Emerging Therapies Mimicking the Effects of Amylin and Glucagon-Like Peptide 1

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Current therapies for type 2 diabetes are frequently associated with inadequate control of postprandial hyperglycemia, weight gain, and, in the case of oral agents, loss of efficacy over time. A better understanding of physiological responses to meals is leading to the development of new agents whose therapeutic action is based on the enhancement of gastrointestinal hormone action. These therapies are associated with slowing of gastric emptying, stimulation of insulin and inhibition of glucagon secretion, improved control of postprandial hyperglycemia, and control of body weight. This review summarizes several limitations in the treatment of type 2 diabetes and describes the mechanisms of action and clinical data obtained with amylin and glucagon-like peptide 1 (GLP-1) receptor agonists and dipeptidyl peptidase IV (DPP-IV) inhibitors for the treatment of diabetes.

Despite considerable effort by patients and physicians, the results of treating type 2 diabetes are often disappointing. This review examines the limitations of current antihyperglycemic therapies and assesses the potential of the emerging class of agents that mimic or enhance the actions of amylin and GLP-1, which are both gastrointestinal peptide hormones that in concert with insulin and glucagon regulate fuel homeostasis and eating behavior (1–4). Several agents from this class have been recently approved for clinical use or are in the advanced stages of development. Their mechanisms of action and therapeutic effects, as described in peer-reviewed publications, will be discussed.

BARRIERS TO NORMALIZING GLYCEMIA

The evidence-based target for glycemic control is an HbA_{1c} (A1C) ≤7% (5), although several groups have proposed an A1C target of 6.5% based on epidemiological analyses (6,7). In clinical trials, patients have achieved A1C values near 7% using basal-bolus insulin treatment in type 1 (8) or type 2 (9) diabetes or combinations of oral treatments and insulin for type 2 diabetes (10). However, routinely maintaining glycemic control at <7% remains an elusive goal in medical practice (11,12).

Several limitations of currently available antihyperglycemic medications are important barriers to diabetes management, and these will be discussed in detail in this review. Economic factors and limited access to providers experienced in managing diabetes are also important barriers, but the consideration of these is beyond the scope of this review.

Postprandial hyperglycemia

In the trials cited above (8–10), good control of fasting and between-meal glucose levels was achieved, but postprandial glucose values remained high. Several agents have been developed specifically to reduce hyperglycemia after meals. α-Glucosidase inhibitors reduce postprandial hyperglycemia by limiting the digestion of complex carbohydrates in the upper small intestine, leading to delayed absorption from the distal small intestine (13,14). Use of these agents is associated with increased secretion of GLP-1, which may contribute to their therapeutic effects (15,16). The nonsulfonylurea secretagogues repaglinide and nateglinide provoke rapid secretion of endogenous insulin with meals (17,18). However, neither of these agents consistently eliminates postprandial glycemic increments. For example, Fig. 1 shows the patterns of plasma glucose and insulin during treatment with placebo, nateglinide, or a sustained-release form of the sulfonylurea glipizide after overnight normalization of fasting glucose by a basal insulin infusion (19,20). Nateglinide increased the postprandial insulin responses but did not completely control postprandial increments in glucose excursion. Even systematic use of rapid-acting analogs of insulin has yielded mixed results, with complete prevention of postprandial hyperglycemia rarely being achieved (21). In general, studies show that these agents reduce increments of glucose after meals by ≤50%. Residual postprandial hyperglycemia contributes to the abnormal glycemic exposure of tissues and limits efforts to reduce A1C from ~7% to the normal 4–6% range (22). Furthermore, both epidemiological evidence and physiological findings have led to the hypothesis that increments of glucose after meals may have harmful metabolic effects beyond simply contributing to glycosylation of proteins and may increase the risk of cardiovascular events (23). Interventional trials testing whether targeting postprandial hyperglycemia is effective in reducing medical outcomes will probably require methods of treatment that are more effective than those currently available.

Hypoglycemia

Hypoglycemia may occur during treatment with any insulin or oral secretagogue, especially when glycemic control

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Abbreviations: CNS, central nervous system; DPP-IV, dipeptidyl peptidase IV; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; RAMP, receptor activity–modifying protein.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

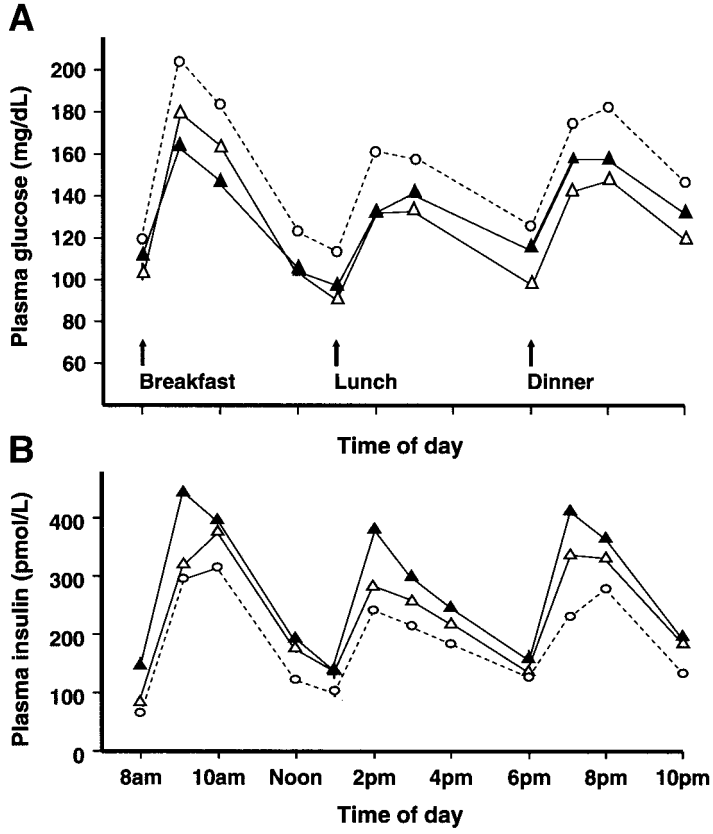

Figure 1—*Persistence of postprandial hyperglycemia during treatment with a rapid- or long-acting secretagogue. Shown are daytime profiles of glucose* (A) *and insulin* (B) *in type 2 diabetes during ongoing treatment with placebo before each meal (○, dashed line), extended-release glipizide (average dose 10 mg) before breakfast (△), or nateglinide 120 mg before each meal (▲). The same 15 patients were studied for each treatment. For clarity, an additional study in which twice-daily conventional glipizide was given was omitted from this figure. On the night before each study, subjects received an intravenous infusion of regular insulin to standardize fasting glucose levels. Neither secretagogue greatly reduced the increments of glucose after meals. Adapted with permission from Carroll et al. (20).*

approaches normal levels. Intensively treated type 1 diabetic patients in the Diabetes Control and Complications Trial collectively experienced assistance-requiring hypoglycemia at a rate of 0.61 events per patient-year (i.e., more than one event every 6 months) (24). These events increased markedly at A1C levels <7% (24). Similarly, in the U.K. Prospective Diabetes Study, major hypoglycemia occurred in 2.3% of insulin-treated patients yearly (25). Although long-term metabolic injury from hypoglycemia is not well proven, acute hypoglycemia can cause motor vehicle accidents or other types of injuries, and fear of hypoglycemia may limit the willingness of both patients and physicians to intensify treatment (26).

Weight gain

Metformin and α-glucosidase inhibitors do not cause weight gain, but insulin, secretagogues, and thiazolidinediones are all associated with variable degrees of weight gain. The excess weight gain with intensive versus standard insulin treatment of type 1 diabetes in the Diabetes Control and Complications Trial was ~2.1 kg in the first year and 4.8 kg after a mean follow-up period of 6.5 years (27). A susceptible subgroup gained much more weight and had worrisome increases in cardiovascular risk factors (28). In the U.K. Prospective Diabetes Study, treatment with insulin or a sulfonylurea was associated with significantly more weight gain over 10 years than treatment with metformin or diet alone (25,29). Weight gain with thiazolidinediones in large trials ranges from 2 to 5 kg (30,31).

Intensively treating type 2 diabetes with multiple injections of insulin can cause as much as 8 kg of weight gain (32), but this effect can be greatly reduced by concurrent treatment with metformin (33,34). When hypoglycemia occurs or is suspected, patients may eat defensively to avoid or treat symptoms of hypoglycemia, so that some of the weight gain during insulin treatment may result from this behavior. Awareness of these difficulties may influence both patients and physicians to delay starting insulin therapy and avoid more aggressive adjustment of insulin dosage.

FASTING VERSUS PRANDIAL MECHANISMS OF CONTROLLING BLOOD GLUCOSE

— The persistence of postprandial hyperglycemia, together with the hypoglycemia and weight gain accompanying efforts to use insulin for better control of postprandial glycemia, have engendered renewed interest in the prandial physiology of glucose homeostasis. The traditional description of diabetes focuses on abnormalities of insulin secretion and insulin action (35,36). This model is most accurate in the fasted state, when plasma glucose levels are determined mainly by insulin concentrations and hepatic sensitivity to insulin (37–39). Glucose uptake in the periphery during fasting occurs mainly in tissues not requiring insulin for entry of glucose into cells, especially the brain, and the relatively low concentrations of insulin during fasting contribute little to the clearance of glucose by other tissues (40). However, modest increases of systemic insulin concentrations can suppress free fatty acid mobilization from adipose tissue and thereby increase hepatic sensitivity to portal insulin. Also, concentrations of insulin are higher in portal than in systemic plasma, and the liver may be more sensitive than muscle to the effects of insulin (41,42). Modulation of insulin secretion during fasting consequently regulates hepatic glucose production and thus the concentration of glucose in plasma. Small changes in plasma glucagon also modulate hepatic glucose production, in effect by altering hepatic responses at given levels of insulin (43,44).

The mechanisms regulating plasma glucose after eating are more complicated (38,39,45,46). An ordinary meal contains 50–100 g of carbohydrate, which is 10–20 times the amount of glucose in the blood. Several factors beyond increasing insulin secretion in response to rising glucose levels combine to prevent the dramatic hyperglycemia that would otherwise occur after meals.

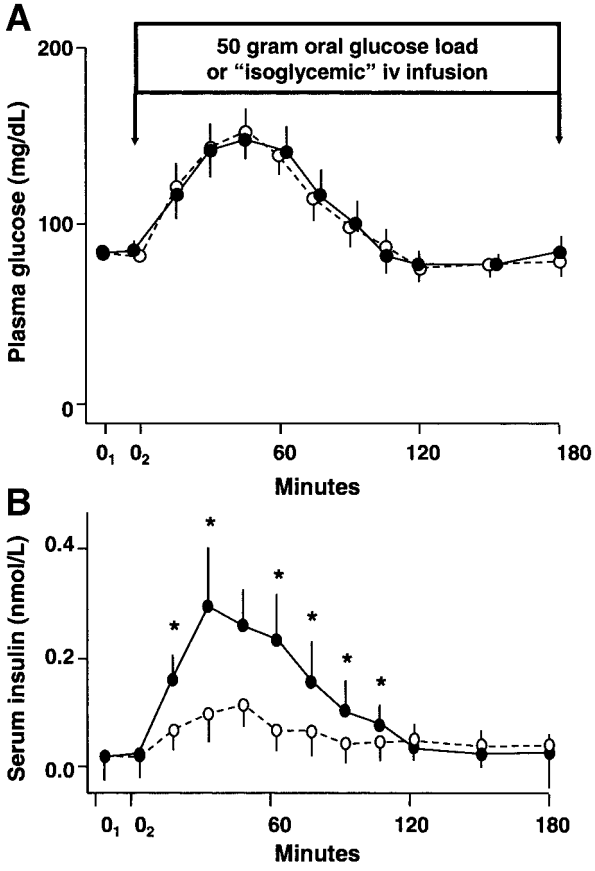

Figure 2—*Illustration of the "incretin effect." In this study, six healthy subjects received 50 g of oral glucose (●, solid line) or, on another occasion, an intravenous infusion of glucose (○, dashed line) to achieve the same profile of plasma glucose. Data for plasma glucose (*A*) and serum insulin (*B*) levels are shown.**$P < 0.05$ *for greater levels of plasma insulin after oral administration of glucose compared with intravenous glucose. Data are means ± SE. Adapted with permission from Nauck et al. (53).*

Incretin effect on insulin secretion

Rapid and sustained secretion of insulin during and after eating is necessary to limit postprandial hyperglycemia (47). Insulin concentrations in the peripheral circulation increase very rapidly (by fivefold or more) with meals (48); this rapid increase in postprandial insulin secretion depends only partly on rising levels of plasma glucose. Over 65 years ago, it was postulated that additional gut-related factors (incretins) contribute to the control of postprandial glycemia (49); this hypothesis was later confirmed when a sensitive assay for insulin became available (50–52). Figure 2 illustrates the "incretin effect," showing a two to three times greater secretion of insulin after oral administration of glucose compared with after intravenous administration, leading to the same profile of plasma glucose concentrations (53). More recently, two specific peptides, glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 have been shown to account for a substantial portion of the incretin effect (1,2).

Suppression of glucagon secretion

Basal secretion of glucagon produces blood levels that lie on the steep part of the dose-response curve for its hepatic effects. Under normal conditions, orally administered glucose sharply reduces glucagon levels, and a mixed meal typically causes no change, a small decline, or a modest increase in glucagon (38,39,45, 46,54–56). Thus the normal hormonal response to eating includes important coordinated changes in plasma levels of both insulin and glucagon. As a result, hepatic glucose production can decline by as much as 60%. Glucagon secretion appears partly regulated by intraislet factors, including endogenous insulin secretion (57), as well as by other neural and endocrine factors.

Neural mechanisms

Neural mechanisms influence the secretion of both insulin and glucagon (58,59). A long-recognized example is the "cephalic phase" of insulin secretion (47,60). A very early increase of insulin secretion occurs before food is swallowed, provoked by visual, olfactory, or oral mechanisms leading to efferent neural signals to the islets. A quantitatively more important process involving neural regulation is the effect that rising concentrations of plasma glucose have on the efficiency of glucose production and uptake by tissues, independent of islet hormones (61,62). Glucose sensors in the portal vein or elsewhere transmit signals to areas in the central nervous system (CNS) that in turn send efferent signals to the liver, muscle, and probably other tissues (63,64). The delivery of gastric contents to the small intestine is at least partially neurally regulated, and variations in the rate of delivery contribute importantly to peak postprandial glucose levels (65). Plasma glucose levels affect gastric emptying, with hyperglycemia generally associated with slower emptying in healthy persons (66).

Abnormalities of prandial responses

As described above, a complex integrated process involving increased secretion of insulin, reduced secretion of glucagon, slowing of gastric emptying, and neural regulation of various tissues normally limits the rise in plasma glucose levels after meals. Many components of this prandial response are abnormal in individuals with diabetes or impaired glucose tolerance. Prandial insulin secretion is absent in type 1 diabetes and delayed and reduced in type 2 diabetes. In type 2 diabetes, both direct glucose-induced insulin secretion (67,68) and incretin-mediated potentiation of insulin secretion are reduced (69). The incretin effect may also be diminished in some patients with early type 1 diabetes (70). Glucagon secretion is typically high during fasting and is not suppressed by an oral glucose challenge, and it increases more than normal after a mixed meal in diabetic individuals (44, 55,57,71,72). Uptake of absorbed glucose by the liver may be reduced, and suppression of endogenous glucose production is markedly impaired (46,55,73). Clearance of glucose entering the systemic circulation is also reduced (73). The impaired ability of hyperglycemia itself to regulate glucose production and clearance contributes to these abnormalities (74,75). Gastric emptying is slowed in patients with diabetic gastroparesis, but early in the course of diabetes emptying may be more rapid than normal (76). These changes all contribute to a failure of postprandial glucose regulation. Figure 3 illustrates some of the most important abnormalities after a glucose challenge in type 2 diabetes (55).

These abnormalities are not entirely corrected by exogenous insulin (77–79). For example, Fig. 4 illustrates an early study in which a high-carbohydrate meal

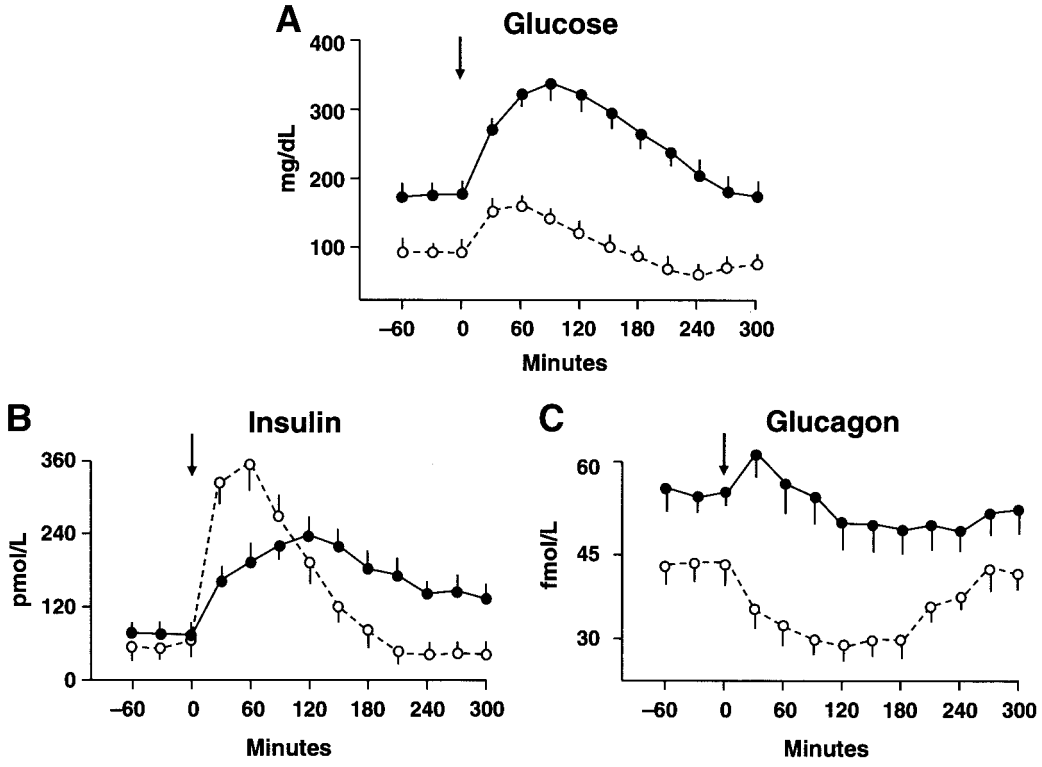

Figure 3—*Profiles of glucose* (A)*, insulin* (B)*, and glucagon* (C) *after an oral glucose load* (↓) *in 10 healthy* (○, *dashed line) and 10 type 2 diabetic* (●, *solid line) subjects. An excessive and prolonged increase of plasma glucose, delayed and reduced response of insulin, and lack of suppression of glucagon are apparent in the diabetic subjects. Data are means ± SE. Adapted with permission from Mitrakou et al. (55).*

was given to type 2 diabetic patients (77). An intravenous insulin infusion that produced very high plasma concentrations of insulin with appropriate timing after the meal improved the postprandial hyperglycemia only moderately, and the abnormal postprandial increase of glucagon was not altered. Furthermore, the difficulty with weight gain experienced by most patients with type 2 diabetes and some with type 1 diabetes may be related to abnormalities that are also not corrected by administration of insulin. Recent data illustrate the importance of multiple gastrointestinal peptide hormones for the integrated control of gut motility, satiety, and postprandial islet hormone responses in individuals with normal glycemic regulation and in those with diabetes.

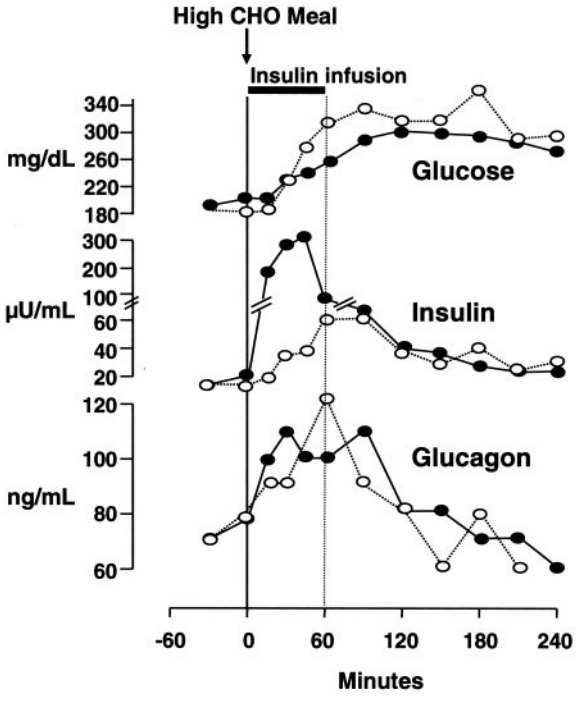

Figure 4—*Profiles of glucose, insulin, and glucagon after a diabetic patient ate a carbohydrate (CHO) meal. A study without* (○) *and one with* (●) *an intravenous infusion of insulin for 1 h during and after the meal are shown. Adapted with permission from Unger (77).*

GASTROINTESTINAL PEPTIDES AFFECTING POSTPRANDIAL GLYCEMIC CONTROL

Amylin

Islet amyloid polypeptide, or amylin, was originally identified as a major constituent of pancreatic amyloid deposits and subsequently shown to be a 37–amino acid peptide cosecreted together with insulin from islet β-cells. Amylin is derived from a larger 89–amino acid preproamylin precursor, and amylin immunoreactivity and mRNA are also present in islet somatostatin-producing δ-cells; in the lung, stomach, duodenum, jejunum, ileum, colon, and rectum; and throughout the CNS (80). Mature bioactive amylin undergoes posttranslational modifications essential for bioactivity, including formation of an intramolecular disulfide bond and COOH-terminal amidation (80). Circulating amylin also exists in nonglycosylated and glycosylated forms in normal and diabetic human subjects.

Plasma levels of amylin increase in response to nutritional stimuli; islet β-cells appear to be the predominant source of circulating amylin (80). Amylin secretion is also stimulated by glucagon, GLP-1,

and cholinergic agonists and is inhibited by somatostatin and insulin.

Amylin-binding sites have been detected in pancreatic β-cells, skeletal muscle, kidney, lung, and brain (81). Functional amylin receptors are generated by coexpression of the G-protein–coupled calcitonin receptor gene and receptor activity–modifying proteins (RAMPs) (82). The potential for the combination of calcitonin receptor isoforms and different RAMP proteins gives rise to at least six different subtypes of amylin receptors that display unique pharmacological properties (83). RAMP-1 and -3 mRNAs are colocalized with calcitonin receptor gene mRNA in mouse pancreatic β-cells (84).

The acute glucoregulatory actions of exogenous amylin include inhibition of gastric emptying and glucagon secretion, with sustained amylin administration leading to reduced food intake and weight loss (4). Amylin potently inhibits glucagon secretion, and reduction of amylin activity using antiamylin antiserum has been shown to dose-dependently increase arginine-stimulated insulin, glucagon, and somatostatin secretion from isolated rat islets, linking endogenous intraislet amylin to inhibitory effects on pancreatic β-, α-, and δ-cells (85). Antagonism of amylin activity has no effect on insulin levels under basal conditions but significantly augments glucose-stimulated insulin secretion in normal but not in diabetic subjects (86).

Exogenous amylin inhibits gastric emptying and gastric acid secretion and reduces short-term food intake. Furthermore, chronic peripheral or intracerebroventricular infusion of amylin reduces food intake, leading to weight loss in rats (87,88). Conversely, amylin−/− mice exhibit increased body weight relative to control mice, and chronic inhibition of central amylin signaling increases food intake and total fat mass in rats (89). Hence endogenous amylin contributes to the long-term control of satiety and body weight.

Amylin−/− mice are healthy and exhibit enhanced glucose clearance and increased sensitivity to the diabetogenic effects of alloxan (90). The physiological importance of the satiety effect of amylin is reflected by enhanced weight gain of amylin−/− mice, which also exhibit reduced responsivity to the anorectic actions of exogenous cholecystokinin and bombesin (91). Amylin also exerts an inhibitory effect on bone resorption, and amylin−/− mice exhibit reduced bone mass, increased numbers of osteoclasts, and increased rates of bone resorption (92).

GIP

GIP is a 42–amino acid peptide produced in the duodenum in enteroendocrine K cells. GIP contains an alanine residue at position 2 and is a physiological substrate for the enzyme dipeptidyl peptidase IV (DPP-IV) (93), which clips and inactivates full-length GIP, thereby generating inactive GIP(3–42). GIP is secreted after nutrient ingestion, functions predominantly as an incretin, and enhances glucose-dependent insulin secretion (94). The actions of GIP are transduced via a seven-transmembrane G protein–coupled receptor predominantly expressed in islet β-cells and to a lesser extent in peripheral sites such as adipose tissue and bone (95). Consistent with the known actions of GIP, blockade of GIP action reduces insulin secretion in rodents (96,97), and genetic elimination of the GIP receptor leads to mild glucose intolerance after enteral glucose loading in mice (98). Most GIP actions in normal or diabetic rodents have been elucidated in short-term studies of GIP administration, and little information is available on the consequences of repeated or continuous GIP administration in experimental models of diabetes. In an intriguing finding, GIP receptor−/− mice exhibit decreased adipose tissue mass, improved insulin sensitivity, and resistance to diet-induced obesity (99). Furthermore, treatment of diabetic *ob/ob* mice with a GIP receptor antagonist markedly attenuates diabetes over an 11-day period, which illustrates the potential importance of GIP receptors on adipocytes for the control of insulin sensitivity (100).

Although GIP exerts potent stimulatory effects on insulin secretion in normal rodents and human β-cells, the diabetic β-cell is relatively resistant to GIP action (101). The mechanisms underlying the markedly diminished GIP responsivity in experimental or clinical diabetes are not completely understood (102) but may involve downregulation of GIP receptor expression (103) or receptor desensitization. Hence the available data suggest that continuous administration of GIP receptor agonists alone may be submaximally effective for the treatment of type 2 diabetes (102). However, whether the GIP-resistant diabetic β-cell might recover GIP responsiveness after treatment with GLP-1R agonists or DPP-IV inhibitors remains to be determined.

GLP-1

GLP-1 is a 30–amino acid gut peptide produced in enteroendocrine L cells located in the distal ileum and colon. GLP-1 is rapidly secreted from the distal gut within minutes of food being ingested. GLP-1 secretion is controlled through a combination of neural and endocrine stimulatory factors that promote initial rapid GLP-1 release. Subsequent direct nutrient contact with GLP-1–secreting L cells in the distal small bowel and colon (104) also stimulates GLP-1 secretion. GLP-1 also contains an NH_2-terminal alanine at position 2, rendering it a substrate for cleavage by DPP-IV (105). Both enzymatic inactivation and renal clearance contribute to a very short circulating $t_{1/2}$ of several minutes for native GLP-1 (106). DPP-IV activation results in the inactivation of GLP-1(7–36) amide and the generation of the metabolite GLP-1(9–36) amide, which does not activate the GLP-1 receptor (107).

GLP-1 controls blood glucose via multiple actions, principally stimulation of insulin secretion and inhibition of both glucagon secretion and gastric emptying (1). GLP-1 also activates regions in the CNS important for control of satiety (108). Hence short-term administration of intracerebroventricular or peripheral GLP-1R agonists reduces food intake, whereas chronic GLP-1R agonist administration has produced weight loss in preclinical studies (109). Moreover, even larger GLP-1R agonists that do not readily cross the blood-brain barrier are able to signal the CNS and promote satiety and weight loss (110), consistent with the importance of ascending vagal afferents for transmission of the GLP-1R signal. GLP-1 also promotes expansion of β-cell mass via stimulation of β-cell proliferation and inhibition of apoptosis (111,112) in multiple preclinical models of experimental diabetes (1). The cytoprotective actions of GLP-1R agonists have also been observed in human islets cultured in vitro (113, 114).

GLP-1 exerts extrapancreatic actions independent of effects on glucoregulation, including activation of the hypothalamic pituitary axis and induction of an aversive stress response in rodents (115,116). Moreover, GLP-1R agonists enhance learning and memory and promote neuronal survival in experimental models of neurotoxicity (117,118). Fur-

thermore, short-term GLP-1 administration activates cytoprotective pathways in vulnerable cardiomyocytes (119) and improves myocardial contractility in preclinical studies (120) and in human subjects after myocardial infarction and revascularization (121).

The physiological roles of endogenous GLP-1 have been identified in studies that have interrupted GLP-1 action using immunoneutralizing antisera, the GLP-1R antagonist exendin(9–39), and GLP-1 receptor−/− mice. These experiments have determined that GLP-1 is essential for control of both fasting and postprandial glucose in rodents (122) and human subjects (123,124). Furthermore, blockade of GLP-1 action results in reduced insulin and increased glucagon secretion, disruption of signals emanating from the portal glucose sensor, as well as an increased rate of gastric emptying (125,126). The actions of GLP-1 are also essential for control of β-cell mass, as GLP-1R−/− mice exhibit a reduced number of large islets (127) and enhanced susceptibility to apoptotic β-cell death (112).

DPP-IV

The observation that both GIP and GLP-1 are rapidly degraded by the action of DPP-IV (93) has fostered interest in determining the role of this enzyme in glucose homeostasis. DPP-IV inhibitors lower blood glucose in normal animals as well as in experimental models of diabetes (2,128–130). DPP-IV inhibition also improves glucose control, reduces A1C, and enhances insulin action in experimental models of diabetes but has no effect on satiety or body weight regulation (131–135). Treating diabetic rodents with DPP-IV inhibitors improves islet survival and maintains β-cell mass and islet function (136,137).

Conversely, genetic inactivation of the DPP-IV gene results in mice with improved glucose tolerance in association with increased levels of GIP and GLP-1 and enhanced insulin secretion after glucose loading (138). Similarly, rats with a naturally occurring inactivating mutation in the DPP-IV gene exhibit enhanced glucose-stimulated insulin secretion and improved glucose clearance (139). Moreover, rats and mice with DPP-IV gene mutations exhibit resistance to diet-induced obesity (140,141). Hence the DPP-IV gene is an essential determinant regulating incretin degradation and the control of glucose-stimulated insulin secretion in rodents. Although the precise substrates important for DPP-IV action in diabetic human subjects remain unclear, disruption of GLP-1 and GIP receptors in mice completely eliminates the glucose-lowering properties of DPP-IV inhibitors (142).

AMYLIN AND GLP-1 ACTION IN NORMAL AND DIABETIC HUMAN SUBJECTS

Amylin

Plasma levels of endogenous circulating amylin in healthy humans correlate with levels of insulin, are lower in the fasted state (~4–8 pmol/l), and rise to 15–25 pmol/l after meal ingestion (143). The kidney is an important determinant of amylin clearance, and human subjects with renal failure exhibit increased circulating levels of plasma amylin (144). Type 1 diabetes is an amylin-deficient state (143), whereas amylin levels are often elevated in patients with impaired glucose tolerance, insulin-resistant obesity, and type 2 diabetes (145).

The potential for native human amylin to form amyloid fibrils prompted the development of synthetic amylin analogs resistant to fibril formation. Pramlintide has three amino acid substitutions at positions 25, 28, and 29 that do not impair the biological potency of the molecule. Pramlintide lowers meal-related glucose excursions in normal subjects and in patients with type 1 and type 2 diabetes (4). In contrast, pramlintide has little effect on blood glucose levels in subjects with type 1 diabetes after intravenous glucose administration (146). The acute glucose-lowering actions of pramlintide are dependent on the inhibition of gastric emptying and suppression of the abnormal postprandial rise in circulating glucagon levels in diabetic subjects (147,148). Conversely, repeated administration of pramlintide does not impair the counterregulatory response to insulin-induced hypoglycemia in normal or diabetic human subjects (149,150).

GLP-1

Acute infusion or subcutaneous administration of native GLP-1(7–36) amide or GLP-1(7–37) lowers meal-related glucose excursions in human subjects via inhibition of gastric emptying and glucagon secretion and potentiation of glucose-dependent insulin secretion (1,2). GLP-1 has also been found in short-term studies of normal and diabetic subjects to enhance satiety and reduce food intake (151). Although acute (5-day) administration of GLP-1 produces weight loss in healthy obese subjects (152), from short-term studies it appears that GLP-1R agonists do not increase energy expenditure in normal (153) or diabetic (154) human subjects.

Repeated daily injections (155) or continuous subcutaneous administration of native GLP-1 lowers blood glucose in subjects with type 2 diabetes (156,157). In one study, continuous infusion of GLP-1 for 6 weeks produced significant improvements in fasting and postprandial glucose and A1C in association with increases in insulin sensitivity and a reduction in body weight (156). Nevertheless, because most of GLP-1 is rapidly degraded after exogenous administration (106), pharmaceutical strategies for enhancing GLP-1 action in human diabetic patients has focused on developing degradation-resistant GLP-1R agonists or potentiating endogenous levels of circulating GLP-1 via inhibition of DPP-IV activity (1,2,130).

Exenatide (exendin-4) is a 39–amino acid synthetic GLP-1R agonist that has been shown in preclinical and clinical studies to mimic the entire spectrum of GLP-1R–dependent actions. Exendin-4 was originally isolated from the venom of a lizard, *Heloderma suspectum* (158). It is encoded by a distinct gene not present in the human genome (159); the corresponding residues of exendin-4 exhibit 53% amino acid identity relative to the 30–amino acid human GLP-1 sequence. Exendin-4 contains a glycine at position 2, is resistant to DPP-IV cleavage, and is considerably more potent than native GLP-1 in vivo due in large part to less rapid inactivation and clearance (109). Dosing range studies have identified an optimal glucose-lowering dose range of 0.05–0.2 μg/kg for exenatide when injected subcutaneously in human diabetic subjects (160). Current antidiabetic regimens for exenatide administration involve twice-daily dosing. However, an injectable long-acting release preparation suitable for weekly dosing, exenatide LAR (161), is under clinical development.

Liraglutide is an acylated human GLP-1 analog that has completed phase 2 clinical trials. Liraglutide binds noncovalently to albumin, may be administered once daily, and exhibits a more prolonged pharmacokinetic profile (162) relative to native GLP-1 or exenatide (154,163). Other albumin-based GLP-1R agonists

Table 1—*Contrasting and overlapping actions of GLP-1R and amylin agonists and DPP-IV inhibitors*

	Amylin agonists	GLP-1R agonists	DPP-IV inhibitors
Enhance insulin secretion	−	+	+
Inhibit glucagon secretion	+	+	+
Slow gastric emptying	+	+	−
Induce satiety and weight loss	+	+	−
Improve β-cell function	−	+	+

under investigation include CJC-1131, a DPP-IV–resistant GLP-1 analog modified with a reactive chemical linker that forms a covalent bond with a single amino acid residue within human serum albumin (164), and Albugon, a recombinant albumin/GLP-1 hybrid protein (110). The ability to link a GLP-1 peptide domain conferring GLP-1R activation to albumin or other proteins that exhibit a more prolonged circulating $t_{1/2}$ should enable the development of longer-acting GLP-1R agonists suitable for once daily or even weekly administration.

DRUGS APPROVED OR IN LATE STAGES OF CLINICAL DEVELOPMENT

— The effects of various agents targeting these gastrointestinal peptide systems, as shown in preclinical studies, are compared in Table 1. Enough experience from large clinical trials has been obtained to permit a preliminary assessment of the clinical potential of several specific agents. Pramlintide and exenatide have been approved for clinical use in the U.S., and the GLP-1R agonist liraglutide and the DPP-IV inhibitors vildagliptin and sitagliptin are well along in clinical development.

Pramlintide

The amylin analog pramlintide (Symlin) has been approved for treatment of type 1 and insulin-requiring type 2 diabetes. When injected 15 min before a meal, it slows gastric emptying, suppresses plasma levels of glucagon, increases satiety, and blunts postprandial hyperglycemia (165). Pramlintide also reduces appetite in diabetic and obese nondiabetic human subjects (166). Appropriate doses for pramlintide administration have been determined in clinical studies: 15 μg before major meals, increased slowly to 60 μg for type 1 diabetes, and 60 μg before major meals, later increased to 120 μg for type 2 diabetes (167–169). Slow titration to the full dose over ≥4 weeks can reduce nausea, vomiting, and insulin-induced hypoglycemia, the drug's main side effects. Reduction of prandial insulin dosage by 50% is advised to minimize the risk of hypoglycemia after initiation of pramlintide therapy.

Published results from four 52-week trials examining the efficacy of pramlintide therapy are available, two in type 1 and two in type 2 diabetes (170–173). In the two trials in type 1 diabetic subjects (170,171), treatment with pramlintide 60 μg q.i.d. with meals led to modest, placebo-adjusted reductions of A1C (0.27 and 0.30%, respectively) at study end point. Significant placebo-adjusted reductions of weight also occurred (1.5 and 1.2 kg). In insulin-requiring type 2 diabetes, treatment with pramlintide 150 μg t.i.d. or 120 μg b.i.d. resulted in similar placebo-adjusted A1C reductions (0.44 and 0.40%, respectively) and placebo-adjusted weight reductions (2.5 and 2.1 kg). Patients with both type 1 and type 2 diabetes reported nausea about twice as frequently with pramlintide as with placebo, although this problem declined markedly with time. In one of the type 1 trials, a worrisome increase in severe hypoglycemia occurred with pramlintide in the first weeks after treatment was begun (171). However, in these trials pramlintide was not started at a lower dose and then increased, and prandial insulin doses were not decreased with commencement of pramlintide therapy.

Figure 5*A* shows the results of a study of 40 patients with type 1 diabetes taking usual dosages of regular insulin with or without pramlintide before a meal (174). In this research setting, pramlintide en-

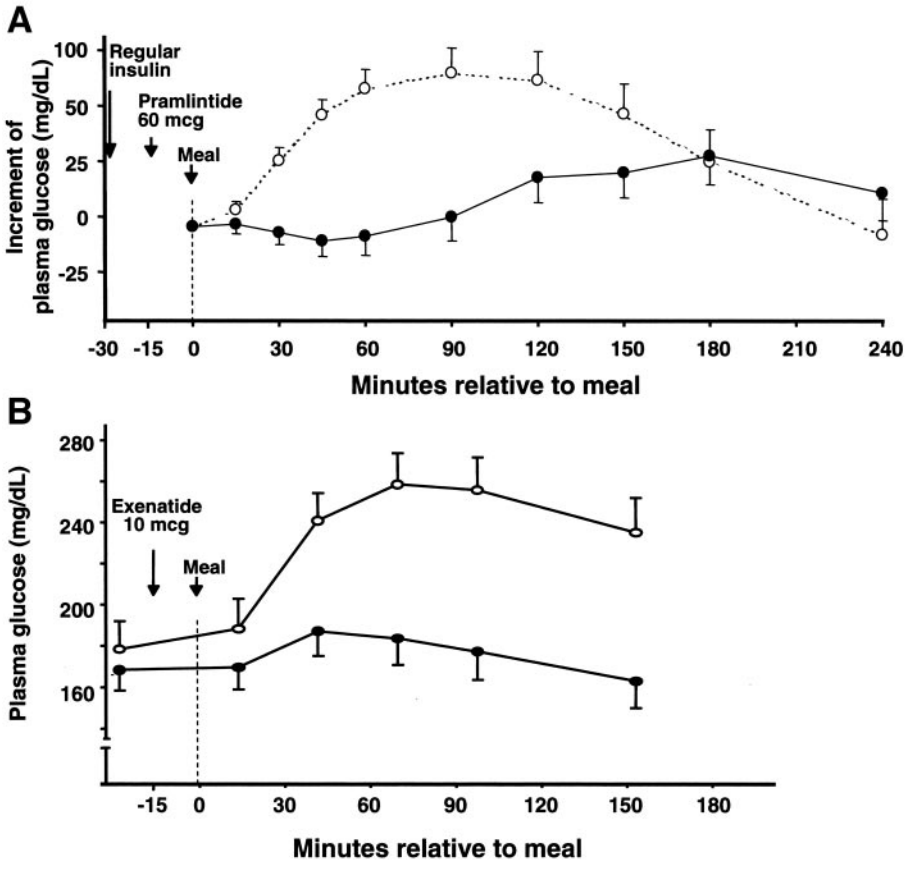

Figure 5—A*: Plasma glucose increments after a meal in 19 subjects with type 1 diabetes taking regular insulin alone (○, dashed line) or with 60 μg pramlintide (●, solid line) before the meal.* B*: Glycemic responses to a meal in patients with type 2 diabetes treated with placebo* (n = 23; ○) *or exenatide 10 μg* (n = 27; ●) *along with metformin and a sulfonylurea. Data are means ± SE. Adapted with permission from Weyer et al. (174) and Kendall et al. (178).*

tirely prevented the postprandial hyperglycemia ordinarily seen in subjects with type 1 diabetes. However, these profiles illustrate how strongly pramlintide can accentuate the effects of prandial insulin, making early postprandial hypoglycemia easily possible if the insulin dose is not decreased appropriately.

Exenatide

Exenatide (Byetta), a GLP-1 receptor agonist, has been approved for use in patients with type 2 diabetes who exhibit unacceptable glycemic control while using metformin and/or a sulfonylurea. Exenatide shares several clinical features with pramlintide despite binding to different receptors. Exenatide slows gastric emptying, suppresses glucagon, and promotes satiety. In addition, it potentiates nutrient-stimulated insulin secretion (175). Studies have defined an appropriate dosing strategy: 5 μg injected twice daily for the first month, followed by 10 μg twice daily thereafter (175–178). Nausea and vomiting can occur, especially at the beginning of treatment, but this is less frequent when treatment is started with the 5-μg dose.

Published results from three large 6-month trials testing the addition of exenatide to metformin alone, sulfonylurea alone, or metformin and a sulfonylurea together are available (176–178). The placebo-adjusted decline of A1C from baseline levels of 8.2–8.6% was ~1.0% in each trial. A mean placebo-adjusted weight loss of 2.5 kg occurred when exenatide was added to metformin, 1.0 kg when the drug was added to a sulfonylurea, and 0.9 kg when it was added to metformin plus a sulfonylurea. As with pramlintide, initiation of exenatide resulted in at least a doubling of the incidence of nausea compared with placebo. Severe hypoglycemia was uncommon, but mild-to-moderate hypoglycemia increased initially when exenatide was added to a sulfonylurea (177,178).

Exenatide therapy has been compared with insulin glargine in patients failing to achieve optimal glycemia control on metformin and a sulfonylurea. After 26 weeks of therapy, the mean A1C reduction (1.1%) was comparable in both groups (179). The incidence of gastrointestinal side effects and the dropout rate was higher in the exenatide-treated patients. However, patients treated with insulin glargine experienced a mean weight gain of 1.8 kg, whereas exenatide-treated subjects had a mean weight loss of 2.3 kg. Rates of reported hypoglycemia were similar in the different treatment groups (179).

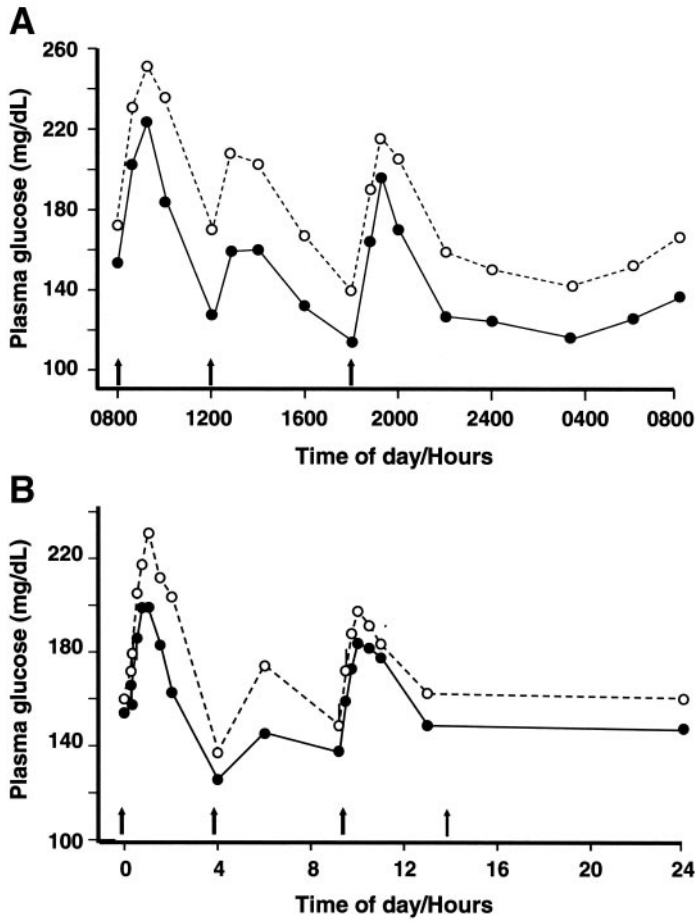

Figure 6—A*: Plasma glucose profiles over 24 h in 13 subjects with type 2 diabetes after 1 week of treatment with placebo (○, broken line) or liraglutide (●, solid line).* B*: Plasma glucose profiles over 24 h after 4 weeks of treatment in type 2 diabetic patients treated with placebo* (n = *19;* ○*, broken line) or vildagliptin 100 mg/day* (n = *18;* ●*, solid line). The timing of meals is shown by arrows. Adapted with permission from Degn et al. (180) and Ahren et al. (182).*

Figure 5*B* shows glucose profiles after a standard meal in a subset of patients from the trial in which exenatide was added to metformin and a sulfonylurea (178). Although fasting glucose was reduced by ~25 mg/dl with the 10-μg dose of exenatide, the more dramatic effect was the ~90% reduction of the incremental glycemic area compared with that found with placebo.

Liraglutide

Liraglutide (NN2211) is a GLP-1 receptor agonist that is administered by a single daily injection. As with pramlintide and exenatide, nausea is the most common adverse effect associated with liraglutide administration. Although the optimal dose has not yet been identified, a 12-week trial including 193 patients with type 2 diabetes showed that 0.75 mg liraglutide daily caused equivalent placebo-adjusted reductions of A1C compared with the sulfonylurea glimepiride (0.75 and 0.74%) from mean baseline values of 7.4–7.9% (163). However, liraglutide treatment was associated with a placebo-adjusted weight reduction of 0.39 kg, whereas patients treated with glimepiride experienced a mean weight gain of 0.94 kg.

The 24-h glycemic profile of 13 patients treated with placebo or liraglutide 6 μg/kg (~0.6 mg) for 1 week is shown in Fig. 6*A* (180). Most of the reduction in the profile consisted of lower basal and preprandial values, with only a small reduction of postprandial increments. A significant reduction of the glucagon profile occurred as well, but insulin levels were not different between treatments (180).

Table 2—*Summary of study findings with pramlintide, exenatide, liraglutide, and vildagliptin*

	Treatment maximum dosage	Type of diabetes	Other treatment	*n*	Duration (weeks)	Baseline A1C (%)	Placebo-adjusted ΔA1C (%)	Baseline BMI (kg/m^2)	Placebo-adjusted Δweight (kg)
Whitehouse 2002	Pramlintide 60 μg q.i.d.	Type 1	Insulin	480	52	8.7–8.9	−0.27	25.2–25.8	−1.5
Ratner 2004	Pramlintide 60 μg q.i.d.	Type 1	Insulin	651	52	8.9	−0.30	26.3–26.8	−1.2
Ratner 2002	Pramlintide 150 μg t.i.d.	Type 2	Insulin	538	52	9.2	−0.44	30.1–30.4	−2.5
Hollander 2003	Pramlintide 120 μg b.i.d.	Type 2	Insulin	656	52	9.1	−0.40	34	−2.1
DeFronzo 2005	Exenatide 10 μg b.i.d.	Type 2	Metformin	272	30	8.2	−0.9	34.2	−2.5
Buse 2004	Exenatide 10 μg b.i.d.	Type 2	Sulfonylurea	377	30	8.6	−0.98	33	−1.0
Kendall 2005	Exenatide 10 μg b.i.d.	Type 2	Metformin/ sulfonylurea	733	30	8.5	−1.0	33.6	−0.9
Madsbad 2004	Liraglutide 0.75 mg once	Type 2	Diet	193	12	7.6	−0.75	30.3–31.9	−0.39
Ahren 2004	Vildagliptin 50 mg once	Type 2	Metformin	107	12	7.7–7.9	−0.7	29.4–30.2	−0

Vildagliptin

Vildagliptin (LAF237), an orally administered DPP-IV inhibitor, at a dosage of 50 mg/day was compared with placebo for 12 weeks in 107 patients with type 2 diabetes while the patients continued to take metformin (181). The placebo-adjusted reduction of A1C from mean baseline values of 7.8% was 0.7%. No significant between-treatment differences in change of weight occurred. Despite concern about the lack of specificity of vildagliptin's action, no notable safety issues emerged in this or other early trials.

The 24-h glycemic profiles of 37 patients treated for 4 weeks with vildagliptin 100 mg/day or placebo are shown in Fig. 6*B* (182). As was seen during treatment with liraglutide, vildagliptin treatment resulted in lower fasting and preprandial glucose values and modest reductions in postprandial increments. Insulin and glucagon profiles showed no change in the absolute levels of insulin but did show lower concentrations of glucagon (181, 182) after vildagliptin therapy. Furthermore, mathematical modeling studies have suggested that an improvement in β-cell function occurs after vildagliptin treatment (183,184).

POTENTIAL CLINICAL ROLES OF THESE AGENTS

— The findings of the studies reviewed above are summarized in Table 2. Each of these agents causes clinically relevant reductions in A1C when starting from relatively low baseline values, with either no weight gain or a significant weight loss. In some cases, important effects on postprandial hyperglycemia have been shown. Because these candidate drugs address abnormalities of prandial physiology in diabetes that are inadequately treated by other therapies, they are all likely to contribute to the treatment of diabetes.

However, there is much more to learn about the optimal use and efficacy of these agents. The development of pramlintide was hampered by the lack of individualized reductions of insulin dose when the drug was added in early safety and efficacy trials, leading to some cases of severe insulin-induced hypoglycemia. Reducing the dosage of prandial insulin by 50% when pramlintide is started should greatly reduce the risk of hypoglycemia. However, more information is needed about which patients are most likely to benefit from the drug, how best to titrate pramlintide dosage to minimize nausea, and how to teach patients to adjust basal and prandial insulin doses during ongoing use of pramlintide. Studies documenting that excessive rates of severe nausea and severe hypoglycemia can be avoided during use of pramlintide in routine clinical practice are needed. At present, successful use of this agent appears to require an experienced physician and a highly motivated patient.

Exenatide is less likely to cause hypoglycemia if used by patients taking metformin, but the risk of hypoglycemia is significantly increased in patients treated with both exenatide and a sulfonylurea. The patient population that is most likely to respond well to exenatide has not yet been identified, and information as to whether twice daily fixed dosing is always optimal and how to individualize dose titration to minimize nausea has not yet been forthcoming. Whether concurrent treatment with secretagogues can be made safe by reducing the dosage of exenatide or the secretagogue or by using secretagogues least likely to cause hypoglycemia must be further established. Studies are needed to verify that these measures can limit the frequency of nausea and hypoglycemia in the same way that experience has led to acceptable rates of side effects with metformin.

Similar practical concerns apply to liraglutide and vildagliptin. Because these agents are in an earlier stage of development, the optimal doses and extent of safety concerns are still unknown.

There are both similarities and differences among these agents that may influence their clinical applications. For example, although pramlintide and exenatide bind to separate receptors, their clinical effects have substantial overlap. Both can blunt or even abolish postprandial increments of glucose, and both more often cause weight loss than weight gain while improving glycemic control. These features directly contrast with the failings of prior therapies and indicate the potential for very effective use of these agents in combination regimens. However, the populations suited to each of these two agents are different: pramlintide has been approved for use only by patients already taking both basal and prandial insulin, and subsequently the risk of insulin-induced hypoglycemia when starting pramlintide appears significant. In contrast, exenatide is approved for use by type 2 diabetic patients not yet requiring insulin, so that the addition of exenatide to prior oral therapies promises a lower rate of hypoglycemia than that achieved with the addition of insulin.

Although liraglutide and vildagliptin enhance GLP-1 receptor occupancy in different ways, they also have similarities in their effects. Both reduce fasting and 24-h profiles of glucose effectively but have relatively less effect on postprandial increments than pramlintide or exenatide. This effect on basal glycemic control seems to depend more on a suppression of glucagon than potentiation of insulin secretion, although that must be present as well. In studies con-

ducted to date, they have differed in their effects on weight: liraglutide induces weight loss while improving glycemic control, like pramlintide and exenatide, whereas vildagliptin has not yet been shown to induce weight loss in clinical studies

The differences in actions of the three agents targeting the GLP-1 system highlight the limitations of our understanding of gastrointestinal peptide physiology. For example, it is still unclear to what extent the various effects of GLP-1 are mediated through actions directly on islet cells, the brain, or peripheral sites (e.g., intestinal mucosa, portal vein) and whether the differing effects of exenatide, liraglutide, and vildagliptin are due to differences in pharmacokinetics or mechanisms of action. Similar questions apply to amylin, which is currently thought to act mainly at the brain but which may have peripheral effects as well. Also it remains to be determined whether pramlintide might have different effects if delivered by a sustained-release formulation.

Scientific questions such as these are relevant to important clinical issues. What is the potential for long-term benefits or risks independent of glucose control and body weight with these new agents? Can the weight-loss benefits of pramlintide, exenatide, and liraglutide be sustained over time? Will these injectable peptides be associated with immunogenicity and the development of neutralizing antibodies that may diminish the efficacy of therapy over time in selected patients? Will there be cardiovascular benefits independent of the improvement in glycemic control with some or all of these agents? Will exenatide, liraglutide, and vildagliptin protect β-cells or promote their regeneration in clinical use, as appears to be the case in animal studies? Conversely, the recent description of hyperinsulinemic hypoglycemia and nesidioblastosis together with increased circulating levels of GLP-1 in a few patients after gastric bypass surgery further emphasizes the importance of understanding the long-term consequences of prolonged activation of the GLP-1 receptor in human subjects (185,186). The answers to these questions will determine, to a large extent, the future role of these agents in the treatment of type 2 diabetes.

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