NEUROPHARMACOLOGY OF THE PANCREATIC ISLETS

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INTRODUCTION

Following the discovery that insulin and glucagon are produced by the pancreatic islets, a vast literature has accumulated concerning the role of substrates, nonpancreatic hormones, and intracellular factors in the control of islet hormone release. The results of such work are the subject of several reviews (1–4) and therefore are not covered in-depth in this paper. More recently, however, it has been appreciated that the nervous system is also intimately involved in the regulation of endocrine pancreatic secretion. The purpose of this review is to discuss the anatomic, physiologic, and pharmacologic evidence for the role of neural factors in the regulation of insulin and glucagon secretion, and to demonstrate that the endocrine pancreas is an important neuroendocrine organ.

CYTOLOGY AND ORIGIN OF THE PANCREATIC ISLETS

In most species the principal components of the endocrine pancreas are the A, B, and D cells. Although variations of islet cytoarchitecture may have evolutionary and functional implications (5–7), an equally important cytologic consideration is the hormonal content of each of the islet cell types. It has been accepted for some time that the A cells contain glucagon and that the B cells contain insulin (8). However, there is no general agreement as to the hormonal product of the D cell. Some investigators have reported that these cells contain gastrin (7), but subsequent studies have pointed to the absence of this hormone in the islets of a large number of species (9). Renewed interest in the D cell has been sparked by the recent immunocytochemical evidence that this cell type contains somatostatin (10, 11), a hormone originally isolated from the hypothalamus. This cytologic finding is the latest of a series that indicates a close functional relationship between the pancreatic islets and the nervous system. Further, this observation is compatible with the hypothesis that some, if not all, of the islet cells originate from the ectoderm, an

embryonic source different from that of the pancreatic exocrine cells. As discussed below, this hypothesis is markedly different from the commonly held view that the pancreatic islet and exocrine parenchyma are both derived from the endoderm.

Within the framework of a common origin of islet and exocrine cells there are a number of theories regarding the genesis of the endocrine pancreas. Some early investigators (see 6) postulated that there might be multiple generations of pancreatic islets formed by the transdifferentiation of exocrine cells into functional endocrine cells. The possibility that islet cells and the adjacent exocrine tissue are in a dynamic state of flux and interconversion has been supported by a few recent studies (12). Several investigators, however, have failed to find evidence that acinar-islet cell transformations play a role in the formation and maintenance of the pancreatic islet mass (see 6, 13). In fact, most studies of islet development suggest that islet formation takes place in regions adjacent to the pancreatic ducts. The process whereby islets might develop from pancreatic ductal cells has been recently reviewed by Pictet & Rutter (6). These workers have shown that during early phases of histogenesis the cells of the pancreatic diverticula undergo a series of divisions forming numerous closed acini. In general, these acini elongate and form the elaborate structure of the adult pancreas by cell divisions that are parallel to the developing duct lumen; each daughter cell thus maintains the same contacts as its parent via junctional complexes with the neighboring cells. Pictet & Rutter also observed, concomitant with acinar development, certain mitoses that were in a plane perpendicular to the axis of the lumen. They have hypothesized that as a result of this type of division, one of the daughter cells is no longer in contact with its former neighbors and begins to differentiate into an endocrine cell, thereby initiating the formation of a pancreatic islet. Pictet & Rutter (6) have additionally shown that pancreatic rudiments (endothelium plus the associated mesenchyme) develop normally in vitro and produce approximately 90% exocrine cells and 10% endocrine cells. However, when the mesenchyme is removed from the rudiment, 90% of the cells develop as endocrine cells, with A cells in the majority. Studies such as these are suggestive of how the pancreatic islets are formed and show that pancreatic rudiments contain all of the cells necessary to form the adult pancreas. Unfortunately, these studies have not clearly defined the precise cellular ancestry of the endocrine pancreas. In their review, Pictet & Rutter (6) suggested several hypothetical mechanisms to account for exact differentiation of islet cells and exocrine tissue from a common endodermal source. In contrast to the view that the pancreatic islets are derived from the endoderm, evidence has been presented by Pearse (14) that suggests that the islets originate from the neuroectoderm. Because various polypeptide-secreting endocrine cells share certain ultrastructural and cytochemical characteristics, Pearse has classified them as the APUD series (Amine Precursor Uptake and Decarboxylation). Pearse has further suggested that all cells having this property arise from the neural crest. While some cells derived from the neural crest are known to produce and secrete amines as their primary function (e.g. adrenal medullary cells), many endocrine cells secrete peptides and retain the ability to convert enzymatically specific precursor molecules [dihydroxyphenylalanine (DOPA) or 5-

hydroxytryptophan (5-HTP)] to the corresponding biogenic amine (dopamine or serotonin, respectively). Such APUD cells have been shown to migrate from the neural crest of the developing embryo to colonize several areas (15, 16) including the foregut and its derivatives (17). In an experiment using fetal mouse pancreas, Pearse et al (13) observed "clear cells" that did not have recognizable secretory granules and displayed no insulin and glucagon immunoreactivity. These cells, however, displayed APUD characteristics. At a later stage of development these same APUD cells had ultrastructurally identifiable secretory granules and immunocytochemical reactions to insulin and glucagon antibodies. Studies of this type show that presumptive islet cells can be readily identified using specific cytochemical criteria prior to the onset of hormone synthesis and storage. Unfortunately, they do not prove beyond doubt that the islets are of neural crest origin. In fact, recent studies have been performed in which the ectoderm has been removed from chick and mouse embryos prior to the formation of the neural crest; in each case, fully differentiated APUD cells were found in the gut (18) and pancreatic islets (19). Thus, by strict definition the presumptive islet cells may not arise from the neural crest per se. It is possible, however, that some ectodermal cells populate the endoderm prior to the formation of the neural crests. If so, such cells could migrate with the developing exocrine and ductal cells and be responsible for the formation of the pancreatic islets in the fashion suggested by Pictet & Rutter (6). Although the neuroectodermal origin of the pancreatic islet cells remains to be proven, the validity of this theory is supported by the fact that some polypeptide-secreting endocrine cells of known ectodermal origin (e.g. thyroid C cells) share common cytochemical characteristics with the APUD cells of the endocrine pancreas.

INNERVATION OF THE PANCREATIC ISLETS

There is abundant anatomic and physiologic evidence that the endocrine pancreas of most vertebrates is innervated by both the sympathetic and parasympathetic components of the autonomic nervous system (for reviews see 8, 20, 21). The notable exceptions are cyclostomes (5), certain reptiles (5, 22), and birds (23). The only mammal not known to have innervated islets is the spiny mouse, *Acomys cahirinus* (24).

The nerve supply to the pancreas is derived from the vagal and splanchnic trunks and enters the gland in association with the arteries. Nerves arising from within the pancreas reach the level of the spinal cord via the splanchnic nerves. These latter fibers presumably mediate only pain due to distention (25), but it is not known whether any of them arise within the islets. Nerve fibers that reach the pancreatic islets also accompany the blood vessels (26, 27). Depending upon the species, these fibers are arranged as the so-called peri-insular plexus (28), but may also form an intra-insular plexus at the more central portions of the islet (29). In addition to an extrinsic nerve supply, nerve cell bodies are sometimes observed within the islets (6, 20). These intrinsic neurons are likely to be postganglionic parasympathetic nerves, but their identification as such is yet to be determined. Therefore, the precise

function of these nerves is unknown. However, as discussed below, the functional significance of the extrinsic islet nerves is fairly well established.

Cytochemical and ultrastructural studies have shown that nerve endings within the endocrine pancreas can be divided into three types according to their synaptic vesicles. The terminals of cholinergic nerves contain 30–50 nm agranular vesicles, whereas those of adrenergic nerves contain mainly 30–50 nm dense-cored structures (30). Both types of terminals also contain a few 80–100 nm electron-opaque vesicles of unknown function. A third type of islet nerve terminal contains 60–200 nm dense-cored vesicles that are histochemically distinct from those of the other autonomic nerves. Since these endings are structurally similar to the "purinergic" terminals of mammalian smooth muscle recently described by Burnstock (31), it is possible that they might release ATP as a neurotransmitter. This latter type of nerve has not been reported in mammalian islets, but it does appear to be the predominant ending in the endocrine pancreas of certain teleosts (32). While there are different ratios of adrenergic to cholinergic endings in the islets of various species, there seems to be no preferential relationship between one type of ending and any given islet secretory cell. There are, however, important differences in terms of nerve secretory cell structural relationships between the various vertebrate groups. Typical pre- and postsynaptic complexes have been observed between neurons and islet cells in some lower vertebrates (5), but in mammals the islet nerve terminals end blindly at a distance of 20–30 nm from the secretory cells. This suggests that, at least in the case of mammals, neurotransmitters may be released into the islet intercellular space and simultaneously stimulate or inhibit a large number of islet cells. Another means of dispersing a neural signal to the general population of islet cells might occur via a more anatomically direct mechanism. Orci et al (33, 34) have recently shown gap junctions between various cells within the islets, and between nerves and islet B cells. These intercellular junctions are known to be areas of low electrical resistance (e.g. as in cardiac muscle) that also allow the passage of molecules of low molecular weight. It is, therefore, possible that the secretory response of a functionally coupled islet could be rapidly altered by electrical and/or chemical signals from the nerves. Further, such a signal could be markedly amplified because of the numerous gap junctions that presumably exist between the cells of the islet. The possibility that electrical signals play a role in the regulation of islet function is further suggested by electrophysiologic studies demonstrating that islet cells have spontaneous electrical activity, and that their firing pattern is altered when challenged with different glucose concentrations (35, 36).

Other features of the endocrine pancreas are indicative of the close structural and functional coupling of islet cells and nerve endings. For example, the relationship between the Schwann's cells of the intra-islet nerve terminals and the endocrine parenchyma of the pancreatic islets of the dog (37) suggests that the islets of this species are structurally similar to autonomic ganglia. Thus, if the theory of neuroectodermal origin of the islets proves correct, this observation would indicate that the endocrine pancreas is cytologically analogous to the adrenal medulla. The islets, therefore, could be considered as specialized metabolic ganglia having both cholinergic and adrenergic innervation.

INTRACELLULAR MONOAMINES AND ISLET FUNCTION

Fluorescence microscopy, using the technique of Falck and Hillarp, has clearly demonstrated that amines are present not only in the islet nerve endings but also within the A and B cells (38). These amines have been specifically identified as serotonin and dopamine by their spectral characteristics and by extraction and chemical analysis of either whole pancreas or isolated islets. The type and amount of these monoamines vary considerably according to several factors. They are not normally found in albinos but are detectable in the islets of pigmented animals. Further, the amines are far more concentrated in the islets of younger animals, including man (38). Although the A and B cells of the albino mouse do not normally contain monoamines, the administration of DOPA or 5-HTP leads to the appearance of amines within these endocrine cells. This ability of islet cells to concentrate and decarboxylate DOPA to dopamine and 5-HTP to serotonin has been found in all species studied thus far (39, 40) and forms part of the evidence that these APUD cells are of neuroectodermal origin.

Autoradiographic studies have shown that following the injection of radiolabeled DOPA or 5-HTP, the corresponding amine is present within the cytoplasm of the islet cells for several hours. Using quantitative grain-counting techniques at the ultrastructural level, it has been shown that the labeled amines are localized within the halo of the secretory granules of the A and B cells (41; I. Lundquist, personal communication). While it is clear that islet cells incorporate the immediate precursors of dopamine and serotonin, the circulating levels of DOPA and 5-HTP are ordinarily quite low. Therefore, the existence of intracellular amines in the islets of certain species implicates amino acids such as tyrosine and tryptophan as the precursors of dopamine and serotonin, respectively. These amino acids are part of the normal intraneural biosynthetic pathway of dopamine and serotonin. The concept that they may serve as precursors in islets cells is supported by preliminary studies (42) that transplantable islet cell carcinomas of hamsters contain tyrosine hydroxylase and L-aromatic amino acid dicarboxylase, two enzymes necessary for the synthesis of dopamine and serotonin respectively. From the studies using labeled precursors, it is evident that once formed the amines turn over rapidly whether or not insulin is released. Since pretreatment with the monoamine oxidase (MAO) inhibitor, pargyline hydrochloride, increases the grain count over islet cell secretory granules (41), amine degradation is also an important determinant of the functional role of intracellular serotonin and dopamine. Of the two catecholamine degrading enzymes, MAO and catechol-O-methyl transferase (COMT), the former appears primarily responsible for the breakdown of islet cell amines. This is due to the fact that although COMT is important in the degradation of extracellular amines, this enzyme has not been identified in pancreatic islet cells. On the other hand, MAO levels have been found to be approximately three times higher in islet cells than in the surrounding exocrine pancreas (43, 44).

As described in the next section, the administration of serotonin and dopamine alters insulin secretion in vivo and in vitro. However, because of their poor penetration into the islet cells, it has been generally concluded that serotonin and dopamine

when applied extracellularly do not necessarily elicit the same effect that they would as intracellular substances. Therefore, the role of intracellular amines as regulators of islet cell secretion has been assessed by either examining the effects upon insulin release produced by the administration of amine precursors, or by inhibiting the breakdown of intracellular serotonin and dopamine.

The in vivo infusion of amine precursors such as 5-HTP or DOPA induces a state of reduced insulin responsiveness to glucose when isolated islets or pieces of pancreas from these animals are subsequently incubated with glucose in vitro (45–47). With this approach, in vivo responses to glibenclamide and isoproterenol are reduced, while glucose-stimulated insulin release remains normal (48). Antagonists known to block the inhibitory effects of extracellular monoamines such as phentolamine and dibenzyline do not block the inhibitory effects of 5-HTP or DOPA (47), suggesting that the intracellular presence of serotonin and dopamine is a critical factor in their inhibitory effects upon insulin release. This concept is supported by in vitro studies of islets from obese hyperglycemic mice that clearly demonstrate the local uptake of 5-HTP and its conversion to serotonin within the islet cells (45). Furthermore, blockade of the decarboxylating enzyme prohibits the inhibitory effect of 5-HTP or DOPA upon pancreatic islet secretion (45, 47, 48).

These inhibitory effects have also been studied using the serotonin antagonist, methysergide. Although some of the inhibitory effects of 5-HTP administration are reversed by pretreatment with methysergide (47), this drug by itself produces roughly the same increase of insulin secretion in controls, leading to results that are difficult to interpret. Further, methysergide also blocks the inhibitory effect of extracellular amines by acting as an α-adrenergic blocking agent (49). Attempts to elucidate the role of intracellular serotonin using other drugs have been conflicting. Parachlorophenylalanine (PCPA), an agent that depletes brain serotonin, has no effect on in vivo glucose-stimulated insulin release from rabbits and fed hamsters (50). However, PCPA does increase the in vitro secretion of insulin from islets of fasted animals (51). Two other serotonin antagonists, cyproheptadine and cinanserin, also increase insulin secretion (50). Again there is complexity, since cyproheptadine stimulates insulin release in the absence of glucose. This latter effect is incompatible with a direct action of cyproheptadine upon serotonin activity because serotonin has been found to inhibit only glucose-induced insulin secretion (50).

There is also conflicting data concerning the in vivo and in vitro effects of MAO inhibitors such as nialamide, pargyline, tranylcypromine, and mebanazine upon insulin secretion. The conflicting data may be related to the ability of these drugs to interact with adrenergic receptors or to interference with enzyme systems other than MAO (see 52–54). Aleyassine & Gardiner (44) have found that the effects of many MAO-inhibiting drugs are concentration dependent. At low doses these agents stimulate insulin secretion, and at high doses they inhibit the release of insulin. All of the MAO inhibitors were found to be effective at relatively low concentrations but neither the stimulation nor inhibition of insulin secretion could be directly correlated with the degree of MAO inhibition. Furthermore, when given in association with 5-HTP some of these drugs elicit hypoglycemia unrelated to insulin secretion (48). Studies using MAO-inhibiting drugs, therefore, cannot be

used to support or refute the hypothesis that intracellular monoamines alter insulin secretion.

In summary, pancreatic A and B cells of many species normally contain monoamines, and in all species the islet cell stores of these monoamines can be either increased or induced by the administration of DOPA and 5-HTP. The responsiveness of the insulin-secreting cells is usually reduced when amine precursors are given. However, many of the compounds used to evaluate intracellular monoamines interact in complex ways with extracellular catecholaminergic and serotonergic receptors. Despite these limitations, Lundquist (48) and Lebovitz (39) have proposed attractive theories that suggest an important inhibitory control of insulin secretion by intracellular monoamines. To date, no convincing evidence has been presented that refutes these theories; nevertheless, most of the evidence for them is circumstantial, and many of the studies upon which they are based have been contradictory.

EXTRACELLULAR AMINES AS REGULATORS OF ISLET FUNCTION

Extracellular amines also control islet secretory rates. These amines originate from three primary sources: (*a*) intra-islet nerve endings secreting acetylcholine or norepinephrine, (*b*) enterochromaffin cells within the islets that contain serotonin and possibly dopamine, and (*c*) amine-secreting cells in other areas of the body (i.e. adrenal medulla and extra-islet nerve endings) whose products reach the islets via the blood. The role of various amines has been approached experimentally by stimulation or sectioning of the pancreatic nerves in vivo, or by the administration of amines and their analogs either in vivo or in vitro. In the course of such studies it has been found that certain other substances related to the nervous system, including somatostatin and prostaglandins, also modulate the effects of amines and their control of islet function.

Electrical stimulation of the vagus nerve (54–58) or the ventrolateral hypothalamus (59, 60), a parasympathetic center, has been found to enhance insulin release. Glucagon secretion following vagal stimulation has also been observed in some species (58, 61) but not in others (62). Under these conditions islet hormone secretion is a direct neural effect, and the response is dependent upon the prevailing glucose level (63, 64). Bilateral destruction of the ventrolateral hypothalamic nuclei (VLH) produces a decrease of plasma insulin levels (60, 65). Vagotomy abolishes conditioned insulin secretion in rats (66) and impairs both insulin and glucagon secretion in man (67). Another important role of the parasympathetic system has been recently suggested by the work of Powley & Opsahl (68) who reported that the hypothalamus may regulate the numbers of pancreatic islet cells via the vagus nerves.

Confirming studies using parasympathomimetic drugs have shown that these agents increase both insulin and glucagon (69–72) secretion indicating that these cells are well supplied with muscarinic receptors. Nicotinic acid does not increase insulin secretion in vitro (73), and this finding has been used to suggest that its

stimulatory effect in vivo (74) is mediated by postganglionic neurons (73). However, there is no evidence that nicotinic acid stimulates nicotinic receptors, and therefore the role of ganglionic stimulating agents remains unexplored. Atropine blocks the increased secretion of insulin and glucagon produced by cholinergic agents (55, 61, 70, 75, 76) and also blocks conditioned insulin secretion (66). Although gut hormones may be released by vagal stimulation or administration of cholinergic drugs, the increased levels of insulin observed under these conditions has been found to be a direct effect upon the B cell (57, 77) and not related to the known insulinotropic effects of gut hormones.

Sympathetic stimulation of the islet cells initiates a dual effect. Stimulation of the splanchnic nerves (57) or the ventromedial hypothalamus (78) elicits a decline of insulin secretion and a concurrent increase of glucagon levels. This effect is complicated by the differing activities of the α-and β-adrenergic receptors of the islet cells. As reviewed elsewhere (20, 79, 82), norepinephrine or epinephrine inhibits glucose-mediated insulin release both in vivo and in vitro. α-Adrenergic blockers such as phentolamine or phenoxybenzamine have been found to reverse the inhibition of insulin secretion during a catecholamine infusion. No reversal of this inhibition, however, has been observed when propranolol, a β-blocker, is administered. In contrast, β-adrenergic agonists stimulate insulin secretion and are blocked by propranolol. Thus, α-receptor activation inhibits insulin secretion, whereas β-stimulation increases insulin secretion. A number of reports also credit β-adrenergic stimulation as the factor responsible for the elevation of glucagon levels (83, 84). However, in one case α-blockers reportedly abolished this effect (85). At present it would seem that there is clear evidence that β-receptors mediate an increase of both insulin and glucagon secretion and that α-adrenergic stimulation inhibits insulin release.

There is now convincing evidence that the opposing effects of α- and β-receptor stimulation upon insulin secretion can occur simultaneously. This is due to the fact that although glucose is ineffective in stimulating insulin release during a catecholamine infusion because of α-stimulation, insulin levels nonetheless rise over time as a function of β-receptor activation (86). This response indicates that two separate phenomena are occurring. First, there is an abrupt stoppage of insulin release by activation of the α-adrenergic receptor that remains operative even in the face of glucose stimulation. Second, there is a tonic, albeit slow, rise of insulin secretion related to the β-adrenergic receptor. Turtle & Kipnis (87) made the observation that, in the presence of theophylline, islet levels of cyclic AMP were elevated by β-adrenergic stimulation and decreased by α-adrenergic stimulation. It was therefore assumed that the inhibition of insulin release by α-agonists was a function of lowered cyclic AMP within the B cell, and that the β-stimulated elevation of cyclic AMP was responsible for the increase of insulin secretion. Under this concept a simultaneous inhibition and stimulation of insulin release by epinephrine, due to changes of cyclic AMP, would be impossible (81). The evidence for cyclic AMP as the second messenger for β-adrenergic activity remains overwhelming. However, two studies have shown that the insulinotropic effect of exogenous cyclic AMP is blocked by simultaneous incubation with catecholamines (88, 89). Thus it appears

the α-adrenergic stimulation initiates a signal that is responsible for the inhibition of insulin secretion independently of its ability to lower cyclic AMP levels. The precise nature of this signal is unknown and warrants further investigation.

The dual effects of catecholamines upon insulin secretion can be illustrated using isoproterenol as a prime example. This amine is a mixed α- and β-agonist that stimulates insulin release in vivo (90), but inhibits insulin secretion in vitro (91). These effects are undoubtedly related to a number of factors including the doses used, which provide different degrees of α- and β-adrenergic receptor stimulation, and interactions, or lack thereof, with other amines. Thus in one system isoproterenol may lead to a net stimulation of insulin secretion, but in the other it would produce a net inhibition. Likewise, other amines including serotonin (92) and histamine (80) have been found to stimulate, inhibit, or have no effect upon insulin secretion. These differences must be viewed in relation to changes of capillary blood flow within the islet as in the case of histamine (80), or the ability of serotonin to enhance the release of catecholamines (93, 94) independently of its direct action upon islet cells. However, the known ability of these amines to activate simultaneously receptors that stimulate (β) and those that inhibit (α) insulin secretion is likely to be the principal factor in these conflicting results.

The molecular configuration of amines is a determinant of their interaction with the islet cells. In a recent review, Lebovitz & Feldman (39) have pointed out that while the ammonium ion (NH_4^+) inhibits glucose-stimulated insulin release, only aromatic amines have the ability to inhibit insulin secretion to other stimuli. This pharmacologic action of monoamines appears to be dependent primarily upon the presence of a terminal amine on the aliphatic portion of the molecule and partially dependent upon the hydroxyl groups of the aromatic ring (91). Insulin secretion is inhibited by serotonin (5-hydroxytryptamine), but its deaminated breakdown product 5-hydroxyindoleacetic acid has no such effect. On the other hand, 5-hydroxytryptophan, an aminated serotonin precursor, does not inhibit insulin release as an extracellular agent, presumably because of the presence of its carboxyl group. The importance of the hydroxyl group is further indicated by the greater potency of dopamine compared to tyramine as an inhibitor of insulin secretion (the former has one additional hydroxyl group). Methylation of the hydroxyl group abolishes the inhibition of insulin release as indicated by the lack of effect of 5-methoxytryptamine or metanephrine. The hydroxyl group is not an absolute requirement for pharmacologic action because β-phenylethylamine and tryptamine both decrease insulin secretion in vitro. The stimulatory properties of β-receptor agonists, however, are more closely related to the hydroxyl groups and can be found almost selectively when the amine group is blocked but not removed (e.g. isopropylnorepinephrine).

There is evidence that many of the monoamines share receptors. The α-receptor antagonist phentolamine blocks the inhibition of insulin secretion produced by serotonin, tryptamine, catecholamines, and histamine. All of these, except histamine, are also blocked by methysergide (39). The blockade of catecholamine and serotonin action by either phentolamine or methysergide strongly supports the concept that these different groups of amines inhibit insulin secretion via the same or similar membrane receptors. Because serotonin also enhances catecholamine

release from nerve endings in addition to its direct effect upon the B cell, its properties may be altered by the release of norepinephrine (94). Alternatively, serotonin may stimulate insulin secretion via β-receptors either directly or in conjunction with catecholamines. This kind of modulation probably explains why serotonin inhibits or stimulates insulin secretion depending upon the particular circumstances.

The ability of amines to stimulate insulin secretion by activation of the β-adrenergic receptor is blocked by agents such as propranolol. Using selective agents such as practolol and salbutamol, Loubatieres et al (95) found that amine-induced insulin secretion is dependent upon stimulation of the β_2-receptor and does not require simultaneous activation of the β_1-receptors. Therefore, the differential stimulation of the two kinds of β-receptors is also important.

Recently, the fatty acid derivatives known as prostaglandins (PGs) have attracted wide attention because of their diverse effects in a number of endocrine systems. Of particular interest is their pharmacologic action upon islet hormone release because of their known interaction with catecholamines in other systems (96, 97). Much like serotonin, PGs have been found to stimulate, inhibit, or have no effect upon insulin secretion (98, 99). Robertson et al (100) found that PGE_1 inhibits basal and stimulated insulin secretion of the dog in vivo. This inhibitory response was not blocked by phentolamine, suggesting the PGs act independently of catecholamine receptors. In contrast, Bressler et al (52) observed a stimulation of insulin secretion by PGE_1. In this in vivo system, β-adrenergic blockade reversed the enhanced release of insulin. Both findings were confirmed by Burr & Sharp (101) who reported that insulin secretion in vitro was increased by PGE_1 at low glucose concentrations, but that at high glucose levels PGE_1 produced an inhibitory effect. In the same study, the concurrent administration of epinephrine and PGE_1 at high glucose concentrations resulted in a paradoxical stimulation of insulin secretion (i.e. the drugs alone would have inhibited insulin secretion with these conditions). These authors suggested that this response could be due to a prostaglandin-mediated change of α- or β-receptor activity, or to an alteration of calcium flux by PGs that is modulated by adrenergic stimulation.

Another recently discovered neurohormone, somatostatin, has been found to have pharmacologic actions upon islet hormone secretion. This cyclic polypeptide was originally isolated from the ovine hypothalamus (102) and was found to inhibit somatotropin (growth hormone) release. It thus became known as somatostatin or SRIF (Somatotropin Release Inhibiting Factor), although it also acts upon other pituitary hormones (103). Somatostatin became of interest to islet physiologists when it was observed to induce hypoglycemia in vivo (104). Further investigation has proven that this hormone is a potent inhibitor of both insulin and glucagon secretion (105–110). The islet hormone responses to somatostatin administration are clearly unique when compared to those of the agents previously discussed. First of all, somatostatin is the only naturally occurring hormone known to inhibit the secretion of both islet hormones simultaneously. Further, at appropriate doses, somatostatin obliterates insulin and glucagon release to all known secretagogues and reduces the basal rates of islet hormone secretion to nearly undetectable levels in

the plasma. Because of these properties, somatostatin has recently been employed as an experimental tool in many phases of pancreatic islet research.

We have observed that this hormone inhibits basal and glucose-stimulated insulin secretion in the perfused dog pancreas in vivo (82, 105). At a dose of 1.7 μg/min, somatostatin inhibited insulin secretion when infused into the pancreatic artery, portal vein, or femoral vein. Surprisingly, when somatostatin was infused into the pancreatic artery at one tenth of this dose, there was no reliable inhibition of either insulin or glucagon, even though this particular dose was calculated to be approximately twice that reaching the pancreatic artery when a dose of 1.7 μg/min was infused into either the portal or femoral vein. We have therefore postulated that somatostatin may act indirectly. To test this theory, we have compared the effects of somatostatin upon insulin secretion during an infusion of either glucose or phentolamine (111). When somatostatin was infused alone (via the femoral vein) at a rate of 1.7 μg/min, the levels of both plasma insulin and glucose fell and remained depressed over a 30 minute interval. This same dose of somatostatin also inhibited the insulin secretion stimulated by glucose infusions ranging from 1 to 6 mg/kg per min. However, with this same protocol, the elevated levels of plasma insulin observed during a phentolamine infusion were not inhibited by somatostatin. During these experiments the phentolamine and glucose infusions increased basal insulin output to comparable steady state levels and produced no significant changes of pancreatic blood flow. Since the steady state insulin output was inhibited by somatostatin during the glucose infusions but not in the presence of the α-blocker, we conclude that phentolamine was responsible for the lack of somatostatin-mediated inhibition of insulin release. This implicates the α-receptor as a site of interaction between somatostatin and the sympathetic nervous system. In contrast with our findings, Efendic & Luft (112) reported that α-blockade had no effect on the ability of somatostatin to inhibit insulin secretion in humans. Some investigators have found that the in vitro inhibition of insulin secretion by somatostatin is reversed by elevating the levels of extracellular calcium (113, 114). Since calcium uptake by the B cell has been implicated as a trigger for insulin release (115), it is possible that a somatostatin-α-receptor interaction would elicit an alteration of calcium flux leading to an inhibition of insulin secretion.

The recent immunocytochemical localization of somatostatin in the pancreatic islet D cells (10, 11) suggests the possibility that this hormone plays a role in the normal and pathophysiologic regulation of islet hormone secretion. This finding also lends support to the hypothesis that the pancreatic islet cells are derived from the neuroectoderm, since it seems unlikely that cells producing somatostatin in the hypothalamus would be of different embryonic origin from those containing the same hormone in the pancreatic islets.

INTERACTING SYSTEMS IN ISLET FUNCTION: A SUMMARY

The complex nature of insulin and glucagon secretion makes an integrated discussion of the role of amines and other neural factors in the regulation of islet hormone secretion quite speculative at present. Nevertheless, the majority of papers cited in

this review can be placed into a coherent framework. The authors offer in Figure 1 a schematic diagram of the potential interactions that have been covered.

We have concentrated on the B cell and have followed the suggestions of Lacy (8, 116) for the fundamental synthesis and storage of insulin. That is, insulin is synthesized in the endoplasmic reticulum and transported to the Golgi complex where it is packaged into granules. These granules are subsequently stored in the cytoplasm and are available for release when the appropriate signal is provided. Insulin is believed to be secreted via two different mechanisms. Some investigators (see 117, 118) have observed that insulin is released simply by the intracytoplasmic breakdown of the secretory granules and the diffusion of the hormone through the plasma membrane of the B cell. However, others (8, 116, 119, 120) have found that the granules are pulled toward the membrane of the B cell by an interaction with the microtubules and microfilaments within the cytoplasm. The membrane of the secretory granules then fuses with the plasma membrane and the insulin granules are thus released into the extracellular space via emiocytosis. Recent studies have shown that during emiocytotic release, the membranes of several granules fuse with one another in a process called *vesicular binesis* (121); thus a single channel can serve as a focal release point for many secretory granules. This fusion of secretory

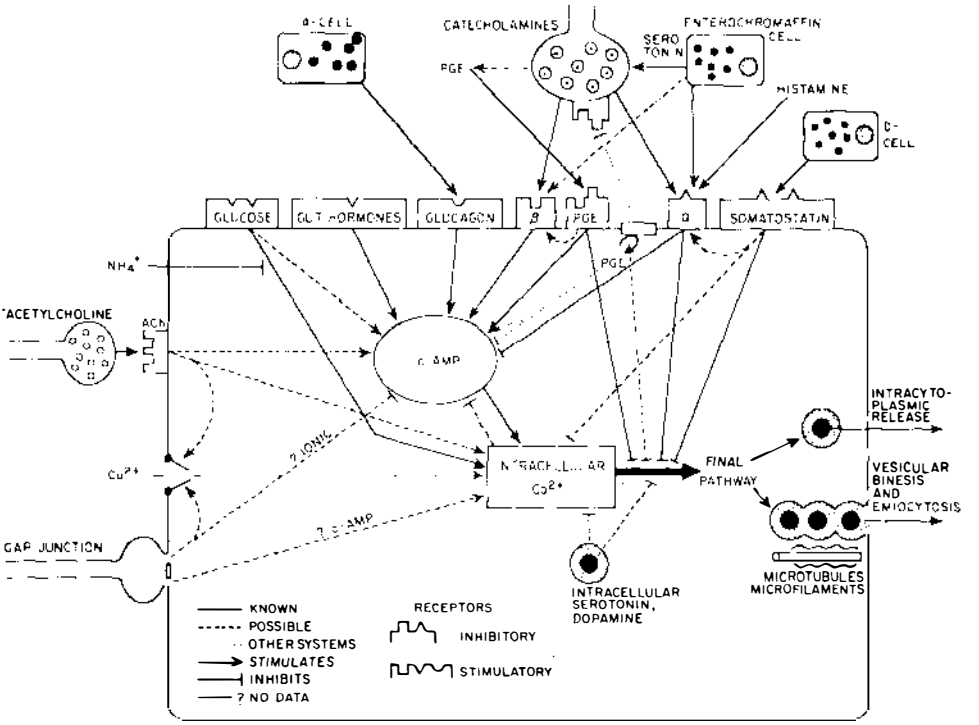

Figure 1 A hypothetical model of interacting systems in insulin secretion.

granules has also been observed during the intracytoplasmic dissolution of the B-cell granules (23, 118) indicating that it may play a wider role in insulin secretion.

Intracellular calcium plays a major regulatory role in the process leading to the triggering of insulin secretion. The availability of intracellular Ca^{2+} is primarily regulated by the concentration of substrates in the blood (particularly glucose and amino acids). Glucagon and gut hormones in conjunction with locally released neural factors may also regulate Ca^{2+} flux of the B cell. Intracellular amines also influence insulin release, and some interaction between them and intracellular calcium seems likely.

One possible interaction is that intracellular amines and Ca^{2+} compete for the same negatively charged ligands within the B-cell granules (122, 123; I. Lundquist, personal communication). This is separate from the regulatory role of extracellular amines, since some blocking agents that effectively alter insulin responses to extracellular serotonin and dopamine are ineffective when the intracellular concentration of these same amines has been elevated.

Extracellular amines, somatostatin, and possibly prostaglandin influence insulin secretion by interactions with cell surface receptors. Although some agents are purely stimulatory (e.g. acetylcholine) and others are purely inhibitory (e.g. somatostatin), the catecholamines, indoleamines, and prostaglandins have been found to be both stimulators and inhibitors. All of these compounds bind to cell surface receptors that in turn interact with the basic secretory processes of the cell to regulate insulin secretion. Some of them bind to more than one receptor. The net effect upon insulin secretion will depend on the receptor stimulated, the pathway regulated, and the endogenous activity of the cell. All of this must be viewed in light of recent evidence (124, 125) indicating that the number of cell surface receptors is not constant, as was previously thought, but varies from time to time as a function of the agonist concentration and the metabolic state of the B cell. Therefore, it is not surprising that different results have been reported for many of the pharmacologic agents used to study insulin secretion. The reader must be wary when a compound is reported to be either stimulatory or inhibitory upon insulin secretion unless a wide range of drug concentrations has been studied over a variety of conditions. Further, there are compounds that interact both with cell surface receptors and with the basic cellular machinery of the islet. Glucose is one such example. Since the net product of B-cell function is insulin secretion, any compound that alters intracellular glycolytic rates, protein synthetic rates, or oxidative metabolism may be expected to change insulin secretion.

Despite these complexities, one can make the following generalizations: 1. Acetylcholine is representative of neurally related compounds that stimulate both insulin and glucagon secretion, have no inhibitory properties, and do not appear to interact with islet metabolism. 2. Somatostatin is representative of a class of compounds that inhibits insulin and glucagon release and neither alters islet cell metabolism nor elicits any stimulatory effect. 3. The other neurally related compounds that influence insulin secretion (indoleamines, catecholamines, and prostaglandins) possess the ability to regulate stimulatory and inhibitory pathways of insulin release simultaneously. Stimulation of insulin secretion by these latter agents appears to involve the activation of adenylcyclase. It is conceivable, therefore, that all of them interact

either directly with the β-adrenergic receptor or alter its sensitivity, thereby changing intracellular cyclic AMP levels. Indoleamines and catecholamines inhibit insulin release via activation of the α-adrenergic receptors and primarily affect glucose-stimulated insulin release. Prostaglandins also inhibit glucose-induced insulin secretion, but do so by a mechanism not requiring adrenergic receptor activation. Somatostatin inhibits insulin and glucagon secretion regardless of the stimulus; but even with this agent there are complex interactions because α-adrenergic blockers modulate the ability of somatostatin to inhibit insulin release. While it was originally hypothesized that α-adrenergic inhibition of insulin secretion is due to reduced adenylcyclase activity and a subsequent decrease of cyclic AMP formation, this explanation is now quite unlikely. This is due to the fact that even though α-adrenergic stimulation lowers adenylcyclase activity, insulin release cannot be stimulated by exogenous cyclic AMP in the presence of epinephrine. Whether cyclic GMP will eventually become identified as an inhibitory second messenger for insulin and glucagon secretion, or whether basic electrolyte transport processes involving calcium flux will be shown to be related to α-receptor activation is not clear at the present time.

Interactions between neuropharmacologically active agents occur because so many of them are present within the islet (i.e. acetylcholine and the catecholamines are found within nerve endings, somatostatin and serotonin are stored in islet cells, and prostaglandins are presumably synthesized by the islet cells). The release of autonomic transmitters is known to be sensitive to the concentration of prostaglandins, and transmitter release is also altered by exogenous serotonin and circulating catecholamines. The stimulus for the release of serotonin stored in enterochromaffin cells of the islet is unknown at present. Nevertheless, a complete picture of islet function must consider the possibility that intra-islet serotonin plays a role in the regulation of insulin and glucagon secretion. Somatostatin present in islet D cells may also be expected to regulate insulin and glucagon release, just as intra-islet glucagon alters insulin secretion. Finally, the purinergic nervous system and gap junctions should be mentioned. Purinergic nerves have been observed in the islets of teleosts and are known to exist in the smooth muscles of mammals. This portion of the autonomic nervous system is believed to be an inhibitory system whose transmitter is ATP. The effects of ATP release upon serotonin, somatostatin, catecholamines, acetylcholine, or islet cells themselves are unknown, but the finding of such nerves in one mammalian system suggests their possible presence in others. The physiologic role of gap junctions observed between islet cells and between nerves and islets cells is also unknown at present. However, it is conceivable that such junctions serve to transmit and amplify stimulatory or inhibitory messages throughout the islet parenchyma.

In conclusion, the islet should be looked upon as a metabolic computer that is capable of adjusting the output of insulin and glucagon according to direct substrate levels and that is modulated by a wide variety of hormonal and neural messages. These hormonal and neural signals often arise within the islet and in some cases are directly connected to the central nervous system. This system of hormonal and neural controls provide for both short- and long-term regulation of metabolic processes.

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