Amylin Agonists: A Novel Approach in the Treatment of Diabetes

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Amylin is a peptide hormone that is cosecreted with insulin from the pancreatic β-cell and is thus deficient in diabetic people. It inhibits glucagon secretion, delays gastric emptying, and acts as a satiety agent. Amylin replacement could therefore possibly improve glycemic control in some people with diabetes. However, human amylin exhibits physicochemical properties predisposing the peptide hormone to aggregate and form amyloid fibers, which may play a part in β-cell destruction in type 2 diabetes. This obviously makes it unsuitable for pharmacological use. A stable analog, pramlintide, which has actions and pharmacokinetic and pharmacodynamic properties similar to the native peptide, has been developed. The efficacy and safety of pramlintide administration has been tested in a vast number of clinical trials. Aproximately 5,000 insulin-treated patients have received pramlintide and ~250 for ≥2 years. The aims of this review are to *1*) briefly describe actions of amylin as demonstrated in animal and human models and *2*) primarily review results from clinical trials with the amylin analog pramlintide. *Diabetes* 53 (Suppl. 3): S233–S238, 2004

A reduced HbA_{1c} is closely linked with decline in late diabetic complications in both type 1 diabetic patients, as shown in the Diabetes Control and Complications Trial (DCCT) (1), and type 2 diabetic patients, as shown in the U.K. Prospective Diabetes Study (UKPDS) (2) and the Kumamoto Study (3). Consequently, the recommendations regarding HbA_{1c} for diabetic individuals in satisfactory glycemic control is currently <7% and therefore even more stringent goals (i.e., HbA_{1c} <6%) should be considered in individual patients (4,5). However, due to the shortcomings of the antihyperglycemic therapy currently available, novel approaches to lowering glycemia are clearly needed in many patients.

The glucagon-like peptide-1 (GLP-1) approach looks promising for the treatment of type 2 diabetes (6). In insulin-treated diabetic patients (type 1 or type 2), it is also difficult to achieve the HbA_{1c} target because blood glucose is often characterized by unpredictable fluctuations; hypoglycemia and weight gain are additional difficulties. Certainly, insulin analogs have proven to be efficient in terms of plasma glucose excursions and hypoglycemic events in several insulin-treated patients. However, another novel approach for lowering glycemia in some insulin-treated diabetic subjects could be the addition of amylin agonists.

Amylin (also known as islet amyloid polypeptide, or IAPP) is a 37–amino acid peptide hormone that is costored and cosecreted with insulin and shares the same processing enzymes. The hormone has a signal transduction pathway similar to calcitonin (CT), calcitonin gene–related peptide (CGRP), and adrenomedullin (3), and the specificity of the amylin receptor has been characterized. Since the description of amylin almost two decades ago (7,8), a large body of data has documented the physiological actions of this peptide. Amylin is secreted in response to nutrient stimuli and displays a 24-h profile similar to that of insulin (9). Also, amylin has been shown to be secreted in a high frequency pulsatile manner, similar to insulin (10). The peptide circulates in a nonglycosylated (50%) and a glycosylated form (11), of which the former is the biological active compound. In healthy humans, fasting plasma amylin concentrations are in the range of 4–25 pmol/l, and amylin is distributed equally to insulin in plasma and interstitial fluids. In opposition to insulin, it is not eliminated significantly in the liver (12) but mainly through renal metabolism.

Amylin agonists display some of the beneficial effects of GLP-1. Below we will not focus primarily on amylin actions but mainly on clinical data utilizing an amylin agonist. Bearing this in mind, it should be emphasized that it has been firmly established that deposits of amylin as amyloid are frequently found in the islets of type 2 diabetic individuals and could represent a visible pathological feature that disrupts islet structure and contributes to the islet dysfunction. Discussion of the latter is beyond the scope of this article but is reviewed in several recent articles (e.g., 13,14).

AMYLIN ACTIONS

Animal studies. In vitro and in vivo studies, mainly performed in rodents, have demonstrated effects of amylin on glucose metabolism (rev. in 15). In the rat skeletal muscle, amylin leads to insulin resistance by impairing glycogen synthase activity and increasing glycogen breakdown and subsequently glycolysis, the latter resulting in increased lactate output. Furthermore, it has been demonstrated that amylin is required for normalization of liver

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GLP-1, glucagon-like peptide-1; IAPP, islet amyloid polypeptide.

glycogen content in streptozotocin-diabetic rats in which liver glycogen stores are depleted.

A selective amylin antagonist has been found to augment insulin secretion from isolated rat pancreatic β-cells (16). Moreover, the occurrence of amylin binding sites in β-cells (17) could point to a direct effect on insulin secretion. Amylin inhibits arginine-induced glucagon secretion in rats in a dose-dependent manner (18). A study has demonstrated that the glucagon inhibiting effect of amylin in rats is six times more potent than that of GLP-1 (19). However, amylin exhibits no glucagon inhibition on the isolated perfused pancreas (20), probably implying that this action depends on extrapancreatic pathways.

Amylin also delays gastric emptying (21). Dose-response studies have demonstrated that amylin is more potent in postponing gastric emptying than other gastrointestinal hormones like GLP-1 and cholecystokinin (22). Moreover, in rats the lack of amylin accelerates gastric emptying (23), supporting the theory that this action represents a physiological effect of the hormone. This specific effect of amylin is apparently mediated through the central nervous system and depends on an intact vagus nerve (24). The ability of amylin in slowing gastric emptying has, however, been shown to be overridden by hypoglycemia (25). Finally, amylin reduces food intake and body weight in rodents (26–28). The inhibitory effect on food intake is present after both intracerebral and peripheral administration of amylin (27). This effect appears to be mainly mediated via central pathways that include high-affinity binding sites in the area postrema in the hindbrain (24,29–31). The importance of amylin as a key player in the regulation of food intake is supported by the finding of increased weight gain in amylin gene knockout mice.

Human studies. Three studies (32–34) have examined the impact of acute administration of native amylin on in vivo glucose metabolism in healthy humans. Despite using high pharmacological doses resulting in circulating amylin levels 50–100 times above normal postprandial concentrations, no effect of native amylin could be demonstrated on glucose uptake, neither as assessed by a hyperinsulinemic-euglycemic clamp (34) nor during an intravenous glucose tolerance test (32,33). This is in agreement with data of Zierath et al. (35) in which no effect on glucose transport in human muscle strips was observed. In terms of impact on β-cell function, only a blunted insulin response was demonstrated at very high doses (33).

AMYLIN ANALOGS

Human amylin has properties (36) that make it potentially unsuitable for pharmacological use. These include the ability to form amyloid fibers; as a result, amyloid plaques self-aggregate and are relatively insoluble in a number of diluents. This is primarily related to the amino acid sequence 25–29 of the peptide (37), but more recent data have also suggested that the amyloidogenic properties of amylin are influenced by other domains within the molecule (38). Whereas human amylin readily forms islet amyloid, this ability is not seen in rodents (36). Preparing the native human molecule for pharmacological use led to the development of an analog in which three proline residues were substituted at positions 25, 28, and 29 (39). This analog, named pramlintide (previously referred to as AC 137), is stable, soluble, nonaggregating, and nonadhesive and has been shown to exhibit beneficial actions similar to those of native amylin (40). Pramlintide has been tested in several clinical studies, all aimed at restoring amylin in absolute or relatively deficient diabetic patients. Like insulin, pramlintide needs to be administered subcutaneously, but pramlintide precipitates above pH 5.5. Therefore, it is not currently suitable for coformulation with insulin. The rationale for replacing amylin in diabetes is that in humans it appears mainly to exert its effects in the postprandial state by *1*) inhibiting the excessive glucagon secretion (three decades ago Unger [41] suggested that "glucagon suppression could be a potentially useful adjunct to conventional antihyperglycemic agents in the management of diabetic hyperglycemia") and *2*) delaying gastric emptying. The importance of postprandial blood glucose control as a specific target of therapy influencing overall glycemic control and perhaps long-term complications in diabetes is currently debated (42). Moreover, the effects of amylin analogs as a satiety factor cannot be emphasized enough in obese diabetic patients.

PRAMLINTIDE STUDIES IN DIABETIC INDIVIDUALS

Acute studies. The first pramlintide study in type 1 diabetic patients explored the effect of an intravenous pramlintide infusion during a Sustacal meal (43) and convincingly demonstrated that the analog was capable of reducing the glycemic response to this challenge, possibly at least partly due to delaying gastric emptying (44). A similar observation was also made in insulin-treated type 2 diabetic patients (45). There is no evidence of an effect of pramlintide administration on insulin sensitivity in either peripheral tissues or the liver in type 1 diabetic patients (46), which reiterates the observations made in healthy individuals using native amylin (34) and which is further supported indirectly using an amylin antagonist (47) in obese type 2 diabetic and nondiabetic individuals. The latter study also demonstrated that the amylin antagonist was able to increase insulin secretion in lean and obese individuals, suggesting a possible physiological role of amylin to restrain insulin secretion. Finally, it should be noted that recovery from hypoglycemia induced by a hyperinsulinemic-hypoglycemic clamp, as well as the glucagon response to it, was not affected by pramlintide (46).

Short-term studies (1–4 weeks). Two-week administration of pramlintide thrice daily to type 1 diabetic subjects has been demonstrated to blunt the glycemic response to a Sustacal meal in a dose-dependent manner (48). Regarding overall glycemic control, 4 weeks of pramlintide tratment as an adjunct to insulin in type 1 diabetes significantly reduced 24-h plasma glucose (49) and serum fructosamine (50,51). The improved glycemic control during pramlintide treatment was not associated with increase in severe hypoglycemic episodes.

Animal models have demonstrated that coadministration of insulin and amylin is necessary to fully restore liver glycogen (52). A study examining endogenous glucose release during glucagon challenges in type 1 diabetic individuals following 4 weeks of pramlintide administration (53) found no perceptible differences between pramlintide and placebo. However, it should be noted that the

study design did not allow conclusions regarding liver glycogen content. Other studies have explored the effect of pramlintide administration on overall and postprandial glycemic control and, in particular, postprandial glucagon secretion in patients with type 1 diabetes. Nyholm et al. (51) demonstrated that pramlintide administration at a dose of 30 μg thrice daily in type 1 diabetic patients reduced circulating concentrations of glucose and insulin as assessed by a daytime profile. Recently, Levetan et al. (54) utilizing a continuous glucose monitoring system (CGMS) have shown that addition of pramlitide to insulin for 4 weeks in type 1 diabetic subjects who were intensively treated with insulin pumps and had fair glycemic control, as assessed by HbA_{1c} values, were also capable of further improving the glycemic control. This interesting study with a 24-h sensor period demonstrated not only reduced daytime glucose excursions in spite of reduced insulin dosage, but also a greater number of glucose readings during nighttime within the euglycemic target. Furthermore, the authors found reduced postprandial triglyceride excursions, probably mainly due to the delay in gastric emptying. Taking the presumed importance of postprandial dyslipidemia and cardiovascular disease into account, further investigations, including longer-term studies, are obviously needed. Given the increasing use of rapid-acting insulin analogs in the treatment of diabetes, it should also be emphasized that pramlintide administered at or just before a meal reduces the postprandial glucose excursion markedly in type 1 diabetic subjects treated with insulin lispro (55).

Nyholm et al. (51) observed that pramlintide treatment led to reduced concentrations of glucagon (~25%) after a carbohydrate-rich breakfast meal. The reduction in postprandial glucagon concentrations has been confirmed by Fineman et al. (56) and, moreover, was present in type 2 diabetic patients (57) (Fig. 1). An even more pronounced reduction in postprandial glucagon was present in intensively treated type 1 diabetic subjects on insulin pumps (54). This glucagonostatic property of pramlintide has indirectly been corroborated by the finding of enhanced glucagon secretion in diabetic individuals following administration of a selective amylin antagonist (47) and by demonstrating an inverse correlation between amylin and glucagon secretion (58).

While the significance of reduced postprandial glucagon secretion for glycemic control cannot be challenged in either type 1 or type 2 diabetes (59–61), it is still not known how amylin agonists modulate postprandial glucagon secretion and, in particular, whether there is a direct effect on the pancreatic α-cell (20). Improved glycemic control (60) and delayed gastric emptying, the latter of which postpones the appearance of nutrients stimulating glucagon release, probably both contribute. However, changes in other substrates, hormones, and peptides currently not well defined may also participate, and furthermore, the autonomic nervous system may be involved. A recent study seems to rule out the contribution of GLP-1 (62).

Long-term studies (26–52 weeks). Several long-term randomized, double-blind, placebo-controlled multicenter trials using pramlintide for 26–52 weeks have been conducted. This review will only concentrate on the studies published at the time of writing (data based on abstracts are not included).

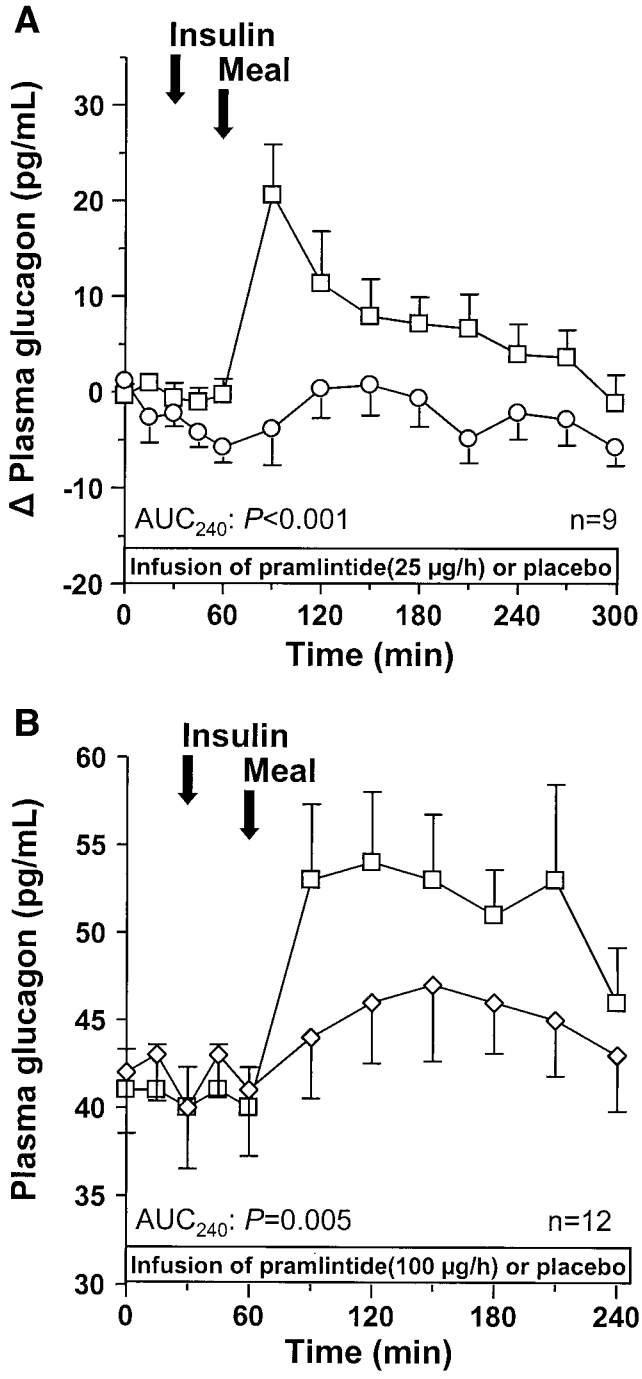

FIG. 1. Plasma glucagon responses (mean ± SEM) to Sustacal meals in type 1 (*A*) and insulin-treated type 2 diabetic subjects (*B*). □ indicates placebo, and ○ and ◇ indicate pramlintide treatment in type 1 and type 2 diabetic subjects, respectively. Data adopted from refs. 56 and 57.

In a 52-week study that included 480 type 1 diabetic patients, the addition of pramlintide (q.i.d.) to the insulin therapy significantly reduced HbA_{1c} (63) (Fig. 2). The reduction was most pronounced during the initial 13 weeks (0.67%), but the difference from placebo was sustained though less pronounced by week 52, as 25% reached the American Diabetes Association (ADA) glycemic target of HbA_{1c} <7%. Of note, the number of severe hypoglycemic events was not increased in the pramlintide group.

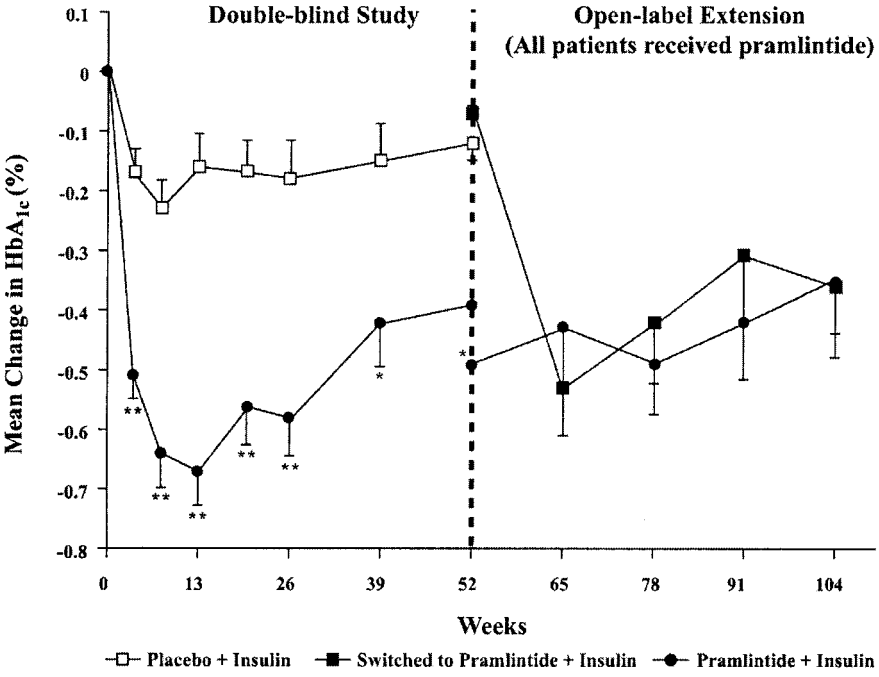

FIG. 2. Change in HbA_{1c} (mean ± SEM) from baseline over time in type 1 diabetic patients. In the left-hand part of the figure (i.e., the double-blind study), ● and □ indicate pramlintide and placebo, respectively. In the right-hand part of the figure (i.e., the open-label extension), ● indicates patients continuing pramlintide treatment, and ■ indicates patients previously treated solely with insulin before the extension period and now being treated with pramlintide as an adjunct. Used with permission from the American Diabetes Association (63).

After 52 weeks, all subjects were invited to participate in an open-label extension. As can be seen in Fig. 2, the reduction in HbA_{1c} was maintained in those who agreed to continue the treatment.

A study of similar duration (52 weeks) was performed in 656 insulin-treated type 2 diabetic individuals (64). However, pramlintide in this design was dosed twice a day and in two different doses (90 and 120 μg). In the 120-μg group, HbA_{1c} was reduced by 0.62% after 52 weeks (Fig. 3). Once again, no increased incidence of hypoglycemia was observed, but as in the aforementioned study there was a transient mild-to-moderate nausea in the pramlintide group. Notably, in these type 2 diabetic patients who were characterized by an initial body weight of ~97 kg, the pramlintide group exhibited a weight loss of 1.4 kg versus a weight gain of 0.7 kg in the placebo group. The latter has been confirmed in a recent post hoc analysis based on two large studies (>1,100 patients) among the subgroup of patients with an HbA_{1c} at the entry between 7.0 and 8.5% (65). The weight loss seemed to be even more pronounced and the percentage of patients reaching the ADA targets in terms of HbA_{1c} was several times higher in the pramlintide group. Recently, Hollander et al. (66) demonstrated in the same population that this weight reduction was most pronounced in the very obese group of type 2 diabetic subjects and that the number of patients experiencing a weight reduction of >5% after 26 weeks was threefold higher in the pramlintide group compared with the placebo- and insulin-treated groups. Interestingly, the insulin- and metformin-treated patients were very positive responders. The daily insulin dose was significantly reduced in the pramlintide group, and it cannot be excluded that this contributed to the weight loss, although it should be emphasized that $HbA_{1c}$was improved (−0.6%).

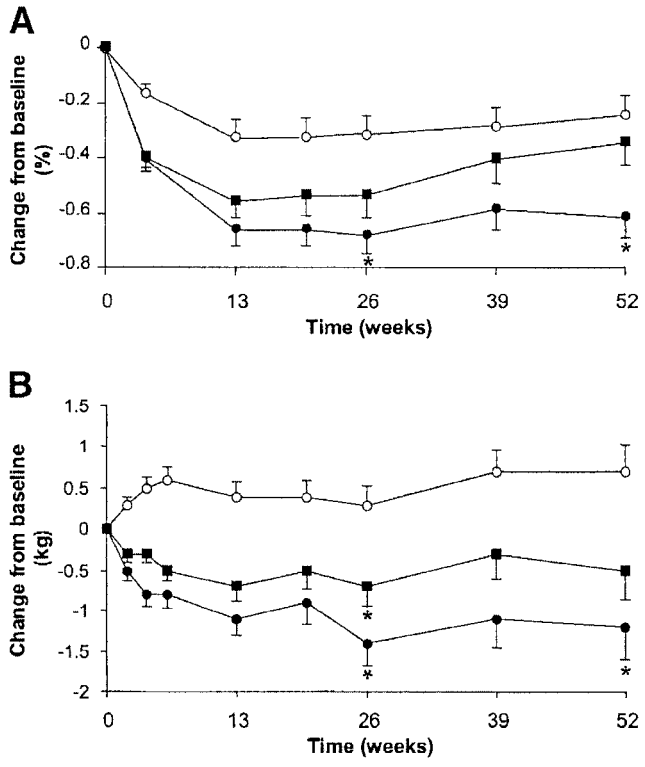

FIG. 3. Change from baseline in HbA_{1c} (mean ± SEM) (*A*) and body weight (*B*) in a 52-week study in type 2 diabetic patients treated with either pramlintide 90 or 120 μg b.i.d. (■ and ●, respectively) or placebo (○). Used with permission from the American Diabetes Association (64). *$P < 0.05$; **$P < 0.001$.

CONCLUSION AND PERSPECTIVES

Several prospective intervention studies have taught us that near-normal glycemia is, in addition to several other pharmaceutical intervention strategies, crucial in minimizing late complications in diabetes. However, the DCCT clearly demonstrated that one of the hindrances is an increased risk of severe hypoglycemic episodes. The UKPDS confirmed that the natural history of type 2 diabetes in terms of β-cell function and insulin resistance is complex, and that none of the used treatment regimens

(metformin, sulfonylurea, and insulin) were outstanding in controlling glucose homeostasis. Moreover, sulfonylurea and insulin were hampered by inducing significant weight gain. During the last decade, new antidiabetic compounds have been introduced on the market (e.g., short-acting and long-acting insulin analogs, thiazolidinediones, and prandial glucose regulators), but we need long-term data to ascertain the efficacy of these compounds.

The worldwide epidemic of type 2 diabetes is of great concern. The pharmaceutical industry is attempting to improve the current treatment concepts, as well as to search for new drugs (rev. in 67) to treat β-cell and α-cell dysfunction, insulin insensitivity, obesity, and so forth. What will probably be on the market within the next few years to primarily lower glycemia and to potentially reduce other features of the metabolic syndrome are new peroxisome proliferators–activated receptor (PPAR) agonists, agents focusing on the GLP-1 concept, and the amylin replacement concept.

One of the novel drugs is pramlintide, a synthetic amylin analog that can be used as a potential adjunctive therapy in patients with type 1 and type 2 diabetes. The rationale for amylin replacement has been defined during the past few years. Several large-scale phase III studies involving more than 3,000 diabetic individuals have demonstrated a beneficial effect of amylin replacement on the HbA_{1c} level in both type 1 and 2 diabetes without an increased number of hypoglycemic events and weight gain. In fact, a significant and sustained weight reduction for 1 year has been observed in type 2 diabetic patients. Pramlintide has been shown primarily to reduce prandial glucose excursions, which have been suggested to play a role in the development of cardiovascular complications, although this still needs to be proven. However, it would be important to further characterize responders to amylin analogs versus nonresponders, in terms of both reduction in HbA_{1c} and weight loss, in order to delineate the group of patients who will profit from treatment. Whether amylin agonists given at bedtime to type 2 diabetic patients could induce "β-cell rest," analogous to somatostatin and potassium channel openers, also remains to be determined. Pramlintide is currently under evaluation for approval as an adjunctive therapy for insulin-treated diabetic patients. In the future, insight into the human genome will hopefully help us tailor the pharmacological treatment in diabetes. In the meantime, all antidiabetic compounds that prove to be efficient and safe should be greeted with open arms.

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