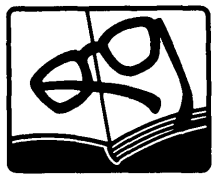
Review



The Role of Gastrointestinal and Neuronal Peptides in the Pathophysiology of Diabetes Mellitus

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The pancreatic islet hormone secretion is modulated by one or more gastrointestinal peptides ("gut-factor") secreted in response to various types of ingested nutrients. Among a number of postulated candidates for the putative "gut-factor", the gastric inhibitory polypeptide (GIP) has recently emerged as a most likely enteric signal of physiologic import, although its precise role in the pathophysiology of diabetes mellitus remains incompletely understood. During the past decade, an avalanche of knowledge has accumulated regarding a number of peptide agents common to the gastro-enteric-pancreatic system and the nervous system. Preliminary evidence indicates a potential role of several of these peptides in the pathophysiology of diabetes. For instance, cholecystokinin and human pancreatic polypeptide (hPP) may be importantly involved in the regulation of appetite and satiety control and the development of obesity whereas somatostatin, "endorphins", and neurotensin may directly or indirectly modulate islet hormone secretion. Finally the significance of the recently demonstrated presence of insulin and glucagon or glicentin-like peptides in the brain requires close scrutiny. DIABETES CARE 4: 435–442, MAY–JUNE 1981.

The presence of a variety of discrete endocrine cells in the gastrointestinal tract has been known for many years. Recently, much work has been done in an effort to understand the intimate endocrine kinship between the gastrointestinal tract and the pancreatic islets. During the past few years, an exciting array of investigations has suggested another intricate but seemingly obligatory relationship between the gastro-enteropancreatic system, on the one hand, and the central and peripheral nervous system on the other. A number of different hormonal peptides have been shown to be present, in common, at various diverse locations in the nervous system, gastrointestinal tract, and endocrine pancreas (Table 1). While the precise significance of this finding in the human physiology and its relevance to abnormal metabolic states are presently far from clear, a number of provocative studies are currently in progress.

This review will deal with (1) our current state of knowledge of the "gut-factors" in the pathophysiology of islet secretion and (2) the potential significance of some of the various peptides common to brain and gut (including pancreas), particularly as they might relate to the regulation of human metabolism and to the pathogenesis of diabetes.

THE ELUSIVE "GUT FACTOR"

A number of years ago it was reported that the magnitude of insulin release following an oral glucose load is much greater than that following an intravenous glucose infusion, despite achieving comparable blood glucose levels.[1,2] Furthermore, it is well documented that the ingestion of a mixed meal results in a diminished rise in blood glucose, when compared with that after carbohydrate ingestion alone, in normal subjects as well as in those with mild diabeties.[3–5] During the past decade, a number of interesting observations have been made in an effort to understand the nature and properties of the putative "gut factor(s)" (or hormones) mediating the facilitated insulin secretion and/or action in response to various types of ingested substances.[6–9] It has become increasingly clear that a variety of hormones released from the gastrointestinal tract during the digestive phase serve not only in the digestive function and exocrine secretion but, in addition, regulate the release of islet hormones, primarily insulin. The relative importance of each of the putative gut hormones of the so-called gastro-entero-pancreatic axis (Table 1) is still incompletely understood. However, it appears that more than one hormone may be involved, de-

TABLE 1
Nomenclature of the gastro-entero-pancreatic system

Cell*	Product	Presence in Pancreas	G.I. tract	Nervous system
B	Insulin	+		+
A	Glucagon	+	+	+†
D	Somatostatin	+	+	+
D_1	V.I.P.-like	+	+	+
PP	Pancreatic Polypeptide	+	+†	—
EC	Serotonin, substance P ?Motilin	+	+	+
P	? Bombesin	+†	+	+
G	Gastrin	+†	+	+
S	Secretin	—	+	—
I	Cholecystokinin	—	+	+
K	GIP		+	—
N	Neurotensin	+†	+	+
L	Enteroglucagon-glicentin	—	+	+†
?	Enkephalin	+	+	+
?	Corticotropin (ACTH)-like	+	+	+
X	Unknown	—	+	—

* In pancreas or G.I. tract.
† Not detected in man.
Adapted and updated from 1977 Lausanne Classification[6] and R. Guillemin.[41]

pending on the nature of the ingested nutrient, besides other factors. For example, gastric inhibitory polypeptide (GIP) appears to be a major candidate involved after glucose or lipid ingestion (Table 2). On the other hand, the release of a different gut hormone, e.g., cholecystokinin, may be more important following protein ingestion.[9,10] The functional overlap between groups of gut hormones may be partly explained by a common phylogenetic origin and consequent chemical structural similarities (e.g., between GIP, secretin, vasoactive intestinal polypeptide (VIP), and glucagon, and between gastrin and cholecystokinin).

It has been postulated that one or more of the gut factors may also be responsible for certain extra-pancreatic effects, e.g., in mediation of insulin action on the liver in facilitating glucose uptake[11] or in modulating the peripheral effects of insulin on lipolysis.[7]

TABLE 2
Gut factors released by enteric signals

	Glucose	Amino acids	Fat
GIP	+	+	+
CCK	0	+	+
Gastrin	0	?	0
Secretin	0	?	0
VIP	0	0	?
Glicentin	0	?	?

+ = release; 0 = no effect; ? = uncertain. See text for details. Adapted from ref. 9.

Secretin. Speculations regarding the possible influence of secretin on islet hormone secretion have been made ever since its discovery in 1902. It is a 27-amino acid polypeptide, produced by the S-cells of the upper small intestine. Studies in the past employed impure preparations and pharmacologic amounts of secretin, leading to the suggestion of an insulinotropic effect. Recent studies employing pure preparations and sensitive radioimmunoassays, however, have revealed no increment of insulin release following either intravenous secretin infusion or intraduodenal acid infusion, a well-known secretagogue for endogeneous secretin release.[12,13] Oral glucose has been shown not to result in secretin release.[14] However, Lerner has reported that in non-insulin-dependent diabetic patients with diminished or absent acute insulin response to i.v. glucose, secretin infusion results in a significant insulin output and augments the insulin response to an intravenous glucose infusion.[15] The levels of secretion in plasma were not measured in Lerner's studies and may well have been much higher than those seen in physiologic circumstances in view of the dose of secretin used. However, it must be pointed out that other investigators have reported elevated levels of plasma secretin in patients with untreated, non-insulin-dependent diabetes and a positive correlation of secretin levels with fasting blood glucose levels.[16] Whether there is indeed a feedback regulation between insulin secretion and secretin release in diabetes is not known. Thus, based on the available evidence, a physiologic role for secretin in islet regulation can not be established.

Cholecystokinin. A 33-amino acid polypeptide cholecystokinin (CCK), like secretin, has not turned out to be an important mediator of endocrine pancreas secretion. It is secreted in response to protein ingestion and fat, but not carbohydrate, and was initially thought to represent an important gut signal for protein or amino acid-induced betacytotropic effect. However, since most of the CCK preparations have been found to contain GIP (see below), the role of CCK per se remains dubious.[6,9] A limiting factor in better understanding the role of CCK has been the lack of a reliable radioimmunoassay for this hormone until very recently. Regardless of the possible effects of CCK on insulin secretion, an interesting observation made by several investigators concerns its possible effects on appetite regulation and the control of satiety. Recently, experiments in several animal species including the rhesus monkey have suggested a decrease in food intake by i.v. or i.p. injections of CCK or the synthetic C-terminal octapeptide of CCK, the predominant form of CCK in brain.[17–19] These observations are similar to those made with intraventricular injections of caerulin, a decapeptide which shares structural homology with CCK. In addition, a markedly reduced content of immunoreactive CCK in brain extracts from hyperphagic, genetically obese mice, compared with their non-obese littermates or normal mice, has been demonstrated, by some workers,[20] but not others.[21] The mechanism of the regulation of satiety by the CCK or CCK-like peptides released from the gut is a subject of current investigation. However,

the presence of a peripheral "satiety receptor" in the gut or liver has been postulated on the basis of the evidence that satiety is elicited by a much smaller dose of CCK when administered peripherally rather than intraventricularly.[19]

Gastrin. Gastrin is another important gut hormone from the metabolic point of view in as much as it shares several amino acid residues with CCK. Like CCK, it has received considerable attention as a likely signal for oral amino acid-induced beta-cytotropic effect.[22] However, the effects seen with gastrin are often relatively modest and poorly sustained unless pharmacologic doses are employed.[6] Patients with tumors producing massive amounts of gastrin and associated Zollinger-Ellison syndrome do not reveal consistent changes in islet secretion or glucose homeostasis.

Gastric inhibitory polypeptide. Gastric inhibitory polypeptide (GIP) is currently thought to be the most important but not the exclusive gut hormone responsible for mediating the entero-insular effects following ingestion of various kinds of nutrients. It is a 43-amino acid polypeptide which was originally isolated from crude preparations of cholecystokinin-pancreozymin. During the past several years, extensive studies have been carried out with this compound and reviewed elsewhere by Brown and co-workers.[23,24] The source of GIP in various species, including man, has been localized to the well-defined K-cells of the upper small intestine.[6] The development of a specific radioimmunoassay has enabled a spate of studies pertaining to the regulation of GIP secretion in healthy as well as in diabetic subjects.

GIP has been clearly shown to be insulinotropic in normal man in several studies.[24] This effect depends on the prevailing blood glucose concentrations with a threshold glucose level of 5.5 mM (100 mg/dl). GIP is released in response to oral glucose, lipid (triglyceride), as well as protein (amino acids) ingestion. The insulinotropic effect in normal subjects is magnified by the induction of hyperglycemia. Glucose-induced increase in GIP is inhibited by acetylcholine and isoproterenol as well as epinephrine in dogs,[25] suggesting a modulation by the autonomic nervous system. In diabetic individuals with autonomic neuropathy, the GIP response to a mixed-meal ingestion was impaired, but gastric emptying time was not studied.[26] In patients with reactive hypoglycemia following vagotomy, an exaggerated early GIP and insulin response has been observed, suggesting a possible role of neural factors in mediating the "late phase" of the dumping syndrome.[27] In the presence of low glucose concentrations, GIP has also been shown to be "glucagonotropic" in isolated perfused pancreas preparations.[28]

A number of reports have shown abnormalities of GIP secretion in patients with obesity and/or non-insulin-dependent diabetes.[28–33] While the fasting GIP levels have been shown to be normal in nonobese diabetic subjects, the post-glucose levels are exaggerated in the presence of relatively diminished insulin response, suggesting a relative insensitivity of B-cells to GIP.[29,30] Moreover, a paradoxical increase in glucagon levels was seen in such patients, commensurate with the peak GIP response,[30] raising the possibility of a stimulation of the islet A-cells by GIP, in contrast to normals. The triglyceride-induced GIP response was interestingly found to be normal in a group of non-insulin-dependent diabetic subjects.[34]

A feedback control of GIP by insulin in normal subjects is suggested by the blunted increment of GIP levels in response to oral fat by the stimultaneous infusion of glucose.[24] Studies of Ebert et al.[33] and Willms et al.[32] have dealt with the impaired feedback control of GIP secretion in obesity. When compared with the responses in normal-weight controls, these investigators reported an exaggerated secretion of GIP in obese subjects following oral glucose, mixed meal, or fat ingestion, and an incomplete suppression following an intravenous glucose infusion administered after an oral fat challenge.[33] However, the abnormalities were restored toward normal following reduced caloric intake or starvation.[32] However, in one study on insulin-dependent, severe diabetic subjects (mean fasting blood glucose = 262 mg/dl), the secretion of GIP in response to oral glucose was found to be significantly impaired and there was little evidence of suppression of GIP by exogenous insulin infusion in both diabetic subjects as well as in normal controls.[35] The question of feedback control by GIP by insulin, therefore, remains unsettled.

In addition to the effects of GIP on the pancreas, indirect evidence indicates that GIP may have certain peripheral effects. In preliminary studies, GIP was found to inhibit glucagon binding and cyclic AMP generation in rat adipocytes.[36] Recently, an anti-lipolytic effect of GIP has been further suggested by a stimulation of lipoprotein lipase activity in cultured mouse preadipocytes.[37] Additional studies are required to explore the possibility of a role for GIP in the adipose tissue metabolism and triglyceride clearance as well as in the postulated hepatic effects of an enteric signal.[11]

Finally, it should be emphasized that GIP is probably not capable of eliciting all the effects ascribed to a putative gut hormone even though evidence for a significant role for several other gut hormones (gastrin, CCK, secretin, VIP glucagon-glicentin) studied thus far is lacking. The insulinotropic effect of "gut factor" is reduced, but not abolished, despite complete neutralization of GIP by GIP antiserum.[9] Similarly, in a recent study, we observed an enhancement of i.v. glucose disposal, when preceded by oral ingestion of certain non-glucose hexoses such as mannose and fructose, in the absence of a significant release of GIP.[38]

PEPTIDES COMMON TO BRAIN, GUT, AND ISLETS

Claude Bernard performed his classic studies of piqûre diabetes more than a century ago.[39] Since that demonstration, speculations have been made of the possible link between a neuronal lesion and consequent metabolic defect leading to the development of at least some instances of diabetes. A precedent for the existence of a peptide in such diverse locations as the central nervous system and the gastroinestinal tract was first established in the case of substance P in the report of von Euler and Gaddum in 1931, a finding which remained dormant for a number of years.[40] This 11-amino

acid peptide, originally described to have hypotensive and smooth-muscle-stimulating properties, was subsequently claimed to be primarily involved with sensory neurotransmission. It was recently shown to inhibit insulin release from the pancreas.[40] The demonstration of an increasing number of peptides in these anatomical locations and the variety of their potential biologic effects in the intact organism have opened new vistas in the field of neuroendocrinology within the past few years. Perhaps most importantly, these peptides serve a neurotransmitter function in the nervous system by specific "peptidergic" pathways, whereas a "paracrine" function is subserved in relation to various endocrine secretory cells in the gut and pancreas.[41–44] These may indeed be more important functions of such peptides in contrast to a less important "hormonal" role.

The following discussion would focus on the present state of our limited but rapidly expanding knowledge regarding the role of various peptides in the regulation of islet secretion and perhaps more importantly in the regulation of food intake and development of obesity, germane to the pathogenesis of non-insulin-dependent (type II) diabetes. A role of CCK was discussed above (see under cholecystokinin).

Somatostatin. Somatostatin, a tetradecapeptide, was isolated and synthesized after many years of extensive search for a hypothalamic factor(s) regulating pituitary growth hormone secretion.[45] Soon after its discovery, the observation of the potent inhibitory effects of somatostatin on endocrine pancreas was confirmed in normal and in diabetic man by several investigators and reviewed elsewhere.[46–48] A burgeoning interest in the possible role of somatostatin in glucose homeostasis followed the discovery that this peptide is widely distributed not only in the nervous system but also in the D-cells of the gastrointestinal tract and pancreatic islets.[47,49–52]

Recent attention has focused on the possibility of somatostatin-producing cells being goverened by a "paracrine" regulation.[48,53] Morphologic studies have shown a characteristic distribution of various types of endocrine cells within the islets and anatomic junctional complexes between adjacent islets cells.[53] Moreover, the somatostatin-containing cells in gastric antrum have been shown to have long, nonluminal processes which terminate on gastrin-producing G-cells and on the other effector cells.[54] Such observations support the hypothesis[48,53] that certain widely distributed "hormones" such as somatostatin might more importantly exert their effects via a "paracrine" effect on the neighboring cells within an organ than via the "endocrine effects," thus obviating the need for release into the circulation and transport to other organs as prerequisites for the physiologic role of such peptides.

Morphometric and biochemical studies of endocrine cells reveal an increase in the number of D-cells as well as somatostatin concentration in the B-cell-deficient (insulinopenic) animals of various species, regardless of whether the diabetes is spontaneous or toxin-induced.[55–59] Conversely, a decrease in the islet D-cell population accompanied by a diminished somatostatin content have been reported in the genetically obese mice.[60–62] The pathogenesis of the syndrome of hyperphagia and obesity has been shown to be due to a hypothalamic defect genetically distinct from that underlying insulin-dependent diabetes.[63] This leads to the speculation of a primary abnormality of somatostatin-secreting cells in the hypothalamus. However, this seems unlikely in view of the recent demonstration of a lack of change in the hypothalamic somatostatin concentration in genetically obese mice,[62] although others have reported an increased concentration in the presence of a diminished islet somatostatin concentration.[60] An inverse relationship between the islet somatostatin content and body weight has been reported in rats made obese by lesions of ventromedial hypothalamus.[64] Further studies are required to elucidate a possible link between alterations in the brain somatostatin and development of obesity, although available evidence does not support this possibility.

Human pancreatic polypeptide. Human pancreatic polypeptide (hPP) is a strand of 36 amino acids localized to a discrete cell type (PP) in the human endocrine pancreas, distinct from A-, B-, and D-cells.[65] In contrast to insulin and glucagon, which are detected in higher concentrations in the body and tail of pancreas, hPP is largely found in the regions of the uncinate process and the head of the pancreas,[66] suggesting a different mode of its embryogenesis. (Some PP-cells are scattered throughout the exocrine pancreas as well.) In contrast to several other gut hormones, no significant concentrations of hPP have thus far been detected in the brain.

The role of hPP in human physiology is at present poorly understood.[67] Its secretion is stimulated by practically all nutrients and most markedly after protein ingestion. Hypoglycemia has been shown to be a potent stimulus for hPP release, probably a vagal effect. A number of cholinergic agents stimulate and atropine inhibits hPP relase.[68] Circulating levels of hPP progressively increase with age and renal failure.

Studies of Floyd et al.[67,68] have revealed elevated plasma levels of hPP in insulin-treated diabetic subjects (both insulin-dependent and non-insulin-dependent types) but not in mild, non-insulin-treated diabetic subjects. It was further suggested from their studies that the increased hPP levels may correlate with the degree of severity of diabetes and that the levels may return toward normal range by improved control. In this regard, however, it should be noted that significant levels of circulating antibody to PP have been shown in the majority of patients treated with insulin thus far due to the contaminant PP in various insulin preparations. The assay of PP in such patients, therefore, may be somewhat unreliable because of methodologic problems. However, studies in a spontaneously diabetic mutant mice strain (C57 BL/6ks) have documented an increase in the islet PP-cell population in the presence of severe B-cell deficiency.[61]

An effect of hPP in the regulation of satiety and appetite control has been suggested by the reports of a diminished hPP response to meals in obese subject.[69,70] These results are in keeping with the earlier reports of a diminished number of

PP-secreting cells and low plasma PP levels in genetically obese mice.[61] However, the significance of the observed alterations in islet morphology and hPP secretion in obesity as well as in diabetes, as in the case of somatosatatin, remains uncertain.

Enkephalins and endorphins. The fascinating story of the "endorphin" family of peptides began during the past decade with the demonstration of receptors for opium alkaloids in brain.[71,72] This was soon followed by two fundamental observations. One was the isolation and characterization of two pentapeptides, Leu-enkephalin and Met-enkephalin,[73] and the other was the discovery of several polypeptide endorphins from the porcine brain.[74] Each of these substances avidly binds to the opiate receptors. The entire sequences of Met-enkephalin and the endorphins (including the most potent β-endorphin) is contained in the 91-amino acid polypeptide, β-lipotropin, which had been earlier isolated from pituitary.[75] However, recent work has revealed that even β-lipotropin is derived from a much larger, mol. wt. 31,000, glycoprotein molecule (pro-opiocortin), which also contains the sequence for ACTH and α-MSH.[76]

The clinical significance of enkephalins and endorphins is presently controversial and under intensive scrutiny.[41,44,77] There seems to be growing consensus that these substances are intimately involved in certain neuropsychiatric processes including the phenomena of pain perception, drug addiction, and emotional behavior. Furthermore, several interesting reports have recently drawn attention to the possible role of the members of the "endorphin" family in metabolic regulation.

In genetically obese mice and rats, a two- to threefold elevated concentration of β-endorphin was demonstrated in pituitary and in plasma.[78] This was associated with a 14-fold elevation of pituitary ACTH content in the obese mice, which is in keeping with the observation of a common precursor for β-endorphin and ACTH and with their known concomitant release from the pituitary.[79] Parenthetically, it is of interest that insulin-induced hypoglycemia has been shown to result in a marked increase in the plasma levels of β-lipotropin and β-endorphin, as well as ACTH.[80] Furthermore, the stress-induced hyperphagia in a rat model is inhibited by the administration of nalaxone, a specific antagonist to opiates.[81]

Polak et al.[82] have shown a significant content of enkephalin-like immunoreactivity in human gastric antrum, upper small intenstine, and pancreas. In experiments employing an isolated, perfused canine pancreas, studies in Unger's laboratory have shown a prompt inhibition of somatostatin release by morphine or β-endorphin, followed by a release of insulin and glucagon.[83] These effects were antagonized by the addition of naloxone to the perfusate. Whether a similar effect of opiate alkaloids, presumably via a specific receptor mechanism, plays a role in the regulation of the human endocrine pancreas is not known. Moreover, somewhat contradictory results were observed in a rat islet cell culture system, wherein the addition of enkephalins was shown to inhibit the secretions of insulin and glucagon, while morphine did enhance the release of both hormones.[84] Stubbs et al.[85] have studied the effects of i.v. administration of a long-acting enkephalin analogue [D-Ala^2, MePhe^4, Met (o)-ol] (DAMME), in normal men. There was a significant rise in levels of growth hormone and prolactin, and a significant fall in FSH, LH, ACTH, and cortisol; no significant effects were observed on the levels of gastrin, VIP, insulin, and glucagon. The latter findings are at variance with the results in isolated perfused pancreas and the islet cell culture system described above.

Recently, a phenomenon of chlorpropamide alcohol-induced facial flushing has been proposed as a genetic marker for non-insulin-dependent (type II) diabetes.[86] Leslie and Pyke[87] showed that this phenomenon can be reproduced by the enkephalin analogue, DAMME, and blocked by prior administration of naloxone. Based on this evidence, it has been suggested that a subgroup of non-insulin-dependent diabetes associated with chlorpropamide-alcohol flush phenomenon may be secondary to an increased sensitivity to endogenous opiates. Perhaps this could be a plausible explanation of the piqûre diabetes?[39]

These various studies underscore the need for further work in exploring the significance of opiate receptors and endogenous enkephalin-like substances in human gut and pancreas.

Neurotensin. Neurotensin is a 13-amino acid peptide, isolated and characterized by Carraway and Leeman.[88] It has been localized in the N-cells of the human intenstine and is widely distributed in the brain. The receptors for neurotensin are distinct from those for endorphins; its actions are not blocked by naloxone. Its properties are somewhat similar to substance P in causing vasodilatation, hypotension, and increased vascular permeability. Intravenous infusion of neurotensin in healthy subjects leads to a significant decrease of gastric acid and pepsin output as well as in a delayed gastric emptying.[89]

Administration of neurotensin in experimental studies has been shown to have metabolic effects, e.g., hyperglycemia, hyperglucagonemia, and variable effects on insulin secretion, the latter depending on the experimental animal.[90] The effects of neurotensin are blocked by somatostatin in the dog.[90] Immunoreactive neurotensin has been detected in the normal pancreas in several animal species and preliminary data suggest an increased content in the pancreas on insulinopenic C57 BL/KsJ mutant mice.[91]

CONCLUDING REMARKS

The number of potentially important agents belonging to the gastro-enteropancreatic system and the nervous system continues to grow. The increasing wealth of information in this area will certainly help bridge the gaps in our understanding of the pathophysiology of diabetes, although much further work is needed. From this point of view, the recent demonstrations of striking concentrations of insulin[92,93] and glucagon or glu-

cagon-like peptides, e.g., glycentin,[44,94] in the brain are of exceeding interest, although not confirmed by all workers.[95] Whether these polypeptides are transported across the blood-brain barrier or synthesized de novo is unclear, although some evidence suggests the latter possibility.[92,93] The potential role of insulin and of insulin receptors in the brain, as related to the pathophysiology of human diabetic syndrome and, perhaps even more importantly, in the genesis of human obesity, are tantalizing issues, particularly when one recalls the previous suggestions of insulin-responsive sites in the central nervous system.[96,97]

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