CHAPTER 38

Normal Function of the Endocrine Pancreas

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The mammalian endocrine pancreas consists of groups of hormone-secreting cells that are dispersed throughout the exocrine tissue. These so-called endocrine islets were first described by Langerhans in 1869, who initially believed that he was observing intrapancreatic lymph nodes (1). However, following the discovery that pancreatectomized dogs develop diabetes mellitus (2), it was suggested that these islets may produce an antidiabetic factor (3,4). This hypothesis was subsequently confirmed, and eventually assumed great clinical significance when Banting and Best obtained an active pancreatic extract of insulin that was used to treat a diabetic patient (5). It is now evident that the islets of Langerhans are very complex structures containing different types of hormone-secreting cells, nerve cells, and a rich vascular supply.

The present chapter will delineate some of the aspects of pancreatic endocrine embryology and functional anatomy, as well as the biochemical and molecular mechanisms that allow the β cell to synthesize and secrete insulin in a physiological manner.

ONTOGENY OF THE ENDOCRINE PANCREAS

The pancreatic rudiments develop in 5-week-old human embryos, appearing initially as dorsal and ventral outgrowths of the abdominal foregut (6). The larger, dorsal component fuses with the smaller ventral region 2 weeks later, as a result of the clockwise rotation of the embryonic gut (6). Endocrine cells, consisting of glucagon-containing α cells, can first be identified in the 9-week-old fetus (7). Somatostatin-containing δ cells and insulin-containing β cells appear shortly thereafter (7). In addition, the fetal endocrine pancreas at this stage exhibits cells that contain pancreatic polypeptide, gastrin, serotonin, norepinephrine, and epinephrine (7,8). It has been suggested that these islets degenerate at the 5th month of gestation in conjunction with a process of lymphocytic infiltration, and that the permanent adult endocrine pancreas arises from a second generation of islets that first appear during the 3rd month of gestation (9). Eventually, in the healthy adult human, the pancreas contains more than 1,000,000 islets.

The exact developmental origin of pancreatic islet cells is not known. Morphologically, they appear to arise from pancreatic ductules (10). Pearse suggested that islet cells derive from the neural crest and are of neuroectodermal rather than endodermal origin (11). He based this hypothesis on the fact that peptide hormone-secreting cells in the gastrointestinal tract, adrenal medulla, the pituitary, the pancreas, and the parafollicular C cells of the thyroid gland share many ultrastructural and cytochemical characteristics and are capable of taking up amine precursors and decarboxylating these precursors (11). According to this concept, the islet cells belong to a family of endocrine and neuronal cells that constitute the so-called APUD system (amine precursor uptake and decarboxylation).

A characteristic feature of APUD cells is the uptake of 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5HTP), and their decarboxylation by an L-amino acid decarboxylase (11). This enzyme is permanently expressed in the endocrine pancreas, in the neuro-
epithelial cells of the basal plate of the brain, and in the spinal cord, but it is only transiently found during fetal development in the exocrine pancreas (12). The endocrine pancreas also expresses other neuronal markers. These include tyrosine hydroxylase, the first enzyme in the pathway of catecholamine biosynthesis, which is transiently expressed in the fetal islets but is never expressed in the pancreatic acinar cells (13), neuron-specific enolase, a nerve cell enzyme (14), receptors for monoclonal antibody A2B5 and tetanus toxin, which delineate gangliosides that are found on astrocytes and nerve cells (15), synaptophysin, a membrane glycoprotein that is expressed in presynaptic vesicles in neurons and in the adrenal medulla (16), PGP 9.5 (17), and glutamic acid decarboxylase (GAD), the enzyme that synthesizes gamma-aminobutyric acid (GABA) from glutamic acid (18). GAD is expressed in GABA-secreting neurons in the brain (18). Within the pancreas, it is found exclusively in the β cells (18). Interestingly, more than 80% of patients with newly diagnosed insulin-dependent diabetes mellitus (IDDM) have anti-GAD antibodies, and the majority of patients with a rare neurological disorder called stiff man syndrome have similar antibodies and a high incidence of IDDM (18).

Pancreatic endocrine cells are highly differentiated and exhibit the phenotypic characteristics that allow these cells to perform their specialized functions. There are four major types of pancreatic endocrine cells in humans. The pancreatic β cell possesses the typical morphology of an endocrine secretory cell, and synthesizes insulin, which is packaged into heterogeneously shaped granules that have electron-dense cores. The α cells are smaller than the β cells, and possess uniform electron-dense granules that contain glucagon (19). The pancreatic δ cells are small, somatostatin-containing cells, and often have a dendritic shape (19). The pancreatic polypeptide- (PP) containing cells have relatively oblong, very electron-dense granules (19).

In spite of this high degree of specialization, studies with transgenic mice have provided additional support for a close embryological relationship between islet cells and nerves cells. Thus, transgenic mice bearing genes encoding either the simian virus 40 (SV 40) large T antigen (Tag) or human placental lactogen (PLA) and coupled to the ostensibly β cell specific regulatory elements of the insulin gene were found, as expected, to express Tag and PLA in developing islet cells (20). However, the transgenics also expressed Tag and PLA in the neural tube and the neural crest (20). Furthermore, within the islet cells, the transgenic markers were found in β cells, α cells, δ cells, and pan-rectic polypeptide-secreting cells (20). Taken together, these observations suggest that the β cell is either of neural origin or shares similar regulatory features with neuronal cells that allow for coexpression of proteins that are dictated by the insulin gene regulatory elements. They also point to the existence of a common precursor cell to the mature pancreatic endocrine cells. The hypothesis of a common progenitor cell in the endocrine pancreas is supported by the finding that islet cell tumors may give rise to cloned cell lines that express several islet cell hormones (21).

ANATOMY OF THE ENDOCRINE PANCREAS

The islets of Langerhans range widely in size and may contain a few dozen endocrine cells, hundreds of cells, or even thousands of cells. As much as 20% of the intrapancreatic blood flow is directed toward the islets, in spite of the fact that they constitute only 2% of the pancreatic volume (22,23). The islets are therefore well vascularized. Blood flow to the islets is further increased in the presence of high glucose levels (24). The larger islets tend to be located near the larger blood vessels (25). The venous effluent from these larger islets appears to bypass the surrounding exocrine tissue (25). In contrast, the smaller and more numerous islets are located near smaller arterioles, and their venous effluent passes through the exocrine tissue and forms an intrapancreatic portal circulation (25).

The existence of a portal circulation within the pancreas along with the dispersal of endocrine islets throughout the exocrine tissue have given rise to the hypothesis that islet cell hormones may participate in the regulation of pancreatic exocrine function (26). In support of this hypothesis, the pancreatic acinar cell has specific high-affinity receptors for islet cell hormones such as insulin and somatostatin (27,28). Furthermore, some patients with insulin-dependent diabetes mellitus exhibit a decreased ability to secrete bicarbonate-rich fluid and pancreatic digestive enzymes in response to pancreatic secretagogues, and the severity of these alterations is more pronounced with decreased β cell secretory reserve and with increased duration of diabetes (29–33). Animal studies indicate that insulin deficiency is associated with decreased epidermal growth factor (EGF) binding, enhanced cholecystokinin (CCK) binding, and altered responsiveness to the divalent cation manganese (34–36). By comparison with pancreatic acini from normal rats, acini from insulin-deficient rats also exhibit attenuated increases in cytosolic calcium transients and markedly depressed levels of biologically active inositol phosphates following stimulation with CCK (37,38), and a threefold shift to the right in the dose-response curve for CCK-stimulated digestive enzyme secretion (35). The role of insulin in the regulation of pancreatic exocrine function is supported by the observations that this hormone enhances pancreatic glucose transport, glucose oxidation, and protein synthesis, potentiates the secretory effects of CCK and its analogues, and increases pancreatic amylase mRNA levels (27,39–46).

As a result of the intrapancreatic portal circulation, many perinsular acini are exposed to extremely high insulin levels (47,48). This anatomical arrangement
means that the pancreatic acinar cell is the first target cell for insulin action once insulin leaves the islet. However, prior to exiting the islet microcirculation, insulin is carried to the α, δ, and PP-cells, thereby having the opportunity to exert direct effects on these islet cells (49). Insulin may also exert local paracrine effects on islet cells. Irrespective of whether insulin exerts its intrapancreatic effects by acting as an endocrine or as a paracrine hormone, it appears that insulin does not act directly to suppress the α cell or stimulate the δ cell (50). Instead, the high insulin levels allow glucose to exert its effects on these cells (50). These observations point toward an interdependence between the actions of glucose and insulin with respect to their regulatory actions within the islets.

The cells that synthesize glucagon, somatostatin, and pancreatic polypeptide are located at the periphery of the islets. They tend to release their hormones into venules that are located on the surface of the islets and that drain away form the centrally located β cells (25). Consequently, the freshly secreted non-β cell hormones are prevented from exerting intrapancreatic effects on the β cell. Instead, in order to regulate β cell function, these hormones must first pass through the liver and the systemic circulation prior to returning to the pancreas. In spite of this apparent vascular separation, it is also possible that islet cells modulate each other’s actions and the actions of the β cell as a result of intraislet cell-to-cell communications through gap junctions (51).

INSULIN SYNTHESIS

The control of glucose and energy metabolism in humans is highly dependent on hormones secreted by the islets of Langerhans, and most notably on insulin. The

![Diagram](https://via.placeholder.com/150)

**FIG. 1.** The pancreatic beta cell. Glucose is carried into the cell through a specific glucose transporter called GLUT-2, or glucose transporter 2. The glucose is metabolized by a rate-limiting glucokinase leading to the generation of glucose metabolites and ATP. This results in the closing of potassium channels, which causes the cell membrane to depolarize, thereby activating voltage-dependent calcium channels and raising cytosolic free calcium (Ca) levels. The rise in calcium induces microtubule contraction and release of insulin. The beta cell also possesses a variety of cell-surface receptors that bind specific ligands (L), leading to the activation of adenylyl cyclase (AC) and phosphoinositides (PIP2) hydrolysis through the actions of phospholipase C (PLC). PIP2 hydrolysis generates diacylglycerol (DG), which activates protein kinase C (PKC), and inositol trisphosphate (IP3), which mobilizes calcium from intracellular stores and which is converted to inositol tetraakisphosphate (IP4). Insulin synthesis is dependent on the availability of proinsulin mRNA, which is transcribed in the nucleus and translated into protein in the endoplasmic reticulum (ER). Preproinsulin is cleaved into proinsulin within the ER. Proinsulin is packaged into coated secretory granules inside the Golgi apparatus. It is converted to insulin, which is released into capillaries and eventually into the pancreatic vein.
human insulin gene is located on chromosome 11 (52). It contains 3 exons and 2 introns (53). The encoded insulin mRNA is translated into preproinsulin, a 110 amino acid peptide that consists of a 24 amino acid signal sequence, the A and B peptides, and a connecting C peptide (54,55). The signal sequence is cleaved into proinsulin in the endoplasmic reticulum by signal peptidases that act in a cotranslational manner (54,55). Proinsulin is then packaged in the Golgi (Fig. 1) and processed into mature insulin in coated secretory vesicles released from the trans-face of the Golgi apparatus (54,55) by two enzymes whose cDNAs were recently cloned (56,57). One is a trypsin-like enzyme that removes the intervening C peptide by cutting at two dibasic amino acid sequences, and the other is a carboxypeptidase B-like enzyme that removes the dibasic peptide from the carboxy terminal end (56,57). The final product, insulin, is a 51 amino acid peptide that consists of A and B peptides that are linked by two disulfide bonds at positions A7 to B7 and A20 to B19 (54).

The regulation of insulin biosynthesis is coordinated by several hormones and nutrients, but most notably by glucose. Insulin synthesis increases markedly within minutes of exposure of the β cell to glucose in vitro, as a result of transcriptional and posttranscriptional events (58,59). Transcriptional activation of the insulin gene is dependent on the presence of 5'-flanking sequences that contain the promoter regions of this gene, including a TATA box, and the FAR and NIR boxes (60–62). These DNA sequences are important regions that function to regulate both the site of initiation of gene transcription and the rate of transcriptional synthesis. The FAR and NIR boxes dictate the cell-specific expression of the insulin gene, and contain the transcription initiation site GCCATCTG that interacts with insulin enhancer binding protein 1 (IEF1), a DNA binding protein (61–63). Cloning and sequence homology analysis indicate that rodent IEF1 is similar to human E12 and E47 DNA-binding proteins, and that they belong to the family of helix-loop-helix transcriptional activators (64,65). Other DNA-binding proteins have also been implicated in the regulation of insulin gene expression. For example, the transactivating protein that binds to the G3 promoter region of the rat 5'-flanking region of the glucagon gene is expressed in α, β, and δ cells (66). It also binds to regulatory elements of the insulin and somatostatin genes (66). Thus, the exact mechanisms by which DNA-binding proteins confer β cell specificity for insulin gene expression in the adult pancreas remain to be determined.

In order for glucose to exert its effects on insulin secretion and synthesis, it must first get into the β cell. This is accomplished through a process of facilitated diffusion, which is mediated by a glucose transporter. Five distinct isomers of the glucose transporter (GLUT-1 through GLUT-5) have been cloned (67). They possess 12 membrane-spanning domains, 5 endoplasmic loops, and 6 exoplasmic loops (67). The 5 human isoforms exhibit considerable amino acid sequence homology that ranges from 39 to 65% (67). The glucose transporter that is expressed in the β cell is also found in the liver, renal tubules, and the small intestine, but is not found in other types of islet cells (68). GLUT-2 has a high Km for glucose uptake, allowing the β cell to transport glucose in proportion to the extracellular glucose concentration (Fig. 1), even at high physiological levels of glucose (69). However, GLUT-2 expression is downregulated by chronic hyperinsulinemia (69). Therefore, in patients with non-insulin-dependent diabetes mellitus (NIDDM), in whom insulin resistance is often associated with high insulin levels, there may be downregulation of GLUT-2 and a failure of the β cell to secrete enough insulin to overcome the resistance (69).

INSULIN SECRETORY DYNAMICS

In the basal state, insulin secretion from the β cell is generally constant. The importance of basal insulin secretion and of diurnal changes in insulin requirements for the regulation of glucose homeostasis is now widely accepted, partly as a result of research performed with artificial pancreas systems (70). However, basal insulin secretion also exhibits small oscillations, with a periodicity of 9 to 14 minutes (71,72). These spontaneous secretory oscillations are attenuated by certain neurotoxins and nicotinic antagonists, suggesting that they are due to regulation that is mediated by intrapancreatic ganglia and nerves (73). It is also possible that these oscillations are intrinsic to the β cell. In support of this hypothesis, isolated human insulinoma tissues secrete insulin, proinsulin, and C-peptide in a pulsatile manner in the absence of innervation and other islet cells (74). Using the artificial endocrine pancreas, investigators have recently shown that insulin oscillations that mimic the periodicity observed in humans (13 minutes) are more efficient at inhibiting hepatic gluconeogenesis than equivalent levels of either continuously administered insulin or insulin administered with a 26-minute cycle (75).

The principal regulator of insulin secretion from the pancreatic β cell is glucose, which also acts by potentiating the insulin-releasing effects of other β cell secretagogues such as CCK (76–79). Glucose stimulated release of insulin is biphasic over the first 2 hours (80). The first phase is rapid but transient, release of insulin occurring in response to an initial change in glucose concentration. It is independent of insulin synthesis, and is due to the secretion of insulin from previously formed granules that are located near the β cell membrane (80). The second phase is associated with a more gradual increase in insulin secretion over a period of about 2 hours, insulin levels rising above those seen during the first phase, and
slowly declining thereafter. This phase is partly dependent on the release of newly synthesized insulin (80,81).

**SIGNAL TRANSDUCTION IN THE BETA CELL**

As in the case of digestive enzyme secretion in the pancreatic acinar cell, insulin secretion in the β cell is dependent on the fusion of secretory granules with the cell membrane, a process termed exocytosis. In most cell types, including the β cell, exocytosis is regulated by changes in cytoplasmic free calcium levels. Calcium is an important second messenger that also participates in the regulation of many other cellular processes. In general, the level of intracellular free calcium is dependent on basal calcium influx and efflux, on effector systems that act to modulate calcium influx and calcium mobilization from intracellular stores, and on the buffering capacity of intracellular organelles that act to sequester the cation (82). In the β cell, these mechanisms maintain the resting cytosolic free calcium levels at approximately 100 nM (81).

The primary modulator of intracellular free calcium levels is glucose (81). It has been appreciated for a long time that the actions of glucose are dependent in a significant manner on the presence of extracellular calcium and on the ability of glucose to enhance calcium influx in the β cell (83–86). The mechanisms by which glucose raises intracellular calcium have been studied intensively. These studies indicate that calcium enters the β cell following activation of voltage-dependent calcium channels (81). The β cell possesses two types of these channels. The preponderant type is dihydropyridine (DHP) sensitive, and is associated with a long-lasting calcium current, hence the designation L-type channel (87,88). The L-channel is inhibited by a rise in intracellular calcium levels, leading to negative feedback regulation (87,88). There is also a DHP-insensitive calcium channel in the β cell, which is associated with a transient calcium current, hence the designation T-type channel (87). Both channels are activated by glucose-induced depolarization of the β cell plasma membrane (89,90). This depolarization occurs as the result of glucose-mediated inhibition of an ATP-sensitive K⁺ channel (89,90). In the resting state, when glucose concentrations are low, this channel is active and allows K⁺ to exit the β cell (Fig. 1), thereby maintaining it in a hyperpolarized state with a resting membrane potential of −60 to −80 mV (89,90). An increase in glucose levels closes this channel, leading to an inhibition of K⁺ efflux and membrane depolarization.

The ATP-sensitive K⁺ channel is not inhibited by glucose directly. Instead, it is inhibited by intermediates generated by glucose metabolism, most notably ATP (91–93). Sulfonylureas, which are used to treat NIDDM, act as β cell secretagogues by inhibiting the ATP-sensitive K⁺ channel (91–93). Other intermediates generated by glucose metabolism, such as NADPH, may also elicit insulin release by inhibiting this channel (94). The β cell also possesses a calcium-activated K⁺ channel (95,96). This high-conductance K⁺ channel is activated by the high calcium levels that are associated with stimulated insulin secretion, thereby allowing for K⁺ efflux and membrane repolarization (95,96).

Glucose also increases insulin secretion by elevating the levels of certain inositol phospholipids either through the activation of phospholipase C (PLC) or by increasing their de novo synthesis (97). PLC is an enzyme that induces the hydrolysis of membrane-associated phosphatidylinositol-4,5-bisphosphate (PIP₂), resulting in the generation of inositol trisphosphate (Ins-1,4,5-P₃) (98). Ins-1,4,5-P₃ raises cytosolic free calcium levels by releasing calcium from intracellular stores (Fig. 1), after binding to a specific receptor (99). Ins-1,4,5-P₃ is either converted by a phosphomonoesterase to a metabolically inactive isomer, or by Ins-1,4,5-P₃-kinase to inositol 1,3,4,5-tetrakisphosphate (Ins-1,3,4,5-P₄), which may act together with Ins-1,4,5-P₃ to enhance receptor-mediated calcium influx (100,101). It is also possible that Ins-1,4,5-P₃ acts directly to increase calcium influx (102). Both Ins-1,4,5-P₃ and Ins-1,3,4,5-P₄ are generated in the β cell (103,104). Furthermore, the Ins-1,4,5-P₃-induced calcium transient in the β cell activates a K⁺ channel that leads to transient bursts of membrane repolarization during glucose-induced insulin secretion (105).

PIP₂ hydrolysis also leads to the generation of a second important second messenger, diacylglycerol (98), the endogenous activator of protein kinase C (PKC). PKC is a serine-threonine kinase that is expressed in the β cell (106). Its activation results in enhanced insulin secretion (107). Conversely, inhibition of PKC activity attenuates the stimulatory effect of glucose on insulin secretion (108). Furthermore, incubation of islets with glucose leads to a rapid increase in diacylglycerol levels (109). Taken together, these observations indicate that PKC participates in the regulation of insulin secretion.

Other serine-threonine kinases, most notably calcium-calmodulin protein kinases and cyclic AMP (cAMP)-dependent protein kinases, have been implicated in the regulation of insulin secretion (82). There are five major types of calcium-calmodulin kinases, which have been classified as I, II, and III, phosphorylase kinase, and myosin light-chain kinase (110). Some of these kinases may play a role in stimulus-secretion coupling in the β cell by regulating the phosphorylation of specific substrates and by affecting the cytoskeleton, which directs the fusion of secretory vesicles to the plasma membrane (82). In contrast, cAMP may enhance secretion by promoting the lysis of insulin-containing secretory granules (111). In addition, by activating the cAMP-dependent protein kinase, which then induces the phosphorylation of voltage-dependent cal-
cium channels, cAMP can lead to increased calcium influx and enhanced insulin secretion (112).

Several additional second messengers may have important roles in enhancing insulin secretion and/or modulating the actions of glucose and other β cell secretagogues. These second messengers include phosphatidic acid, lysophospholipids, and arachidonic acid and its metabolites (82,113). In some instances, signals are generated that inhibit insulin secretion. For example, somatostatin inhibits insulin secretion, in part, by activating ATP-sensitive K⁺ channels, thereby causing β cell hyperpolarization (114). In contrast, adrenaline inhibits insulin secretion by activating a low conductance K⁺ channel that is distinct from the ATP-sensitive K⁺ channel (115). These actions of somatostatin and adrenaline are dependent on GTP-binding proteins (G protein), which couple receptors to effector pathways (81,114,115). G-proteins consist of α, β, and γ subunits and have been implicated in the regulation of many cellular processes, including stimulation of adenyl cyclase, receptor-mediated activation of PLC, and gating of specific ion channels (116). These proteins may function, therefore, to coordinate the variety of signals and second messengers that lead to insulin secretion.

SUMMARY

The endocrine pancreas is a complex system of microdomains that responds to a multiplicity of extracellular signals, which includes hormones, neurotransmitters, and a variety of nutrients. The actions of these extracellular signals are modulated in complex ways. The resultant interactions lead to the activation of many cellular processes that include the induction of ion fluxes, phosphatidylinositol hydrolysis, generation of a variety of second messengers, and phosphorylation of numerous proteins. The dispersal of the islets throughout the exocrine tissue allows islet-acinar interactions and places the endocrine cells in a unique microenvironment whose complexity is yet to be fully understood.

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