.[1,2]

Insulin and glucagon play opposing roles in glucose and nutrient homeostasis. While insulin promotes energy storage, glucagon promotes catabolism, making energy available to tissues when food is not available. In the liver, high insulin:glucagon ratio (such that occurs following a meal) stimulates glycogen synthesis and inhibits glycogenolysis, gluconeogenesis, fatty acid oxidation, and ketone production. In adipose tissue, high insulin:glucagon ratio favors fatty acid and glucose uptake and triglyceride formation. Conversely, low insulin:glucagon ratio signals energy utilization, resulting in glycogenolysis, gluconeogenesis, and fatty acid oxidation. Amylin is cosecreted with insulin and appears to play a role in the regulation of gut physiology. Glucagon-like peptides (GLP) 1 and 2 also play roles in nutrient metabolism. GLP-1 stimulates production and release of insulin and somatostatin, and inhibits glucagon. GLP-1 also has effects on the stomach, brain, and heart. GLP-2 stimulates mucosal growth and nutrient absorption, and inhibits motility in the intestine. Pancreatic polypeptide (PP) levels rise after a mixed meal, and PP is often elevated in patients with pancreatic neuroendocrine tumors, but its physiologic action is not known.

The islets receive a disproportionately large (5–10 times more) amount of blood supply, compared to a similar volume of exocrine tissue. The posterior head is supplied by the superior mesenteric artery, and the remainder of the pancreas is supplied by the celiac artery. Insulin-secreting cells are centrally located within the islets and the direction of blood flow from center to periphery allows insulin-secreting cells to exert a tonic inhibitory effect on glucagon secretion. The islets have a complex innervation and capillary network to receive signals from other hormones, allowing the islet to integrate the hormonal response and function as a coordinated secretory unit. This chapter will discuss the interactions of various factors involved in the regulation of nutrient metabolism by the endocrine pancreas.

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Table 3.1 Islet cell types

Cell type	Percentage of total	Hormone
Alpha (α)	15–20	Glucagon, ghrelin
Beta (β)	65–80	Insulin, amylin
Delta (δ)	3–10	Somatostatin
PP	1	Pancreatic polypeptide
Epsilon (ϵ)	1	Ghrelin

Insulin Synthesis

Insulin is synthesized in the pancreas within the beta cells (β-cells) of the islets of Langerhans. Insulin, one of the smallest proteins in the human body, is built from 51 amino acids. It consists of two polypeptide chains (A and B) linked by disulfide bonds. Another disulfide bond exists within the A chain. Insulin mRNA is translated as a single sequence precursor called preproinsulin in the rough endoplasmic reticulum of β-cells. It is composed of 110 amino acids and is relatively inactive. Almost immediately, preproinsulin is being converted to proinsulin by the removal of its signal peptide (Fig. 3.1).

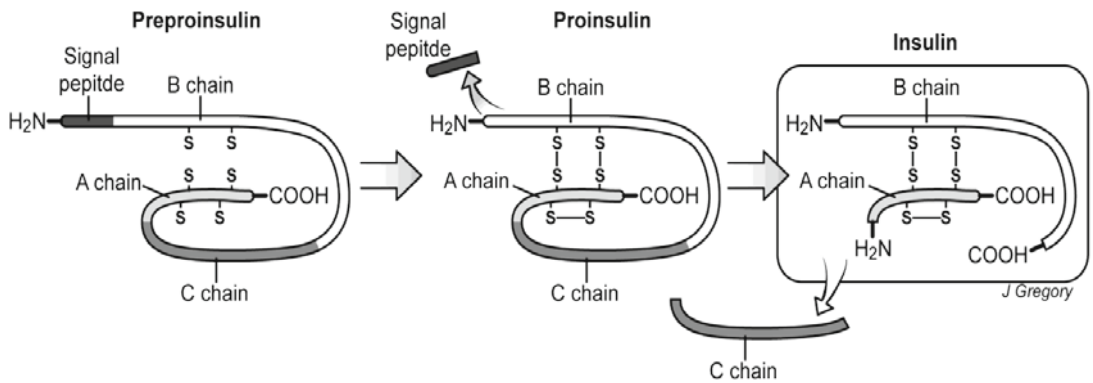

Fig. 3.1 Conversion of preproinsulin to insulin

Theoretically, there are several ways in which proinsulin may be secreted from the β-cell, but none of them involves the regulated secretory pathway. The clathrin-coated microvesicles that bud from the maturing secretory vesicle contain a small fraction of the synthesized proinsulin. Some of these vesicles fuse with endosomes, and their contents are cycled to the cell membrane and released. This is referred to as the constitutive-like (CL) pathway. Other vesicles fuse directly with the cell membrane prior to vesicle maturation and release their contents. This is referred to as the constitutive pathway (Fig. 3.2). The process of secretory vesicle maturation is highly efficient and less than 15% of the total insulin is secreted as proinsulin. This figure is much higher under conditions in which insulin secretion is less well regulated as in patients with type 2 diabetes mellitus or with insulinomas.

Proinsulin is converted to insulin by the action of two prohormone-converting enzymes (PC1/3 and PC2), which become activated in the *trans* Golgi network. These enzymes excise pairs of basic amino acids that are subsequently removed by exoprotease carboxypeptidase E. This results in the formation of an insulin molecule and the C-peptide, a 31-amino acid residue. Partially processed proinsulin, called des-31,32 proinsulin, is secreted to some extent in the process of exocytosis and makes up a large proportion of the circulating proinsulin. As proinsulin processing proceeds, the interior of the granules becomes acidic by the action of vesicular proton pumps, creating conditions for the optimum crystallization of insulin within the granules.

C-peptide is secreted with insulin in equimolar amounts and serves as a useful marker of insulin secretion. It had been presumed that C-peptide had no biological activity; however, reports have appeared describing biologic

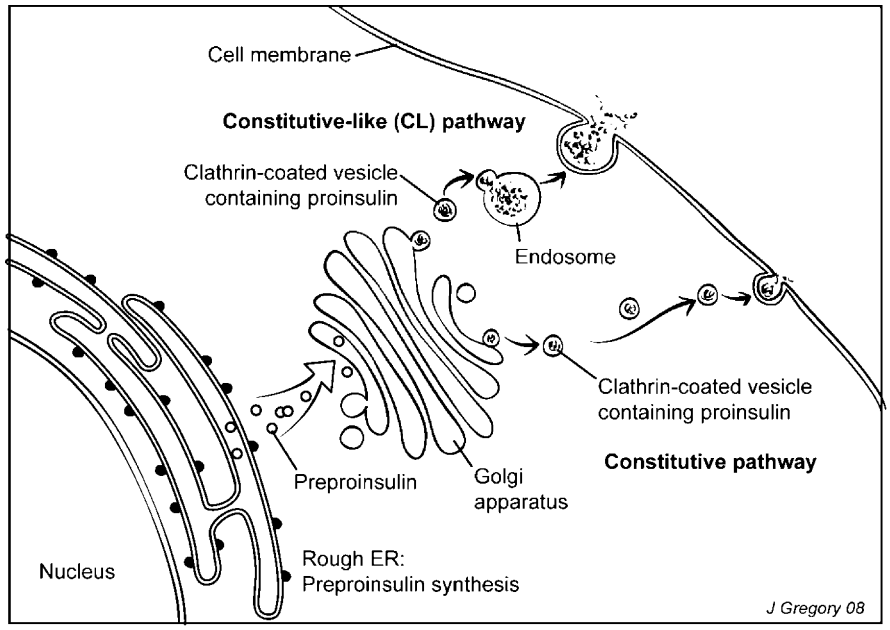

Fig. 3.2 Insulin biosynthesis and secretion

effects of C-peptide. Those include enhancement of glucose transport and utilization, improvements in microcirculation in muscle, skin, retina, and nerve, stimulation of renal tubular Na^+, K^+ ATPase, and stimulation of islet cell proliferation. As a result, C-peptide has been demonstrated to increase renal and nerve blood flow,[3,4] as well as blood flow in both resting and exercising muscles.[5,6] It also affects relocation of skin blood flow from shunt channels to nutritive blood flow.[7] Some clinical trials showed that short-term C-peptide infusion in type 1 diabetic patients exerts beneficial effects on microvascular function by improving both myocardial blood flow and blood volume.[8]

The proposed actions of C-peptide raise the possibility that combined insulin and C-peptide therapy in patients with type 1 diabetes may more effectively alleviate the progression of diabetes-related complications, including the stabilization (or even reversal) of diabetic neuropathy, nephropathy, and retinopathy.[9–11] However, C-peptide receptors have not been demonstrated, and physiologic actions of C-peptide would require novel interactions with membrane bilayers or other cellular constituents. Recently completed clinical trial of C-peptide among patients with type 1 diabetes and diabetic neuropathy showed some improvement in nerve functions, including nerve conduction velocity and vibration perception.[12,13]

The Insulin Gene and Insulinopathies

The human insulin gene is a single-copy gene located on the short arm of chromosome 11 in band 15. Unlike other members of the insulin gene family which include IGF-I and IGF-II and which are synthesized by most tissues, insulin is produced only by islet β-cells. The selective expression of the insulin gene is brought about by the actions of transactivating factors that bind to specific DNA recognition sequences.

Several families in whom structurally abnormal insulin is produced have been identified. The disorder is inherited in an autosomal fashion and presents with mild hyperinsulinemia and glucose intolerance. The hyperinsulinemia is likely due to impaired receptor binding, leading to reduced insulin clearance. In all cases, a single nucleotide substitution leads to a single amino acid replacement. In another type of variant, an amino acid substitution at the proconvertase cleavage site leads to increased proinsulin secretion via the constitutive secretory pathway.[14]

It is unlikely that variations in either coding or noncoding sequences of the insulin gene are associated with a significant number of cases of diabetes. However, it is possible that variants in promoter regions or defects in regulatory proteins will lead to decreased insulin gene expression and to diabetes of the MODY type (see Chapter 14).

Insulin Release and Second Messenger Signal Transduction

Neurotransmitters and hormones bind to specific cell surface receptors activating second messenger systems that regulate insulin secretion (Fig. 3.3). Cyclic AMP generated by binding of glucagon-like peptide-1 (GLP-1), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), and gastric inhibitory peptide (GIP) to their respective stimulatory G protein-coupled receptors magnifies glucose-stimulated insulin secretion. Conversely, norepinephrine binding to its inhibitory G protein-coupled receptor inhibits cyclic AMP formation and consequently inhibits insulin secretion.

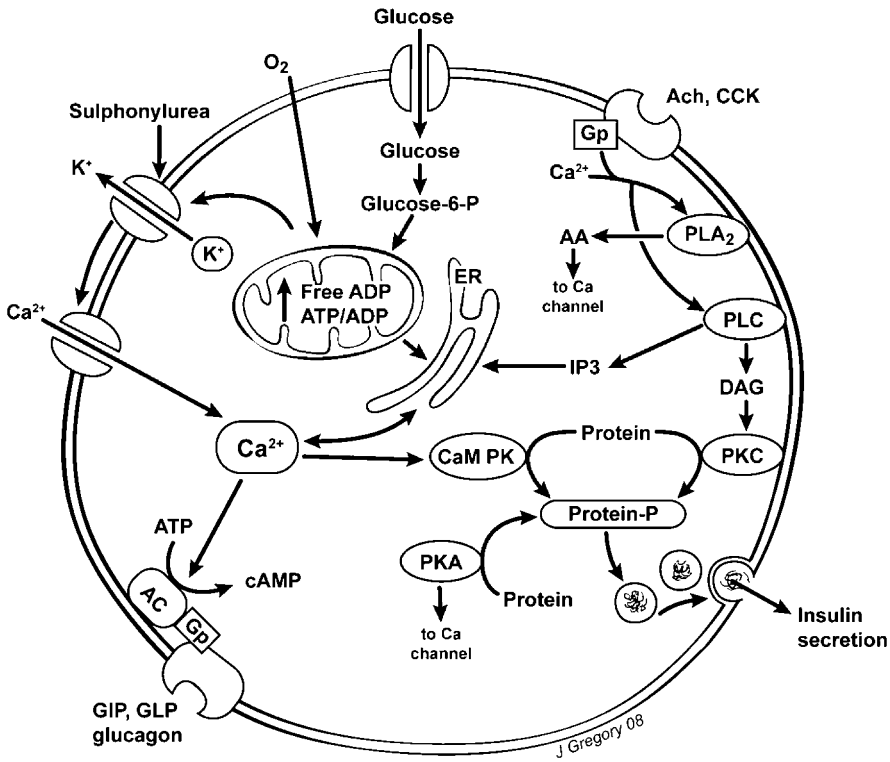

Fig. 3.3 Intracellular pathways involved in insulin secretion (see text). Ach, acetylcholine; CCK, cholecystokinin; Gp, G protein; GK, glucokinase; PLA_2, phospholipase A_2; AA, arachidonic acid; PLC, phospholipase C; CaM PK, calcium calmodulin-dependent protein kinase; ER, endoplasmic reticulum; P, phosphate; GIP, gastric inhibitory peptide; GLP, glucagon-like peptide. Adapted with permission from Liang et al.[44]

Cyclic AMP increases $[Ca^{++}]_c$ both directly, by activating L-type calcium channels, and indirectly, by activating protein kinase A which phosphorylates and closes potassium channels depolarizing the plasma membrane potential. In addition, cyclic AMP sensitizes the insulin secretory machinery by shifting the dose–response curve of calcium-induced insulin secretion to lower calcium concentrations. Protein kinase A also rapidly phosphorylates a set of proteins that potentiate insulin secretion. Finally, cyclic AMP stimulates insulin gene transcription both directly, by binding to a cyclic AMP response element of the insulin promoter, and indirectly, by phosphorylating (via protein kinase A) a cyclic AMP response element-binding protein.

Three phospholipases in β-cells (phospholipase A_2, C, and D) play a role in regulating insulin secretion. Binding of acetylcholine to its G protein-coupled receptor activates phospholipase C that hydrolyzes membrane-bound phospholipids to inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to specific receptors on intracellular membrane-bound structures releasing calcium from intracellular stores and increasing the $[Ca^{++}]_c$. DAG-activated protein kinase C phosphorylates proteins that elicit a variety of cellular responses amplifying glucose-stimulated insulin secretion. In addition, DAG stimulates insulin secretion by increasing the fusogenic potential of cell membranes and by activating DAG lipase, which liberates arachidonic acid from phospholipids.

Arachidonic acid is a 20-carbon unsaturated fatty acid containing four double bonds that exists for the most part esterified in membrane phospholipids. It is released from the plasma membrane by the action of phospholipase A2 upon binding of acetylcholine to its G protein-coupled receptor. This is independent of its release by the action of DAG lipase. Arachidonic acid interacts with the voltage-dependent calcium channels and amplifies insulin secretion by shifting the activation curve of the channels to potentials that are more negative. Arachidonic acid also activates protein kinase C and mobilizes calcium from intracellular stores.

Phosphatidic acid is released from membrane phospholipids upon binding of acetylcholine to its receptor with subsequent activation of phospholipase D. Increased phosphatidic acid levels stimulate insulin secretion by a yet to be determined mechanism.

The resting membrane potential of β-cells is determined primarily by potassium conductance through ATP-dependent K^+ channels. When the cells are exposed to 3 mM glucose (below the threshold for stimulated insulin secretion), the membrane potential is between –60 and –70 mV. As the glucose concentration is increased, the K^+ channels begin to close. This elicits an oscillatory pattern in which periods of more negative potentials are interspersed with plateaus of membrane depolarization upon which spikes of calcium-dependent action potentials are superimposed. As the glucose concentration increases, the duration of the depolarized plateaus increases as well, and the interplateau durations decrease until, at a concentration of 20 mM, the depolarization is continuous. Membrane depolarization opens voltage-gated calcium channels increasing $[Ca^{++}]_c$ and leading to insulin secretion. Two other potassium channels, the delayed rectifier K^+ channel and the Ca^{++}-dependent K^+ channel, function to repolarize the membrane potential. As mentioned above, sulfonylureas bind to and close the ATP-dependent K^+ channels providing the mechanism by which these agents stimulate insulin secretion.

A second source of increased $[Ca^{++}]_c$ is release of calcium from intracellular stores. The endoplasmic reticulum contains a large number of low-affinity calcium-binding sites. Two specific receptors, the IP_3 receptor and the ryanodine receptor, serve as intracellular channels for mobilizing stored calcium. The IP_3 receptor can be phosphorylated by cyclic AMP-dependent protein kinase, protein kinase C, and calcium calmodulin-dependent protein kinase II, providing mechanisms by which several second messenger systems affect insulin secretion. Calcium itself activates the ryanodine receptor, and it has been proposed that this calcium-induced calcium release may be important in the calcium oscillations observed in β-cells.

Insulin Secretion

The total amount of insulin secreted at any given time reflects the sum of the insulin secreted by individual islets. The human pancreas secretes about 30 units of insulin per day in normal adults. The average fasting insulin concentration is 10 μU/ml and rarely rises above 100 μU/ml in normal subjects following a meal. The concept of insulin resistance is demonstrated by Fig. 3.4. Insulin resistance[15] is defined as impaired insulin-stimulated glucose disposal. Obese subjects who are insulin resistant require a higher concentration of insulin to maintain normoglycemia. Insulin-resistant subjects who have beta cell dysfunction and are unable to make this compensatory insulin response will develop hyperglycemia and type 2 diabetes.

Stimulated insulin secretion, either by an ingested meal or by an intravenously administered glucose, results in a biphasic insulin response (Fig. 3.5). The first phase is rapid in onset, has a sharp peak, and lasts for about 10 min. The second phase is a prolonged plateau that lasts for as long as the blood glucose remains elevated. As the figure shows, the first phase of secretion is lost in patients with type 2 diabetes. However, in the same diabetic subjects, the first phase response to intravenously administered arginine is intact, demonstrating that

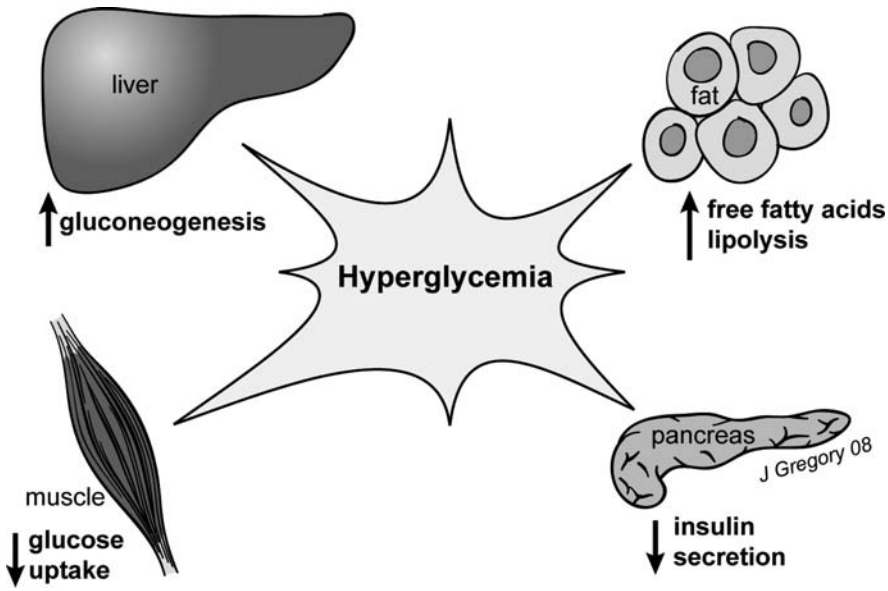

Fig. 3.4 Mechanism of hyperglycemia

the loss of the glucose-stimulated first phase secretion is due to failure to transduce a glucose-associated signal. Sustained levels of high glucose stimulation result in a reversible desensitization of the beta cell response to glucose ("glucose toxicity") but not to other stimuli.

A plausible explanation for biphasic insulin secretion is that the first phase represents release of insulin from a population of secretory vesicles that are "docked" and "primed" at the β-cell membrane and awaiting a glucose-dependent calcium signal for immediate release. The second phase represents replenishment of exocytosis-competent secretory vesicles.

While glucose concentration is the most potent stimulus for insulin secretion, it is not the only determinant. Just as insulin affects the uptake and storage of fatty acids and amino acids (as well as glucose), fatty acids and amino acids also exert an influence on insulin secretion. Extrapancreatic hormones and neural activity also coordinate and magnify the effects of nutrients on pancreatic hormone secretion. Thus, there are four main factors that are responsible for regulating insulin secretion: (1) concentrations of nutrients (including glucose, free fatty acids, amino acids) bathing the islets; (2) activity of autonomic nerves innervating the islets; (3) endocrine hormonal inputs (glucagon, etc); and (4) interactions between the islet cells.

Nutrients and Insulin Secretion

The principal role of the pancreatic hormones is to regulate the uptake and release of metabolic fuels from the hormone-sensitive tissues, liver, muscle, and fat. After meals, when nutrient levels in the blood are high, insulin secretion is stimulated, glucagon secretion is inhibited, and the high insulin to glucagon ratio promotes nutrient storage. At times of fasting, when stored fuel energy is needed, insulin secretion is inhibited, glucagon secretion is stimulated, and the low insulin to glucagon ratio promotes nutrient release from storage.

Glucose and the Fuel Hypothesis of Insulin Secretion

Insulin is secreted at a rate that depends in part on the concentration of glucose in the blood. It was originally theorized that increased blood concentrations of glucose led to greater receptor occupancy on islet cells, which

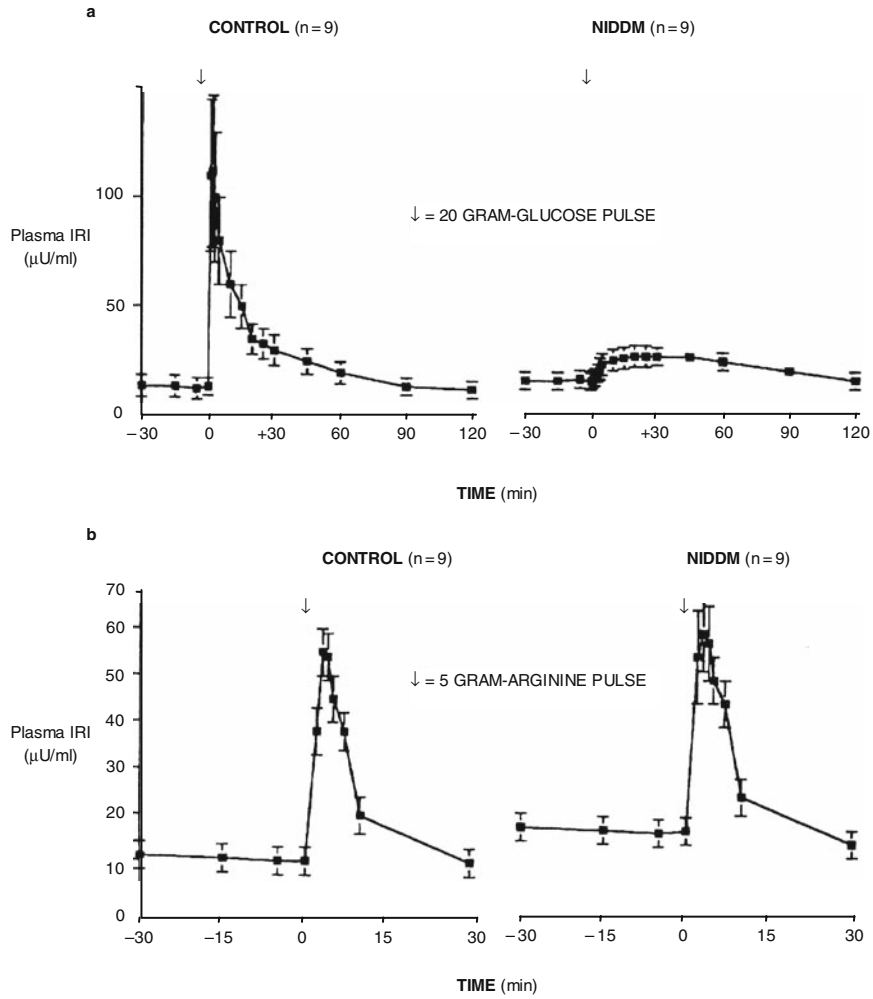

Fig. 3.5 The *top panel* shows the first phase of insulin secretion in response to intravenous insulin in normal subjects (*left plot*) and the loss of the first phase in diabetic subjects (*right plot*). The *bottom panel* shows the intact insulin secretory response to intravenous arginine in normal subjects and individuals with diabetes. From Poitout and Robertson[45] with permission

subsequently resulted in greater insulin secretion. This view was abandoned in light of a large body of evidence, demonstrating that insulin secretion is proportional to the rate at which glucose is metabolized within the islet β-cells.[16] This forms the basis of the well-accepted "fuel hypothesis" (Fig. 3.6), which states that the intracellular glucose concentration determines the rate of glucose metabolism, and the rate of glucose metabolism determines the rate of insulin secretion.

Details of this mechanism have been well worked out. Metabolism of glucose increases the ratio of the concentrations of ATP to ADP. ATP interacts with ATP-dependent potassium channels closing the channels. Potassium channel closure depolarizes the plasma membrane potential, which in turn opens L-type voltage-gated calcium channels. The cytoplasmic calcium concentration, $[Ca^{++}]_c$, rises and calcium activates protein kinases and interacts with the cell's secretory machinery leading to exocytosis of insulin-laden secretory vesicles, i.e., insulin secretion.

This cellular pathway explains the mechanism of action of sulfonylureas, the first class of drugs used to enhance insulin secretion in patients with type 2 diabetes mellitus. Sulfonylureas bind to the ATP-dependent potassium channel complex, closing the channels. Subsequent membrane depolarization and calcium channel opening raises intracellular calcium concentrations and increases insulin secretion.

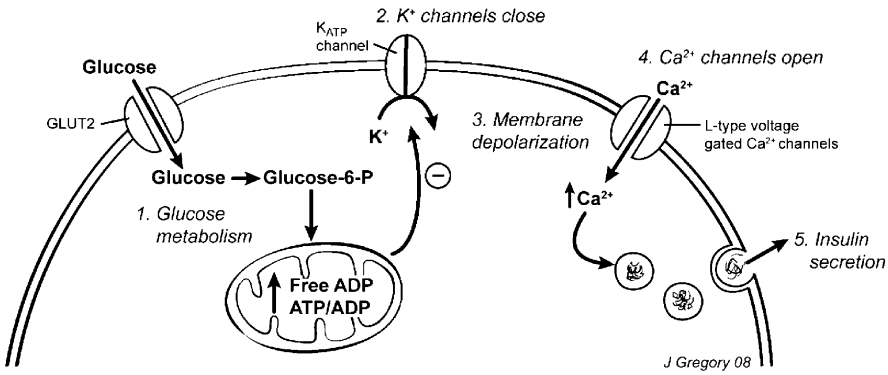

Fig. 3.6 Schematic view of the fuel hypothesis (see text above)

Glucose enters the β-cell through facilitated glucose transporters, GLUT-2, which are constitutively expressed in the plasma membrane of islet cells. As a result, changes in plasma glucose are reflected by changes in the free glucose concentration within islet cells. Glucose is trapped within the β-cell by the first step in glycolysis, the phosphorylation of glucose to glucose-6-P. This reaction, catalyzed by glucokinase, is the rate-limiting step in glycolysis, and since insulin secretion is proportional to the rate of glucose metabolism, it can be said that the combined actions of GLUT-2 and glucokinase form a physiologic "glucose sensor."

The mechanism outlined above does not account for all of the insulin secretion stimulated by glucose. It has been shown that the mitochondrial metabolism of glycolytically derived pyruvate causes insulin secretion independently of increased $[Ca^{++}]_c$.[17] The exact nature of the mitochondrial signals is unknown and is the subject of intensive investigation and debate. There is strong evidence that mitochondrially derived glutamate provides the signal for insulin secretion in insulinoma cell lines. However, several labs have shown that this does not appear to be the case in native islets. It is anticipated that further elucidation of the mechanism of insulin secretion will lead to new therapies.

The total amount of insulin secreted at any given time reflects the sum of the insulin secreted by individual islets. In type 2 diabetes, an inadequate insulin secretory response reflects inadequate insulin secretion from the individual β-cells of the individual islets. This is referred to as beta cell dysfunction. Figure 3.7 shows the concentrations of insulin, C-peptide, and glucose in the blood of normal and diabetic subjects over a 24-h period.[18] The subjects were fed three standard meals a day composed of 50% carbohydrate, 15% protein, and 35% fat. In the normal subjects, insulin and C-peptide concentrations rose to a sharp peak after meals and then

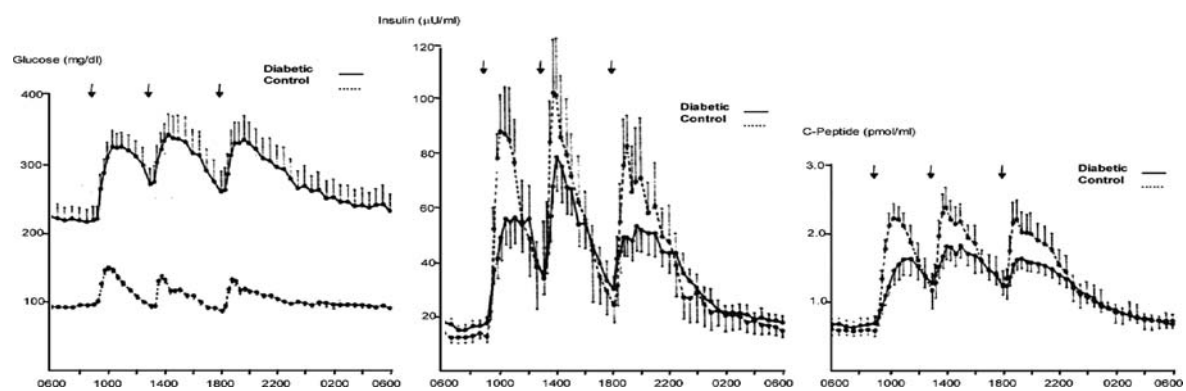

Fig. 3.7 Blood glucose, insulin, and C-peptide levels over a 24-h period in normal subjects and in subjects with type 2 diabetes. Postprandial insulin and C-peptide levels are higher in normal subjects. Glucose levels are lower. From Polansky et al.[46] with permission

rapidly declined. Glucose rose to approximately 50% above basal levels and then returned to baseline within 1–2 h. In subjects with type 2 diabetes, insulin and C-peptide peaked less sharply and rose to lower levels. Glucose levels were higher and their peaks were more prolonged.

Lipids and Insulin Secretion

Nonesterified fatty acids (NEFA), also known as free fatty acids (FFAs), are an important energy source for many tissues of the body. In addition, they are metabolized in β-cells where they also serve as important signaling molecules regulating β-cell function. Acute exposure to free fatty acids increases both basal insulin secretion and glucose-stimulated insulin secretion. Chronically elevated levels of free fatty acids, such as those seen in patients with type 2 diabetes mellitus, may have deleterious effects on β-cell function and may have an etiologic role in both the β-cell dysfunction and the insulin resistance of type 2 diabetes mellitus.[19]

The cellular events leading to the fatty acid-induced enhancement of glucose-stimulated insulin secretion are illustrated in Fig. 3.8. High glucose and insulin lead to Krebs cycle activation, resulting in increased citrate and acetyl-CoA, which are converted to malonyl-CoA via acetyl-CoA carboxylase. Malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase I (CPT-I), the outer mitochondrial membrane enzyme that transports fatty acyl-CoA into the mitochondria, thereby playing a central role in the balance between mitochondrial glucose and fatty acid metabolism. Inhibition of CPT-I results in an increase in cytoplasmic fatty acyl-CoA, which acts as a signaling molecule having several actions that ultimately increase insulin secretion. Fatty acyl-CoA also increases insulin vesicle trafficking, alters ion channel activity, and promotes vesicle docking and fusion with the cell membrane.

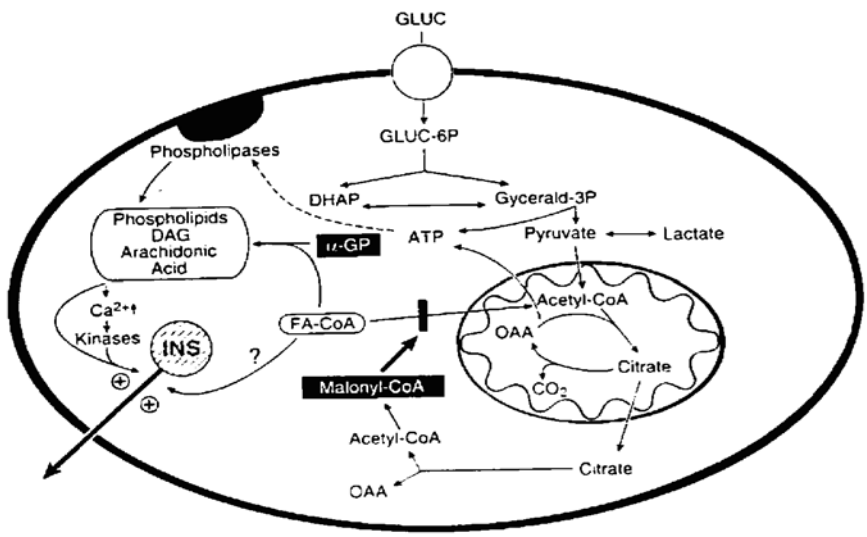

Fig. 3.8 Glucose inhibits the oxidation of fatty acyl-CoA by increasing the production of malonyl-CoA which blocks transport of fatty acyl-CoA into the mitochondria. This ensures that cytoplasmic fatty acyl-CoA is available to enhance insulin secretion. From Newgard and McGarry[16] with permission

The accumulation of lipids in muscle leads to insulin resistance.[20] Since fatty acids enhance insulin secretion, it may be that this enhancement arose as an adaptation to protect against the hyperglycemia that would otherwise have resulted from fatty acid-mediated insulin resistance. The breakdown of this balance may occur in type 2 diabetes mellitus. In early type 2 diabetes, the disease is characterized by prolonged elevation of FFA along with insulin resistance, basal hyperinsulinemia, and exaggerated postprandial insulin secretion. It may be speculated that prolonged exposure to elevated fatty acids causes a decompensation in which β-cell dysfunction cannot overcome the effects of insulin resistance.

Forty years ago, Randle hypothesized that free fatty acids compete with glucose as substrate oxidation and that increased FFA oxidation may cause insulin resistance via elevation of intramitochondrial acetyl-CoA/CoA and NADH/NAD ratios, with subsequent inactivation of pyruvate dehydrogenase.[21] This would lead to increased citrate, inhibition of phosphofructokinase, and increased glucose-6-phosphate (G6P). Increased G6P inhibits hexokinase II, which ultimately decreases glucose uptake. More recent studies have challenged this view. Shulman et al. showed that increased plasma FFA led to 50% reduction in insulin-stimulated rates of muscle glycogen synthesis, which was preceded by a fall (not increase) in G6P. Inhibition of glucose transport and phosphorylation led to reduction in rates of glucose oxidation and muscle glycogen synthesis.[22]

Higher circulating FFA (NA/IL/Hep) produces higher levels of insulin and C-peptide. Experiments using animal models of diabetes support this view. In the male Zucker diabetic fatty rat, there is a pronounced increase in plasma fatty acids, triglycerides, and islet triglycerides that occurs before hyperglycemia appears. Diet restriction as sole therapy reduces hyperlipidemia, islet hypertriglyceridemia and improves β-cell function while preventing hyperglycemia. In another experiment using rats, circulating FFA was rapidly increased by infusing intralipid. It was found that elevated fatty acids enhanced glucose-stimulated insulin secretion at 3 and 6 hours of exposure but suppressed it at 48 h. Carpentier et al.[23] showed essentially the same results in healthy young men. Observations such as these raise the possibility that in diabetes-prone individuals, chronically elevated fatty acids play a role in the β-cell dysfunction of clinical diabetes. However, data are not definitely conclusive that FFAs are the link between insulin resistance and beta cell dysfunction.

Neural Regulation of Insulin Secretion

The pancreatic islets are richly innervated by autonomic and sensory nerves.[24] Insulin secretion is enhanced by stimulation of parasympathetic nerves and inhibited by stimulation of sympathetic nerves. Sensory pathways are for the most part inhibitory. Additional neural pathways mediate direct entero-pancreatic interactions.

The cephalic phase of insulin secretion refers to the first 3–4 min of insulin secretion triggered not by blood-borne nutrients but by the sight, smell, and anticipation of food. The cephalic phase has been demonstrated in a number of ways: by imaginary feeding under hypnosis, by the ingestion of nonnutrient sweeteners, and by the rise in blood insulin levels prior to the rise in blood glucose after ingestion of a glucose load.

The neural effector pathways begin in the ventro-medial hypothalamus and dorsal motor nucleus of the vagus. The cephalic phase is abolished by vagotomy or by ganglionic blockade with muscarinic antagonists, demonstrating that it is mediated by cholinergic neurons of the parasympathetic nervous system (Fig. 3.9).[25]

The question of the physiologic importance of the cephalic phase has been raised since it accounts for only 1–3% of the total insulin response to a meal (or about 25% above baseline). Pancreatic polypeptide, on contrary, is almost entirely under vagal control and increases 100% above baseline during tasting or chewing food. Therefore, the pancreatic polypeptide response during cephalic phase is a sensitive marker of vagal activation by food stimuli.[26] The significance of the cephalic phase of insulin release was demonstrated with the use of trimethaphan, a nondepolarizing antagonist at the nicotinic acetylcholine receptor, that was accompanied by impaired reduction of glucose levels at half an hour to an hour, typical sign of glucose intolerance.[27] On the other hand, replacement of insulin in subjects with type 2 diabetes in the first 15 min after food ingestion improves glucose tolerance. These data imply that cephalic phase plays a role in glucoregulation, causing insulin to lower blood glucose in response to an ingested glucose load. Increase in insulin during the first 10–15 min after meal intake, inversely correlating to the change in glycemia between 25 and 60 min, suggests a relationship between postprandial blood glucose and neurally mediated preabsorptive insulin secretion.

The insulin output of an individual islet derives from the coordinated function of many β-cells. Within islet cells, oscillatory patterns can be seen in oxygen consumption, production of ATP, and concentrations of cytosolic calcium. Electrical coupling by gap junctions serves to help coordinate activity. In addition, insulin secretion from the pancreas as a whole is pulsatile, suggesting synchronization between the islets as well. Blockade of

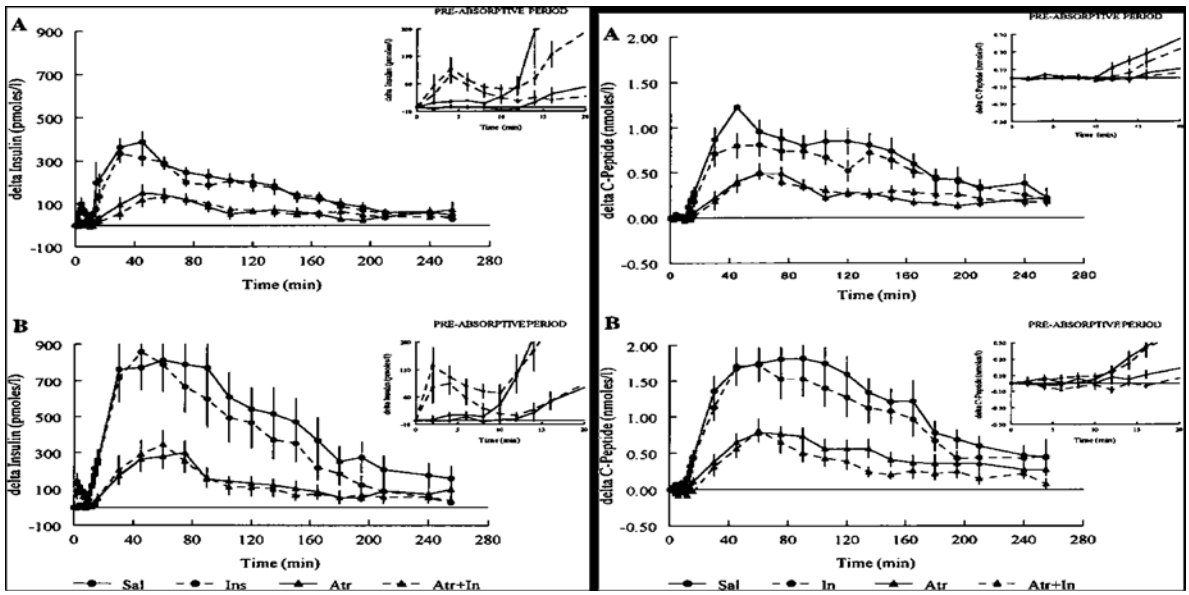

Fig. 3.9 Plasma insulin (*left panel*) and C-peptide (*right panel*) levels expressed as difference from baseline in normal-weight (**a**) and obese (**b**) subjects after ingestion of mixed-nutrient meal during saline (Sal), insulin (Ins), atropine (Atr), and atropine and insulin infusions (Atr + Ins). Adapted with permission from Teff and Townsend[25]

pancreatic ganglia abolishes this synchronization. The clinical importance of oscillatory insulin secretion is suggested by its loss in patients with impaired glucose tolerance and type 2 diabetes.

Parasympathetic Nerves

The parasympathetic nerves innervating the islets originate in the dorsal motor nuclei of the vagus. Preganglionic fibers traverse the vagus in the bulbar outflow tract and the hepatic and gastric branches of the vagus. They enter the pancreas and terminate in intrapancreatic ganglia from which postganglionic fibers emerge to innervate the islets. The postganglionic nerve terminals contain the classical neurotransmitter acetylcholine and the neuropeptides gastrin-releasing peptide (GRP), vasoactive intestinal polypeptide (VIP), and pituitary adenylate cyclase-activating polypeptide (PACAP).[28]

Vagal activation stimulates insulin secretion. Stimulation of the postganglionic fibers releases acetylcholine, which binds to M3 muscarinic receptors on islet cells. The hormones secreted by the other three islet cell types, glucagon, somatostatin, and pancreatic polypeptide, are also stimulated by acetylcholine via M3 receptors. In β-cells, binding of acetylcholine to its receptor stimulates phospholipase C (PLC) activation via a G protein-coupled mechanism. This stimulates phosphoinositide hydrolysis to IP_3 and diacylglycerol (DAG). Phospholipase A_2 (PLA_2) is also activated producing arachidonic acid. Insulin secretion is stimulated by subsequent increase in $[Ca^{++}]_c$ and protein phosphorylation. The mechanisms by which PLC and PLA_2 stimulate insulin secretion are discussed in section "Insulin Release and Second Messenger Signal Transduction". The intracellular pathways by which acetylcholine stimulates secretion of the other islet hormones have not been elucidated.

VIP, PACAP, and GRP stimulate insulin secretion upon binding to their respective G protein-coupled receptors. VIP and PACAP exert their effects by stimulating adenylate cyclase and increasing levels of cAMP. GRP binding to its receptor activates PLC and phospholipase D (PLD). The mechanisms by which cAMP and PLD stimulate insulin secretion are discussed in section "Insulin Release and Second Messenger Signal Transduction."

Sympathetic Nerves

At times of physiologic stress (such as prolonged fasting, exercise, hypoglycemia, or hypovolemia), maintaining blood glucose levels becomes vitally important. Glucose output by the liver plays the main role in this process stimulated in part by the counter-regulatory hormones cortisol, epinephrine, and growth hormone. In addition, activation of local sympathetic nerves stimulates glucagon secretion, while insulin secretion is concurrently inhibited. The decreased insulin to glucagon ratio provides the signal for hepatic glucose production and output.

The adrenergic nerves innervating the islets are postganglionic fibers whose cell bodies are located in the celiac ganglion and paravertebral sympathetic ganglia. The preganglionic nerves originate in the hypothalamus, leave the spinal cord at the level of C8 to L3, and traverse the lesser and greater splanchnic nerves to reach the postganglionic cell bodies. The postganglionic nerve terminals contain the classical sympathetic neurotransmitter, norepinephrine, along with the neuropeptides galanin and neuropeptide Y (NPY).

Norepinephrine inhibition of glucose-stimulated insulin secretion is mediated by α2-adrenoreceptors. It is not known whether the inhibition of basal insulin secretion is also mediated by norepinephrine. Sympathetic activation also stimulates glucagon and pancreatic polypeptide secretion, while somatostatin secretion is inhibited.

The norepinephrine-induced inhibition of insulin secretion is mediated by several signaling pathways: First, α2-adrenoreceptor activation leads to hyperpolarization of the β-cell through opening of the ATP-dependent potassium channels. This prevents opening of the voltage-gated calcium channels, thereby preventing increased $[Ca^{++}]_c$ and subsequent exocytosis of secretory granules. Second, the formation of cyclic AMP is inhibited, and third, there is an inhibitory action on the distal exocytotic machinery.[29]

The concept that sympathetic neuropeptides inhibit glucose-stimulated insulin secretion derives from animal experiments in which sympathetic stimulation leads to inhibition of secretion under conditions in which α2-adrenoreceptors are blocked. The mediators of this inhibition are the neuropeptides galanin and NPY. Binding of these neuropeptides to their respective receptors activates pathways similar to those activated by norepinephrine.

Sensory and Other Nerves

The islets are extensively innervated with sensory afferents containing the neuropeptides calcitonin gene-related peptide (CGRP) and substance P (SP). The afferent fibers leave the pancreas along with the sympathetic fibers of the splanchnic nerve and participate in reflexes whose effectors are the autonomic nerves. CGRP has an inhibitory effect on insulin secretion mediated by a decrease in islet cyclic AMP probably reflecting α2-adrenoreceptor activation. The CGRP neurons also stimulate glucagon secretion and thus likely participate in the islet's reflex response to hypoglycemia. The actions of SP neurons are less well characterized and both stimulatory and inhibitory effects have been demonstrated.

Other nerves that innervate the islets and affect insulin secretion include neurons that contain nitric oxide synthase (NOS) and cholecystokinin (CCK). The NOS neurons stimulate insulin secretion. The CCK neurons stimulate insulin secretion via mechanisms that involve PLC and PL2 pathways. In addition, nerves originating in the duodenal ganglia directly innervate islets, suggesting the existence of direct entero-pancreatic neural mechanisms.

Glucagon and Glucagon-Like Peptides

Glucagon and the glucagon-like peptides, GLP-1 and GLP-2, are the products of a single gene and are derived from differential posttranslational processing of a single proglucagon protein. Glucagon is produced by the alpha cells of the pancreatic islets, and the GLPs are produced by entero-endocrine cells of the small and large intestine. Glucagon and GLP-1 have important roles in maintaining glucose homeostasis.

Glucagon

Glucagon is synthesized in alpha cells of pancreatic islets as a 160-amino acid prohormone (proglucagon), which is encoded by the preproglucagon gene on chromosome 2. The proglucagon is then split into four peptides, of which glucagon, the 29-amino acid polypeptide with the molecular weight of 3485 Da, is biologically active[30] (Fig. 3.10). The whole process takes about 60–90 min.

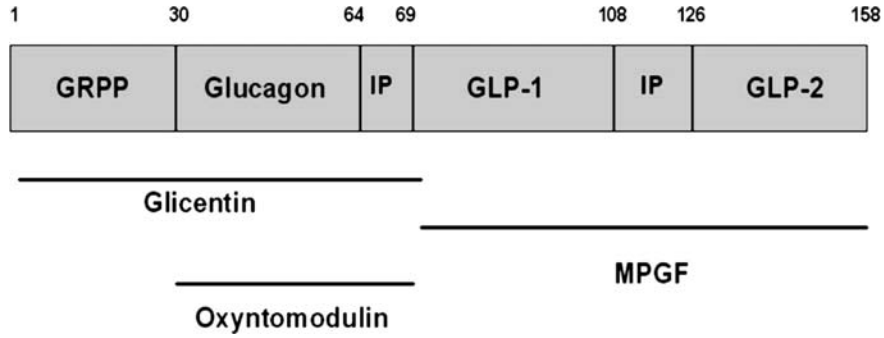

Fig. 3.10 Structure of the mammalian preproglucagon product. GRPP, glicentin-related pancreatic peptide; IP, intervening peptide; GLP-2, glucagon-related peptide-2; MPGF, major proglucagon fragment. Reprinted with permission of Dr. Michael W. King at http://themedicalbiochemistrypage.org/insulin.html

Additional peptides are derived from the preproproteins including glicentin, oxyntomodulin, and the major proglucagon fragment (MPGF) that comprises amino acids 72–158.

Glucagon plays a central role in the maintenance of basal blood glucose levels. Hypoglycemia stimulates and hyperglycemia suppresses glucagon secretion. Glucagon levels rise with fasting and exercise. During times of nutrient need, blood glucose levels are maintained by hepatic glucose production stimulated by low insulin–glucagon ratios. The binding of glucagon to its G protein-coupled receptor on hepatocytes increases intracellular levels of cAMP, leading to activation of protein kinase A, phosphorylase kinase, and phosphorylase. Glycogen synthase is inactivated. The result is stimulation of gluconeogenesis and glycogenolysis and inhibition of glycolysis. Increased hepatic fatty acid oxidation and ketone body formation provide additional energy substrate (Fig. 3.11). In adipocytes, glucagon acts via increased cAMP to stimulate lipolysis, liberating fatty acids into the circulation. In addition, glucose uptake into adipocytes is inhibited, thereby decreasing triglyceride synthesis.

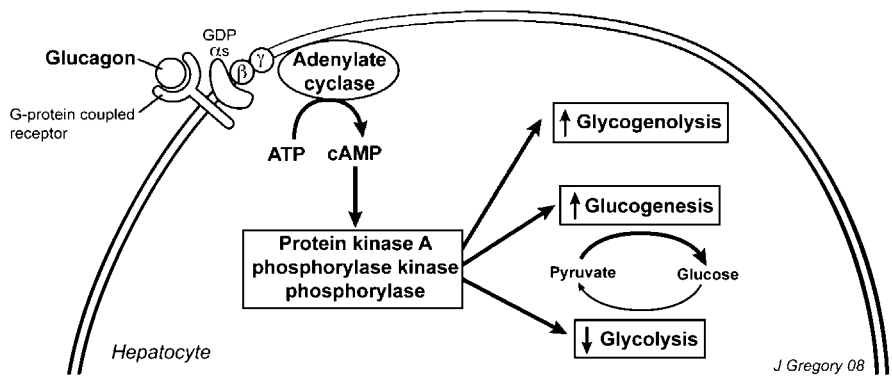

Fig. 3.11 Mechanism of glucagon action

Positive regulators of glucagon secretion include sympathetic nerve stimulation, epinephrine, CCK, PACAP, and GIP. Insulin released by β-cells tonically suppresses glucagon secretion. Conversely, during hypoglycemia, insulin levels are low, releasing glucagon from tonic suppression. Glucagon is one of the first hormones

secreted in response to falling glucose concentrations and is crucial for normal glucose counter-regulation.[31] Additionally, glucose suppresses glucagon secretion by inducing β-cell release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA).

Table 3.2 below provides a summary of both stimulating and inhibiting regulators of glucagon secretion.

Table 3.2 Major regulators of glucagon secretion

Stimulators	Inhibitors
Decreased plasma glucose levels	Elevated plasma glucose levels
Catecholamines	Insulin[a]
Gastrin	Somatostatin[a]
Cholecystokinin	Increased levels of circulating fatty acids
Gastric inhibitory polypeptide	γ-Aminobutyric acid (GABA)
Amino acids (such as arginine,[b] alanine,[c] cysteine,[c] serine,[c] glycine[c])	
Glucocorticoids	
Pituitary adenylate cyclase-activating peptide (PACAP)[47]	
Sympathetic and parasympathetic stimulation	

[a] Direct inhibition of α-cells.
[b] Stimulates both glucagon and insulin release.
[c] Stimulates mainly glucagon release.

Glucagon-Like Peptides

L cells of the small intestine synthesize an identical proglucagon molecule whose alternate processing results in the formation of several polypeptides, of which glucagon-like peptides 1 and 2 are probably of most physiologic importance.

GLP-1

The majority of GLP-1-producing cells are in the terminal ileum and proximal colon. Proglucagon synthesis in the gut is stimulated by nutrient intake, and GLP-1 levels in the blood increase rapidly after a meal. The activity of GLP-1 is largely regulated by its rate of degradation, with its half-life being very short, approximately 1 min. GLP-1 binding to its G protein-coupled receptor on β-cells increases glucose-stimulated insulin secretion via both increased cyclic AMP and increased intracellular calcium.

GLP-1 infused into healthy subjects decreases gastric emptying, causes a sensation of satiety, and decreases appetite. Thus, in addition to enhancing insulin secretion, GLP-1 has effects outside of the pancreas that serve to limit postprandial hyperglycemia. In rodents, intracerebroventricularly administered GLP-1 inhibits food intake demonstrating CNS actions. Infusion of the GLP-1 antagonist exendin into healthy subjects increases blood glucose and reduces glucose-stimulated insulin secretion.[32] The multiple actions of GLP-1 in lowering blood glucose make the development of a GLP-1-like agent modified for a longer half-life, an interesting approach to be used in the treatment of diabetes mellitus. For additional information on GLP-1, please see Chapter 4.

Somatostatin

Somatostatin was originally identified in 1973 in hypothalamic extracts as a 14-amino acid peptide that inhibits the release of growth hormone from dispersed rat pituitary cells. Since then, somatostatin and its receptors have been found in all neuroendocrine tissues, as well as in the central and peripheral nervous systems. A single

somatostatin gene codes for two biologically active peptides of 14 and 28 amino acids, named somatostatin-14 and somatostatin-28, respectively. In addition to acting as hormones, the peptides act as neurotransmitters, neuromodulators, and local paracrine regulators. Their diverse physiologic actions include modulation of secretion, neurotransmission, smooth muscle contractility, and cell proliferation.

There are five different somatostatin receptors designated sst1, sst2A, sst3, sst4, and sst5. All subtypes have been found in the brain.[33] In contrast, peripheral tissues vary in the subtype expressed (Table 3.3).

Table 3.3 Subtypes of somatostatin receptors

Subtype	Chromosomal location	Distribution in tissues
sst1	14	Brain, lungs, stomach, Jejunum, kidneys, liver, pancreas
sst2	17	Brain, kidneys
sst3	22	Brain, pancreas
sst4	20	Brain, lungs
sst5	16	Brain, heart, adrenal glands, placenta, pituitary, skeletal muscles, small intestine

Adapted with permission from Lamberts et al.[48].

All types of somatostatin receptors are members of the G protein-coupled receptor family, and all inhibit adenylate cyclase activity. Other effectors linked to the ssts via G proteins include voltage-sensitive calcium channels, potassium channels, ser/thr phosphatases, and tyrosine phosphatases.

Somatostatin is produced in neurons of the hypothalamic periventricular area that terminate near the pituitary portal capillaries. Release of somatostatin by these neurons inhibits growth hormone secretion by cells of the anterior pituitary. Elsewhere in the brain, somatostatin acts as a neurotransmitter or a neuromodulator. It is stored in synaptic vesicles, released by a calcium-dependent mechanism upon depolarization, and produces postsynaptic hyperpolarization upon its release.

In the gastrointestinal tract, somatostatin is found in the stomach, the duodenum, submucosal neurons, and the mesenteric plexus of the intestinal tract. It is produced both by gastrointestinal endocrine D cells and by visceral autonomic neurons. Thus it has paracrine and hormonal functions as well as act as a neurotransmitter. It inhibits the secretion of a variety of hormones including insulin, VIP, GIP, gastrin, cholecystokinin, secretin, motilin, and GLP-1 and reduces gastrointestinal motility, gallbladder contraction, and blood flow. Its concentration in the blood increases after meals as a consequence of both gastrointestinal and pancreatic secretion.

Intravenous administration of somatostatin inhibits insulin secretion as well as exocrine pancreatic secretion. However, the precise role of somatostatin in islet function has not been determined. Sst2A receptors are present on islet β-cells and α-cells, suggesting that somatostatin may have a direct role in regulating insulin and glucagon secretion.

Islet Amyloid Polypeptide (IAPP)

IAPP is a 37-amino acid protein that is the principal component of islet amyloid deposits. These deposits are formed in normal islets during aging but are more abundant in the islets of individuals with type 2 diabetes. The amino acid sequences of IAPP from normal and diabetic subjects are identical, and consequently the increased deposition of amyloid deposits in diabetes is not due to structural abnormalities in the amyloid protein.

IAPP is localized in the secretory vesicles of β-cells and is cosecreted with insulin. Levels of IAPP are in the range of 0.2–3.0% of that of insulin in islets, and the amount cosecreted with insulin is about 5.0%. Several hormonal effects of IAPP have been proposed, but the data in support of a precise physiologic role for IAPP are far from compelling. Studies showing that amidated IAPP inhibits insulin-stimulated glucose disposal used

non-physiologically high concentrations of IAPP. Other studies showed IAPP having complementary action to insulin. Still other studies showed that extremely high concentrations of IAPP inhibit insulin secretion.

Ghrelin

Recently, a new peptide hormone ghrelin was discovered in alpha cells of the Langerhans' islets as well as in epsilon cells. The latter constitute a newly detected endocrine cell type and originate from neurogenin 3-expressing precursor cells.[34] Ghrelin (meaning "to grow" in reference to the Proto-Indo-European word "ghre") was originally found in a rat stomach as an endogenous ligand for growth hormone secretagogue receptor. It is mainly produced in the stomach with fundus being the predominant harbor of the ghrelin-containing cells. Lower levels of ghrelin were also found in other compartments of the gastrointestinal tract, including the duodenum, the jejunum, the ileum, and the colon. Ghrelin receptors are mainly expressed in the hypothalamus and pituitary, first-trimester human placenta, and germ cells. The ghrelin mRNA expression in the glomeruli of the kidneys, and direct correlation of ghrelin plasma concentration in patients with advanced renal disease, suggests that kidneys are the main organ participating in ghrelin clearance.[35]

Ghrelin and other growth hormone secretagogues (GHSs) kindle the release of growth hormone from the pituitary gland.[36] Additionally, ghrelin stimulates appetite and increases fat mass by activating cells on the hypothalamic arcuate nucleus,[37] a region known to control food intake, and promoting the mesolimbic cholinergic–dopaminergic reward link.[38,39] Plasma ghrelin concentration is increased during fasting and diminished with regular feeding,[40] implicating that either ghrelin may serve as one of the first signals for food intake or its secretion is controlled by some nutritional factors in blood (Fig. 3.12). Plasma ghrelin levels are lower

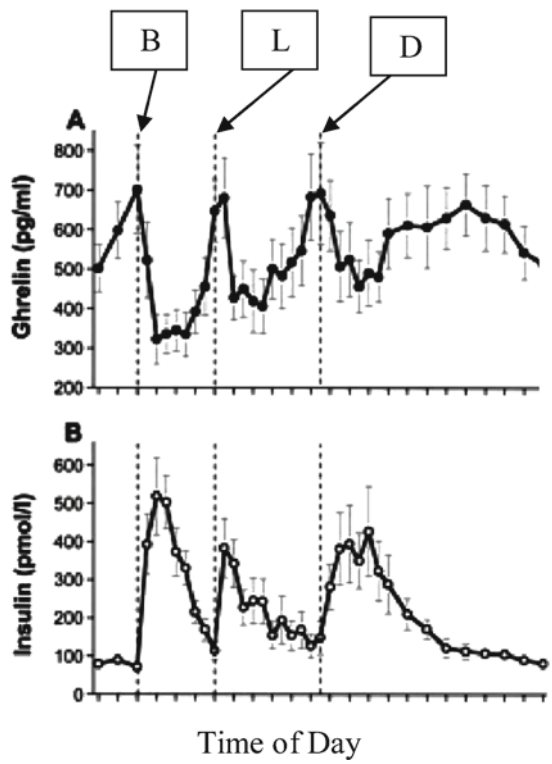

Fig. 3.12 Average plasma ghrelin (**a**) and insulin (**b**) concentrations during a 24-h period in 10 human subjects consuming breakfast (B), lunch (L), and dinner (D) at the times indicated. Blood concentrations of ghrelin are lowest shortly after meal and rise during fast, just prior to the next meal. Adapted with permission from Cummings et al.[40]

in obese patients compared to lean controls.[41] Bariatric surgical procedures, including laparoscopic Roux-en-Y gastric bypass and gastric banding, are associated with significantly suppressed ghrelin levels, possibly contributing to the weight-reducing effect of the procedure.[42] However, patients who underwent gastric bypass, were found to have lower levels of ghrelin and more profound suppression of its fluctuations in relation to meals in comparison to the patients who underwent laparoscopic gastric banding. These findings can explain more sustained long term weight loss in a former group.[43]

Summary

The endocrine pancreas has a central role in maintaining energy homeostasis by regulating nutrient uptake and release by the hormone-sensitive storage tissues, liver, fat, and muscle. When the circulating levels of nutrient fuels, such as glucose and FFA, are high, energy metabolism within islet β-cells is increased, and intracellular signals that increase insulin secretion are generated. At the same time, glucagon secretion from islet α-cells is inhibited. Thus, high insulin to glucagon ratio signals nutrient storage, and a low ratio signals nutrient release. The islet response is further regulated by autonomic and sensory nerves and by blood-borne hormones produced at distant sites of the gastrointestinal tract.

Type 2 diabetes mellitus is a condition marked by both insulin resistance and β-cell dysfunction in which insulin secretion is inadequate to fully signal storage of circulating nutrient fuels. β-cell dysfunction is the descriptive term for the condition in which there is a breakdown in the intracellular chain of events that leads to insulin secretion. This is manifested by blunted peaks of insulin secretion in response to meals and by an inappropriately high concentration of circulating proinsulin. In addition, there is dysregulation involving the autonomic nervous system so that both inter-islet communication and intra-islet stimulation of secretion are lost.

Both obesity and type 2 diabetes are characterized by insulin resistance. However, in non-diabetic individuals, insulin resistance is compensated for by increased insulin secretion. Only when β-cell dysfunction is also present does type 2 diabetes mellitus result.

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