Treatment of Type 1 Diabetic Patients with Glucagon-Like Peptide-1 (GLP-1) and GLP-1R Agonists

Urd Kielgast1,*, Jens J. Holst2 and Sten Madsbad1

1Department of Endocrinology, Hvidovre Hospital and University of Copenhagen, Denmark
2Department of Biomedical Sciences, Panum Institute, University of Copenhagen, Denmark

Abstract: GLP-1 (glucagon-like peptide-1) is a peptide hormone secreted from endocrine cells in the intestinal mucosa in response to meals. The major effects of GLP-1 are to increase glucose-induced insulin secretion and reduce glucagon release, but GLP-1 also inhibits gastric emptying rate and reduces appetite and bodyweight in obese subjects. In vivo studies using animal models of type 2 diabetes and in vitro studies using human islet cells have suggested that GLP-1 or GLP-1 analogues are also able to increase β-cell mass, but in animal models of type 1 diabetes, there is much less evidence for a β-cell preserving effect. This review summarizes the present knowledge of GLP-1 and its analogues regarding its role as a possible treatment in patients with type 1 diabetes. The studies that address the effect of GLP-1 and GLP-1 analogues on β-cell mass in both type 2 and type 1 diabetes, as well as the potential of GLP-1 as an adjuvant therapy in islet cell transplantation, will be reviewed. Suggestions for future studies of GLP-1 treatment in type 1 diabetes may include early treatment in order to preserve β-cell mass and prolong the remission period, but should also take a potential insulin sparing effect and changes in the risk of hypoglycemia into account.

Keywords: Type 1 diabetes, GLP-1, Glucagon, β-cell mass, Residual insulin secretion, Glycemic control.

INTRODUCTION

Several glucagon-like peptide-1 (GLP-1) receptor agonists are currently in clinical development, and the “first in class” agonist, exenatide, (Byetta*), has been approved in US and Europe for the treatment of people with type 2 diabetes. Treatment with GLP-1 receptor agonists has been shown to improve glycemic control and to induce weight loss in overweight subjects with type 2 diabetes. In animal and human studies, GLP-1 receptor agonists have displayed beneficial effects on pancreatic β-cell function that could be of interest in relation to people with type 1 diabetes [1, 2]. A cure for type 1 diabetes may include halting the autoimmune insult to the pancreatic β-cells and restoring insulin secretion by expanding β-cell mass by β-cell-regeneration. Immuno-suppression initiated at the onset of type 1 diabetes has been shown to preserve β-cell function, but is associated with significant toxicities [3, 4]. Other studies using nicotinamide and parenteral insulin have failed to prevent development of type 1 diabetes [5, 6]. The aim of this review is to summarize the present knowledge of the secretion and action of the incretin hormone GLP-1 in people with type 1 diabetes and to suggest a future role for GLP-1 as a possible treatment of subjects with type 1 diabetes.

THE NATURAL HISTORY OF THE RESIDUAL β-CELL FUNCTION IN TYPE 1 DIABETES

Type 1 diabetes is a condition in which pancreatic β-cell destruction usually leads to absolute insulin deficiency [7]. The pathogenesis of type 1 diabetes involves a T-cell mediated autoimmune attack on β-cells as well as a B-lymphocyte response with production of antibodies against the β-cell in susceptible individuals [7]. Type 1 diabetes becomes clinically manifest when the number of β-cells drops below the threshold required for the secretion of sufficient insulin to maintain a normal glucose tolerance [7]. By the time of diagnosis, most patients with type 1 diabetes still have the capacity to secrete insulin in amounts corresponding to 20-30% of that of non-diabetic individuals, indicating that there is a potentially expandable β-cell mass [8]. After initiation of insulin treatment and control of hyperglycemia, there is a short period of improvement in function of the remaining β-cells (the remission period), and insulin treatment can be paused in 10-20% of patients without loss of glycemic control [9]. However, the destruction of β-cells continues, and after 5 years, the prevalence of residual β-cell function declines to about 15%, with a stimulated C-peptide value of about 5-10% of that of non-diabetic individuals [9]. The β-cell destruction is more rapid when onset of diabetes takes place at a young age compared with older individuals [9]. A residual insulin secretion is of great importance in subjects with type 1 diabetes, since the need for exogenous insulin is reduced and glycemic control is better than in subjects without residual β-cell function [9]. Furthermore, the counter-regulatory response to hypoglycemia is more normal than in subjects without β-cell function because of a better glucagon response and thereby, the risk of severe hypoglycemia is reduced [9]. Post hoc analysis of the Diabetes Control and Complications Trial (DCCT) has indicated that diabetic complications may also be improved by therapies that preserve β-cell function [10, 11]. The amount of β-cell function necessary to confer these clinical benefits is relatively small and is present in most patients during the first two years after diagnosis [10, 11]. A clinically meaningful stimulated C-peptide level is about 5-10% of that of non-diabetic individuals [11].
GLP-1 is an incretin hormone, which is secreted from endocrine L-cells in the intestinal mucosa in response to meals. The L-cells are located throughout the entire gut, but the density is highest in the distal ileum and colon [16, 17]. Intact GLP-1 is rapidly degraded by the ubiquitous enzyme, dipeptidyl peptidase 4 (DPP-4), resulting in a plasma half-life of only 1-2 minutes in humans [18]. It acts through interaction with the GLP-1-receptor present in pancreatic islets, kidney, in certain areas of the brain [1] and in the heart [19, 20]. GLP-1 has several actions in the body in relation to glucose regulation and weight control: It increases glucose-induced insulin secretion, suppresses glucagon secretion (also glucose-dependently), inhibits gastric emptying, reduces appetite and promotes satiety [21]. Many hormones are thought to have incretin effect [22], but at present the two most important incretins are GLP-1 and glucose dependent insulinoetric polypeptide (GIP) [1]. The overall incretin contribution to insulin secretion after a meal may be up to 70% of the total response [23]. Furthermore, GLP-1 enhances the biosynthesis of insulin [24] thereby replenishing intracellular stores of insulin. In rodent models of type 2 diabetes or insulin resistance, GLP-1 has been shown to promote β-cell neogenesis, proliferation and differentiation and inhibit apoptosis in vivo, thereby increasing β-cell mass [25-32]. Treatment with GLP-1 delays development of diabetes in animal models of both type 2 and type 1 diabetes [27, 33], and has been shown to increase insulin content and to promote recovery of β-cells. However, so far most studies have failed to show any effect on β-cell mass in animal models of type 1 diabetes [34]; an exception to this comes from a recent study where the combination of GLP-1 and gastrin increased β-cell mass and showed evidence of a down regulation of the autoimmune response in the NOD-mouse [35]. In addition, in vitro studies have shown that GLP-1 is able to inhibit apoptosis of human β-cells [29, 36]. The results of the studies that address the effect of GLP-1 on β-cell mass in diabetes will be discussed later.

Several studies have indicated that the incretin effect in patients with type 2 diabetes is impaired or absent [23]. This is primarily because of a severe defect in β-cell sensitivity to GIP, while the insulinoetric effect to GLP-1 is better preserved, although its potency in this respect is impaired [37]. Accordingly, infusion of GLP-1 resulting in supraphysiological plasma levels, can restore glucose-induced insulin secretion to normal levels [37]. An additional contributing factor to the impaired incretin effect in people with type 2 diabetes may be that the meal-induced GLP-1 secretion is attenuated in these subjects. These defects in both action and secretion of the incretin hormones are now generally considered to be secondary to the development of diabetes [1].

More recently, it has been suggested that GLP-1 also exerts favourable actions on cardiovascular function. Thus, GLP-1 treatment improves outcomes in models of cardiac injury such as experimental ischemia [38] and improves left ventricular function in patients following acute myocardial injury [39] as well as in patients with chronic heart failure [40] and treatment with GLP-1 has a favourable effect on endothelial function in human subjects [19]. In a recent study in mice with experimental myocardial infarction after permanent left anterior descending artery (LAD)-ligation, it was shown that seven days pre-treatment with liraglutide (a long-acting GLP-1 agonist with half life of 12-13 hours [41]), reduced risk of cardiac rupture, reduced infarct size by almost 30% and improved cardiac output and survival rates. These findings were independent of weight loss and were associated with increased expression of proteins involved in cardioprotective signalling pathways in the myocytes [42]. In another recent study of pigs, 75 minutes of experimental left circumflex coronary artery occlusion followed by a reperfusion-period of three days, treatment with exenatide given 5 minutes before start of the reperfusion period, reduced infarct size by 40% and prevented deterioration of diastolic and systolic function [43]. Other potential beneficial cardiovascular effects of GLP-1 include reduction in blood pressure [44] and an improved lipid profile [45]. GLP-1 analogues resistant to degradation by DPP-4, as well as DPP-4 inhibitors, are now used in the treatment of type 2 diabetes, and have been shown to reduce elevated fasting and postprandial plasma glucose levels and to improve long-term glycemic control. Additionally, the higher GLP-1 concentrations which can be obtained with GLP-1 agonists reduce appetite and promote weight loss [2], but are also associated with adverse events which are primarily gastrointestinal in nature, i.e. nausea, vomiting and diarrhoea. The DPP-4 inhibitors are weight neutral and without gastrointestinal side effects, which is most likely explained by the lower GLP-1 concentrations obtained during treatment [46]. Compared to patients with type 2 diabetes, little is known about the secretion, action and pathogenic role of GLP-1 in type 1 diabetes mellitus, and of special interest in relation to type 1 diabetes is the possible effect of GLP-1 on β-cell mass.

SECRETION AND ACTION OF GLP-1 IN TYPE 1 DIABETES

Only a few studies have addressed the secretion of incretin hormones under physiological conditions in people with type 1 diabetes, and their results are ambiguous. In an ICA (islet cell antibody) positive group of people disposed to develop type 1 diabetes (characterized by normal fasting, but elevated 2-hours OGTT glucose values, impaired first phase
insulin-response, lack of glucagon suppression following a glucose load and an impaired incretin effect), both fasting and postprandial plasma levels of GIP and GLP-1 were normal [47]. In 16 lean, primarily C-peptide negative type 1 diabetic patients with longstanding diabetes, fasting GLP-1 concentrations did not differ from those of normal subjects, whereas the GLP-1 secretion to a mixed meal was virtually absent [48]. In contrast, Vilsbøll and co-workers [49] found that the incremental response of both total and intact GLP-1 and GIP following a meal did not differ between C-peptide negative type 1 diabetic patients and a glucose tolerant control group, but that the fasting level of intact GLP-1 tended to be lower in the type 1 diabetic subjects. The authors speculated that this could be due to a negative feedback mechanism from exogenous insulin given 30 min prior to the meal, but also stated that effects of insulin on GLP-1 secretion are not clear. Thus, it has recently been claimed that human L-cells express insulin receptors and secrete GLP-1 in response to insulin in vitro and that a condition of insulin resistance is associated with a loss of this function [50].

GLYCEMIC EFFECTS OF GLP-1 IN THE FASTING STATE IN TYPE 1 DIABETES

In fasting subjects with type 1 diabetes, Creutzfeldt and co-workers found that a continuous infusion of GLP-1 (1.2 pmol/kg/min) resulting in pharmacological plasma levels, reduced fasting hyperglycemia from 13.4 to 10 mmol/l and the glucagon concentration by approximately 50%, whereas insulin levels were only slightly increased [51]. The patients had their normal dose of intermediate acting insulin reduced by 50% the night before the study, resulting in fasting hyperglycemia and hyperglucagonemia. In another protocol where patients were well insulinized and without fasting hyperglycemia, GLP-1 infusion influenced neither glucagon nor insulin concentrations, and glucose levels remained constant at 8 mmol/l in response to both GLP-1 and saline infusion [52].

GLYCEMIC EFFECT OF GLP-1 AND EXENDIN-4 IN THE POSTPRANDIAL STATE

Since type 1 diabetic patients in the remission phase and for the first 1-2 years after diagnosis still have a significant insulin responses to a meal, it is possible that an intact enteroinsular axis contributes to regulation of postprandial glucose excursions in recent-onset type 1 diabetes. Infusion of GLP-1 (1.2 pmol/kg/min) without concomitant insulin injections almost abolished plasma glucose excursions after a mixed meal in C-peptide positive patients [53], possibly due to a delayed gastric emptying and to suppression of postprandial glucagon levels. Plasma C-peptide and insulin levels tended to be lower during the GLP-1 infusion compared with a control day, likely explained by the lower glucose levels when GLP-1 was infused. In type 1 diabetic patients with a wide range of residual insulin secretion, a subcutaneous injection of 0.63 μg/kg (~190 pmol/kg) GLP-1 (a dose presumed to delay gastric emptying) given together with the usual dose of insulin attenuated the rise in postprandial glucose, and the human pancreatic polypeptide (HPP)-response (a marker of afferent vagal activity) was delayed. However, the incremental AUC levels for both glucagon and C-peptide were not different from the control day [54]. This indicates that the ability of GLP-1 to control postprandial glucose excursions in type 1 diabetes mellitus is also dependent on its ability to delay gastric emptying. Evidence of the glucose-lowering effect of GLP-1 in C-peptide negative subjects with type 1 diabetes comes from a study where a closed-loop insulin infusion system (artificial pancreas) was used to estimate the isoglycemic meal-related requirement of exogenous insulin: A continuous infusion of GLP-1 of 0.75 pmol/kg/min decreased the postprandial rise in blood glucose, reduced the calculated isoglycemic meal related insulin requirement by 61%, reduced plasma glucagon and increased glucose utilization [55]. The investigators suggested that GLP-1 might exert insulin-like or insulin sensitising effects on the peripheral tissues, but it is likely, however, that a reduced rate of appearance of nutrients in the gut was also of importance for the observed reduced insulin requirement. In another study of C-peptide negative type 1 diabetic patients, a dose GLP-1 0.63 μg/kg administered subcutaneously together with the usual dose of insulin before breakfast and lunch decreased AUC for plasma glucose, HPP and glucagon. During the following 5 days, treatment with GLP-1 maintained the improved postprandial glucose levels, but did not reduce either fasting blood glucose levels or insulin dosage [56]. Lastly, in type 1 diabetic patients with meal-stimulated C-peptide levels of less than 0.1 nM, a subcutaneous injection of 0.03 μg/kg exendin-4 (exenatide; a DPP-4 resistant GLP-1 receptor agonist with a half life of 3-5 hours) 15 minutes before a meal, together with the usual dose of insulin, normalized postprandial glucose levels. Plasma levels of acetaminophen, (a marker of gastric emptying rate), glucagon and HPP were reduced, whereas insulin levels were unaffected [57]. Therefore, in C-peptide negative type 1 diabetic patients, inhibition of gastric emptying, as well as reduction of glucagon levels, seem to explain the glucose regulating effect of GLP-1 during a meal, whereas in patients with residual β-cell function, the endogenous insulin secretion probably also is of importance.

EFFECT OF GLP-1 ON INSULIN RESISTANCE AND GLUCOSE EFFECTIVENESS IN TYPE 1 DIABETES

In type 1 diabetes, insulin sensitivity is initially reduced, but normalizes after improved glycemic control whereas glucose effectiveness (the ability of glucose per. se to suppress hepatic glucose production and to increase glucose uptake) seems to remain impaired [58]. In the study of Guttiani and co-workers previously mentioned, GLP-1 infusion appeared to increase glucose utilization during a hyperinsulinenic euglycemic clamp [55]. Accordingly, others have also found that insulin sensitivity improves [2, 59, 60] during treatment with GLP-1. However, later studies have indicated that GLP-1 has no effect on extrapancreatic glucose metabolism in type 1 diabetes [61], type 2 diabetes [62] or healthy humans [63]. In conclusion, at present, it is not clear whether the observed improvement in insulin sensitivity associated with GLP-1 treatment in some studies is a direct property of GLP-1 or is an indirect effect due to metabolic changes caused by reduction in bodyweight, glucose- and glucagon-levels or food intake.
EFFECT OF GLP-1 AND GLP-1R AGONISTS ON ß-CELL MASS IN ANIMAL MODELS OF DIABETES MELLITUS OR OBESITY AND IN HUMAN IN VITRO STUDIES

Since the effect of GLP-1 on the ß-cell mass is the most intriguing question in relation to treatment of type 1 patients with GLP-1 receptor agonists, the results from other animal models, in addition to those of type 1 diabetes, will be discussed.

The ß-cell mass reflects the balance between net destruction and regeneration, and has been examined in several animal models of diabetes. It is generally concluded that GLP-1-based treatments increase ß-cell mass in both type 2 diabetic and non-diabetic rodents through enhanced ß-cell proliferation, differentiation of new ß-cells from pancreatic duct progenitor cells and through inhibition of ß-cell apoptosis [1, 52, 64-68]. In vivo animal studies have shown that ten days treatment with exendin-4 in 90% pancreatectomized male Sprague-Dawley rats (a ß-cell deficient animal model) resulted in significantly lower glucose levels, increased insulin content and a 40% increased ß-cell mass compared with control animals when measured 2½ weeks after end of treatment [28]. Perfetti and co-workers [26] found that five days continuous infusion of GLP-1 to 22 month old glucose-intolerant Wistar rats increased ß-cell mass by almost 50%, increased total pancreatic insulin content and showed evidence of increased pancreatic cell proliferation and ß-cell neogenesis. Farilla and co-workers subjected diabetic Zucker Diabetic Fat (ZDF) rats (a rodent model of insulin resistance, hyperglycemia and diabetes) to two days continuous infusion of human recombinant GLP-1, and tested their response to an intra-peritoneal glucose tolerance test (IPGTT) performed four days after removal of the pump. GLP-1 induced a significant decrease of plasma glucose levels and an increase of insulin secretion. Ex vivo studies performed shortly after the IPGTT, showed an increase in islet cell proliferation, a reduction of cellular apoptosis and a 40% increased ß-cell mass [25].

Two weeks treatment with exendin-4 to six week old obese db/db (a rodent model of leptin resistance and obesity) and non-diabetic C57B1/6 mice delayed development of hyperglycemia and increased insulin concentrations in the db/db mice, and increased mean islet size in the C57B1/6 mice, but no other measures of the ß-cell mass were reported [69]. Six weeks treatment with two different doses of liraglutide to (yet) nondiabetic ZDF rats resulted in attenuated diabetes development, and after only two weeks (when animals were still normoglycemic), the ß-cell mass and proliferation were lower than in control animals that had begun to develop diabetes. After six weeks, when the treated animals were no longer completely normoglycemic, ß-cell proliferation was unaffected compared to controls but ß-cell volume fraction was increased. However, this difference disappeared when a second pair-fed control group with similar body weight, but with increased 24-hour glucose profiles, was used as comparator. Therefore, the in vivo effect of liraglutide on ß-cell mass may, in part, depend on the glycemic state of the animal, and the presence of normoglycemia may decrease the ability of liraglutide to increase ß-cell mass. There was no effect of liraglutide on ß-cell volume fraction or proliferation in a subgroup of 60% pancreatectomized Sprague-Dawley rats (a ß-cell deficient animal model) receiving liraglutide for 4 days [32]. Other investigators have treated obese Zuckerrats with exendin-4: After six weeks treatment, a decreased ß-cell mass was observed compared to pair-fed animals comparable with respect to food intake, body weight and glucose levels [30], but when ß-cell mass was related to the concomitant insulin sensitivity of the animals, the ß-cell mass was significantly increased in the exendin-4 treated animals compared with pair fed rats. Thus, exendin-4 increased ß-cell mass to a greater extent than would be expected when related to insulin resistance. In another study, two weeks treatment of liraglutide to ob/ob mice (a rodent model of leptin-deficiency and obesity) induced a borderline significant increase in ß-cell mass, while in db/db mice, treatment with liraglutide resulted in an increased proliferation rate as well as an increased ß-cell mass [31]. When GLP-1 was added to a cell culture of freshly isolated human islet cells, there was evidence of preserved cell morphology and function, decreased apoptosis, increased intracellular insulin content and of a potentiation of glucose-dependent insulin response associated with down regulation of active caspase-3 and up-regulation of bcl-2 [29]. In addition, it has also been demonstrated that GLP-1 prevents ß-cell apoptosis induced by elevated concentrations of glucose (glucotoxicity) and palmitate (lipotoxicity) in a culture of human ß-cells and that this effect was mediated through protein kinase B activation [36]. In a group of sixty-nine patients with type 2 diabetes one-year treatment with exenatide improved ß-cell function resulting in a 2.46-fold increase in glucose plus arginine stimulated C-peptide secretion compared with insulin Glargine [70]. Nevertheless, after a 4-week off-drug period, glycemic control and ß-cell function returned to pre-treatment values. The results indicate that one-year treatment induces functional improvement in ß-cell secretory capacity without any significant changes in ß-cell mass.

EFFECT OF GLP-1 AND GLP-1R AGONISTS ON ß-CELL MASS IN ANIMAL MODELS OF TYPE 1 DIABETES

Relatively few studies address the effect of GLP-1 on ß-cell mass and development/remission of diabetes in type 1 diabetic mice models. The idea in most of these studies is that pharmacological modulation of the ongoing immune attack combined with a purported “growth-enhancer” like GLP-1, could increase ß-cell mass in this disease. The NOD (non obese diabetic) mouse is a widely used rodent model of type 1 diabetes since it develops spontaneous autoimmune diabetes with many similarities to autoimmune type 1 diabetes in humans [71].

In a recent study, a 4-8 week continuous infusion of GLP-1 to pre-diabetic NOD-mice (with no food restriction) induced ß-cell neogenesis (increased number of cells in the duct epithelium and increased number of islet-cell-like-clusters), enhanced ß-cell proliferation and suppressed ß-cell apoptosis [33]. This was accompanied by an improved glucose tolerance test, a delayed onset of diabetes and a transient reduction in insulitis. Animals treated for 8 weeks did not develop diabetes by age 21 weeks compared with a 60% diabetes incidence in control animals. When exendin-4 was given to pre-diabetic, four week old NOD-mice, diabetes
development was delayed from age 21 to 29 weeks, and in all mice that had survived without diabetes until age 30 weeks, exendin-4 treated animals displayed significantly lower insulitis score, an increase in number of islets without infiltration and an increased β-cell mass compared with saline treated animals [72]. In another recent study, where new-onset diabetic NOD-mice were treated with the monoclonal antibody anti-CD3 with or without daily injections of exendin-4, the combination of the two treatments was associated with a significantly higher rate of remission (44%) than anti-CD3 alone (37%) or exendin-4 alone (0%). The effect was most pronounced in animals with lower blood glucose values at diagnosis, and was associated with an increased pancreatic insulin content and recovery of residual islets. However, there was no effect of exendin-4 on either β-cell area, replication or apoptosis, and there was no evidence that exendin-4 could modulate the ongoing auto immune response [34]. The combination of lisofylline (LSF) (a blocker of T-cell activation and cytokine production) and exendin-4 could control hyperglycemia in six out of nine newly diagnosed type 1 diabetic NOD-mice with free access to food [73]. In five of these, the effect lasted for more than six weeks after cessation of treatment. In contrast, neither LSF nor exendin-4 alone affected blood glucose levels differently from saline treated animals. The authors stated that the therapeutic effect was associated with improved glucose-stimulated insulin secretion and reduced β-cell apoptosis, but these effects were shown in vitro with isolated islets from euglycemic BALB/c mice, and not with the NOD-mice in whom the therapeutic effect was seen. Furthermore, exendin-4 plus LSF did not increase insulin secretion more than LSF or exendin-4 alone, whereas this combination reduced β-cell apoptosis to a greater extent than LSF or exendin-4 alone, and exendin-4 alone was no better than LSF alone with respect to insulin secretion, β-cell metabolism or apoptosis. Unfortunately, no direct measure of β-cell mass in NOD-mice was done in this study [73]. In 88% of overtly diabetic NOD-mice, treatment with the "general" immunosupressant anti-lymphocyte serum (ALS) plus exendin-4 induced complete remission of diabetes in 23 out of 26 mice, and these animals exhibited normalised glucose tolerance, increased insulin content of islets and improved insulin response to a glucose challenge. ALS alone induced remission in six of fifteen mice, but exendin-4 alone had no effect on glucose tolerance. Again, β-cell mass was not directly measured [74]. Very recently, it was found that three weeks treatment with gastrin (that induces β-cell neogenesis and growth via induction of PDX-1 in pancreatic duct cells) and GLP-1 in combination restored normoglycemia in seven out of seven mice, increased β-cell mass, pancreatic insulin content, the number of replicating cells in pancreatic ducts, and inhibited β-cell apoptosis in recently diabetic NOD-mice. In contrast, GLP-1 or (gastrin) alone did not affect blood glucose levels, insulin content or β-cell mass [35]. In the same study, syngeneic islet grafts in diabetic NOD mice, treated with vehicle or gastrin plus exendin-4, showed a shift in cytokine expression as well as evidence of a protection against apoptosis in the treated animals. None of the studies referred to above included a pair-fed control group.

The mechanisms by which GLP-1 receptor-agonism possibly could promote β-cell survival have been reviewed in detail elsewhere [65, 75, 76]. The precise mechanisms are poorly defined and several downstream transduction pathways are involved, but it seems that activation of the pancreatic and duodenal homeobox factor-1 (PDX-1) (which is an important transcription factor for pancreas development and β-cell survival) is essential for many of the proliferative and cytoprotective actions of GLP-1 [77]. Thus, it has been shown that exendin-4 directly stimulates the expression of pancreatic PDX-1 homeodomain protein in mice [69]. An in vitro study using rat insulinoma cells found that pre-treatment with GLP-1 strongly inhibited H2O2 induced apoptosis through an increase in cAMP and activation of protein kinase A (PKA), as well as through a phosphatidylinositol-3-kinase (PI3K)-dependent pathway, resulting in expression of several proteins involved in cell survival [78]. Another study, using rat INS-1E cells incubated with IL-1, TNF-α and interferon-γ, showed that pre-treatment with exendin-4 led to reduced cytokine-induced inhibition of protein kinase B (PKB) phosphorylation and was associated with reduced apoptosis and necrosis [79]. Furthermore, this protective effect of exendin-4 was abolished in cells expressing kinase-dead PKB. When β-cells are stressed by exposure to cytokines, activation of the c-Jun NH2-terminal (JNK) kinase pathway (a class of mitogen-activated protein kinases – MAPKs) promotes apoptosis through decreased levels of islet-brain 1 (a blocker of stress induced JNK-pathway) [80]. Accordingly, it has been shown in vitro, that exendin-4 protects β-cells against cytokine-induced apoptosis through inhibition of JNK signalling pathway [80]. Thus, GLP-1 seems to activate protective and to inhibit apoptotic pathways in vitro.

COMMENTS ON THE β-CELL PROTECTION STUDIES

GLP-1 receptor agonists seem to increase β-cell mass through increased proliferation as well as decreased apoptosis in animal models of type 2 diabetes in some, but not in all studies. Interpretation of the studies is generally hampered by the lack of pair-fed control groups with comparable weight loss and glucose levels. In type 1 diabetic animal models such as the NOD-mouse, GLP-1 has been shown to improve the diabetic status of the animals, but only in combination with an immuno-modulating treatment or gastrin. However, GLP-1 alone was able to increase β-cell mass in NOD-mice, if treatment was begun before occurrence of elevated glucose levels. In contrast, in diabetic NOD-mice, GLP-1 alone has no effect on β-cell mass, but has shown an effect on glucose control and on insulin content of existing β-cells. When GLP-1 was combined with gastrin, there was a convincing increase in β-cell mass in diabetic NOD-mice, which was not apparent when GLP-1 was combined with anti-CD3. Whether GLP-1 treatment has a direct effect on β-cell mass in type 1 or 2 diabetic, pre-diabetic or healthy rodents or whether the observed effects in some studies are, in part, based on other metabolic factors perhaps related to differences in body weight, food intake or glucotoxicity is unclear. Furthermore, it may be difficult to extend the results from rodent studies to humans since rodent β-cells have a much larger capacity for replication (5-10 fold increase in rodents after partial pancreatectomy whereas in humans the capacity is much lower) [81]. In addition, there may be different responses to hyperglycemia in terms of β-cell survival,
and this could affect the resulting β-cell mass differently in the two species. Thus, increasing glucose levels from 5.5 mmol/l to 8-11 mmol/l reduced in vitro β-cell apoptosis in rat [82], but induced it in human β-cells [83, 84]. It must also be kept in mind that glucose is the strongest stimulus for β-cell growth in rodents, and that GLP-1 (which reduces glucose levels) under some circumstances, thereby, may diminish glucose as a growth stimulus. In order to determine whether GLP-1 treatment has a direct effect on β-cell mass in the diabetic state, a pair-fed control group with similar changes of glucose levels (obtained with e.g. insulin) and weight should be included. Furthermore, great caution should always be taken when results from animal studies are translated into humans. There are several major incompatibilities in both innate and adaptive immunity between mice and humans [85]. This, as well as the inbred status of the NOD-mouse combined with a probably more heterogenic pathogenesis of the human disease, limits the usefulness of mouse models in the study of human type 1 diabetes [86].

**THE EFFECT OF GLP-1 TREATMENT ON ISLET FUNCTION AFTER ISLET CELL TRANSPLANTATION IN ANIMALS AND IN TYPE 1 DIABETIC PATIENTS**

Because of the anticipated effects of GLP-1 on β-cell proliferation, differentiation and apoptosis, its potential effect on transplanted islets is particularly interesting:

It has recently been shown that adenoviral-induced expression of prohormone convertase (PC) 1/3 (that converts proglucagon to GLP-1 in enteroendocrine L-cells) in the α-cells of the pancreatic islets increases the islet endogenous secretion of GLP-1, improves glucose-stimulated insulin secretion and protects against IL-1β-induced cell death after islet cell transplantation in a mouse model of type 1 diabetes [87]. In another study, there was evidence that exendin-4 could promote revascularisation and the formation of insulin-producing cells in the graft-bearing kidney in mice transplanted with fetal islet-cell clusters [88]. In human fetal pancreatic cells, exendin-4 up-regulates expression of PDX 1 and increases differentiation and maturation [89]. Inhibition of DPP-4 in streptozotocin treated mice undergoing islet transplantation fully regulated blood glucose in contrast to the control mice who rapidly lost their ability to control blood glucose [90]. A recent study, where human islet cells with "low endocrine cell purity" were implanted under the renal capsule of streptozotocin-treated NOD-mice, showed that the combination of GLP-1 and gastrin enhanced the correction of hyperglycemia, increased the insulin content in the human cell grafts, and increased levels of human C-peptide in the plasma of the animals [91]. In order to improve islet cell graft function in sixteen patients with islet allograft dysfunction requiring exogenous insulin, patients were treated with exendin-4 for about seven months and results showed that three patients (requiring low doses of insulin) could discontinue insulin, and the need for insulin was significantly reduced (overall by about 27%) [92]. Furthermore, there was a reduction in postprandial capillary glucose values and mean glucose area under the curve (AUC) after stimulation testing. Unfortunately, four patients (25%) discontinued because of side effects, and no testing of β-cell function was done after the end of treatment. From this study, a trophic effect of GLP-1 receptor agonism on the β-cells in type 1 diabetic patients cannot be determined. Ghofalli and co-workers found that three months treatment with exendin-4 stimulated insulin secretion and reduced insulin requirements (by about 39%) in eleven out of twelve type 1 patients after islet transplantation, but found no evidence of a trophic effect or of a beneficial effect on long-term glycemic control of this GLP-1-analogue since the effect on insulin secretion and insulin dose disappeared one month after end of treatment [93]. Finally, in a recent small non-randomized study of five type 1 diabetic patients treated with islet cell transplantation and exenatide, all were insulin-independent during an 18 month follow-up period. In contrast, only one out of five subjects treated with islet cell transplantation alone was free from insulin treatment. The exenatide treatment improved insulin secretion and suppressed postprandial glucagon secretion compared with control subjects [92, 94].

**TREATMENT OF TYPE 1 DIABETES WITH GLP-1: FUTURE PERSPECTIVES**

Based on the literature, GLP-1 treatment may be beneficial in controlling postprandial glucose excursions and fasting hyperglycemia in type 1 diabetes with or without residual β-cell function Fig (1). Whether treatment with GLP-1 can prevent the loss of β-cell mass in patients with residual β-cell function should be tested in a future clinical randomised trial including type 1 patients with short duration of disease. Optimally, the treatment with a GLP-1 receptor agonist should be started a few days after initiation of insulin treatment, when the acute hyperglycemic crisis has been stabilized. The arguments for the very early initiation of GLP-1 treatment are that GAD treatment in newly onset people with type 1 diabetes initiated six months or more after diagnosis did not show any protective effect on disease progression, whereas treatment initiated earlier improved β-cell function [95]. In the Canadian-European trial of cyclosporine-induced remission of type 1 diabetes, duration of disease of less than six weeks and less than two weeks of insulin treatment were associated with the degree of remission [3]. Another argument for initiating GLP-1 treatment as early as possible after diagnosis is that most type 1 diabetic patients display the maximal remission period one to four months after diagnosis [9], and that the greatest β-cell mass is present at the time of diagnosis. Indeed, the C-peptide response at diagnosis is positively correlated to the degree of remission after the start of insulin treatment [3, 9, 10, 95]. We also suggest that treatment with GLP-1 from the time of diagnosis could induce a faster and longer remission period than treatment with insulin alone, solely explained by the acute glucoregulatory effects of the GLP-1 receptor agonist via alterations of islet function and delayed gastric emptying Fig. (1) [1, 2, 9]. Possibly, during this period, more patients could discontinue insulin, reduce risk of hypoglycemia, and experience a better quality of life. Early intervention is of importance, since the total amount of β-cells is an important variable in relation to effects on glycemic control and the need for insulin. Nevertheless, another study of interest is to treat type 1 diabetes subjects without β-cell function, in order to elucidate whether it may be possible to re-establish some endogenous insulin secretion. The background for the last study is that autopsy studies in type 1 diabetic patients with long duration of disease have indicated that precursor cells to β-cell still
occur in the pancreas [96]. It should also be considered whether GLP-1 treatment could reduce exogenous insulin demand with maintained or improved glycemic control without an increased risk of hypoglycemia Fig. (1).

An interesting question in relation to the treatment of subjects with type 1 diabetes with GLP-1 is what happens during hypoglycemia. Several studies in glucose tolerant individuals and in patients with type 2 diabetes have shown that GLP-1 based therapy has no effect on the glucagon response to hypoglycemia [97, 98]. This is of importance in relation to type 1 patients with residual β-cell function and a preserved glucagon response during hypoglycemia. Some patients without residual β-cell function have no or a reduced glucagon response to hypoglycemia and therefore the effect of GLP-1 on α-cell function may be of minor importance [99]. The delayed gastric emptying is also a consideration since it could result in an attenuated uptake of carbohydrates after food intake during a hypoglycemic event, and thereby increasing the risk of severe hypoglycemia. Another issue is the effect of GLP-1 on appetite and weight. Most type 1 patients have lost weight before diagnosis and are, at time of diagnosis, normal or underweight. However, in clinical studies, treatment of type 2 diabetic patients with GLP-1 analogues has induced a weight loss of 2-3 kg during the first year of treatment [46] with the greatest loss in the patients with highest BMI. The weight loss in patients with a BMI< 25 kg/m2 was only about 1-2 kg, and in lean Japanese type 2 patients, the weight changes during treatment with the GLP-1 analogue liraglutide did not differ from placebo treatment [100]. Lastly, it must also be considered whether GLP-1 treatment could induce an immune attack on newly produced β-cells through increased exposure of antigens on the β-cell surface. Taken together, GLP-1 based treatment of patients with type 1 diabetes is worth testing in future clinical trials, given the present evidence of its glucose lowering properties independent of residual β-cell function, and because of its potential insulin sparing effects and possible concomitantly reduced risk of hypoglycemia.

Appropriate primary outcomes in a future placebo controlled trial with GLP-1 in people with type 1 diabetes could be glycemic control expressed by HbA1c and incidence of hypoglycemia, total daily dose of insulin and endogenous insulin secretion evaluated by plasma C-peptide.

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