Beta-cell-based Therapies for Type 2 Diabetes

a report by
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The World Health Organization (WHO) has predicted that 366 million people will have diabetes by 2030, which is an increase of over 100% from the figure for 2000.[1] Five to 10% of these individuals will have insulin-dependent type 1 diabetes, which is treated by subcutaneous injections or infusions of recombinant insulin to replace insulin lost by autoimmune destruction of pancreatic β cells. The other 90–95% will have type 2 diabetes, which occurs under conditions of insulin resistance coupled with β-cell failure and is often associated with obesity. Thus, by 2030 up to 350 million people worldwide will have type 2 diabetes and many will require therapies to improve β-cell function. This article will consider those therapies and discuss their modes of action.

Beta-cell Dysfunction in Type 2 Diabetes

It is now widely accepted that β-cell failure to secrete sufficient insulin to compensate for peripheral insulin resistance is an important feature of type 2 diabetes. In fact, β cells have a remarkable capacity to accommodate increased insulin requirements and it has been estimated that only 15–20% of obese individuals with severe insulin resistance become diabetic, highlighting the importance of β-cell compensation in maintaining normal glucose homeostasis.[2] An increase in the size and number of β cells to counteract insulin resistance is thought to occur in obese individuals who do not develop diabetes, but this β-cell adaptation does not take place in those individuals in whom type 2 diabetes occurs.[3] In support of this, a direct correlation between reductions in β-cell mass and the occurrence of type 2 diabetes has been observed in pathological investigations of pancreases retrieved from patients with type 2 diabetes.[4,5] Thus, when considering therapeutic intervention in type 2 diabetes, the agents of choice will be those that both stimulate insulin secretion in a glucose-

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dependent manner and maintain or enhance β-cell mass through either increased proliferation or decreased apoptosis.

Regulation of Insulin Secretion

The primary purpose of β cells is to synthesise and secrete insulin, which accounts for over 10% of a β-cell's total protein content. To appreciate how therapeutic agents can act on β cells to stimulate insulin secretion, it is first necessary to understand how β cells normally respond to elevations in blood glucose levels with regulated insulin release. The main elements of this process are shown in *Figure 1*. Thus, glucose is transported into β cells by the high-capacity glucose transporter (GLUT)-2 (GLUT-1 or GLUT-3 in humans), and the first stage of metabolism is via a pancreas-specific glucokinase that generates glucose-6-phosphate. The importance of this enzyme in insulin secretion has been demonstrated by recognising that glucokinase gene mutations are responsible for some cases of maturity-onset diabetes of the young.[6] Further glycolytic and oxidative metabolism of glucose results in the generation of adenosine triphosphate (ATP), which closes ATP-sensitive potassium (K) channels in the plasma membrane. The ensuing reduction in K^+ efflux depolarises the β-cell plasma membrane, leading to an opening of voltage-dependent calcium (Ca^{2+}) channels. This allows Ca^{2+} in the extracellular fluid to enter β cells down its concentration gradient and stimulate exocytotic release of stored insulin through interactions with Ca^{2+}-sensitive proteins such as Ca^{2+}/calmodulin-dependent protein kinases and syntaptotagmins.[7]

Although glucose is the major physiological stimulator of insulin secretion, it has long been recognised that oral glucose administration produces an insulin output three to four times greater than the same glucose load given intravenously,[8] indicating that agents released from the gut ('incretins') can potentiate the glucose-induced secretory response. Two such incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are released from intestinal L and K cells, respectively, and they stimulate insulin secretion in a glucose-dependent manner. GLP-1 and GIP receptors on β cells are coupled to adenylate cyclase, and GLP-1/GIP binding leads to elevations in cyclic adenosine monophosphate (cAMP) and activation of protein kinase A and exchange proteins activated by cAMP (EPACs), which ultimately increase insulin released into the circulation. Cholecystokinin, another incretin hormone released from I cells in the gut, acts at specific receptors to activate phospholipase C. The subsequent hydrolysis of phosphatidyl 4,5 bisphosphate to inositol 1,4,5-trisphosphate and diacylglycerol leads to Ca^{2+} mobilisation from intracellular stores and activation of protein kinase C enzymes. Ultimately, these pathways lead to an enhanced insulin secretory response to elevated blood glucose levels. In addition, the

neurotransmitter acetylcholine, released from islet parasympathetic nerve terminals in response to the sight and smell of food, activates phospholipase C after binding to muscarinic cholinergic receptors, and exerts similar potentiating effects to cholecystokinin.

Thus, it can be seen from *Figure 1* that agents activating pathways downstream of glucose metabolism or generating second messengers – which are coupled to potentiation of insulin secretion – may work effectively as β-cell-based therapies for type 2 diabetes. Appropriate profiles of insulin release will allow tight glycaemic control to minimise the complications that arise from long-term hyperglycaemia. The relative merits and potential disadvantages of the β-cell therapies discussed in this article are summarised in *Table 1*.

Therapeutic Agents that Depolarise Beta Cells

Sulphonylureas

Sulphonylureas (SURs) have a long history as successful therapeutic agents for type 2 diabetes and have been in commercial use for over 50 years. Glimepiride, a third-generation SUR in clinical use, is more potent than earlier-generation SURs, and has essentially the same mode of action as the others. Thus, they all bind to the SUR1 subunits of the octameric ATP-sensitive K^+ channel complex, and this brings about the same chain of events as ATP generation following glucose metabolism to stimulate insulin secretion (see *Figure 1*).

Concerns have been raised about SURs despite their widespread use over the past five decades and the inclusion of glibenclamide as one of only two orally acting diabetes therapeutics in the *List of World Health Organization Essential Medicines* (March 2007). The main problem is that SURs stimulate insulin secretion even in the absence of raised blood glucose levels, and this can lead to unexpected hypoglycaemic episodes. In addition, there is evidence that SURs can increase β-cell apoptosis *in vitro*,[9,10] and if this occurs *in vivo* it would be expected to exacerbate the decline in β-cell mass that has been observed in type 2 diabetes, as described above. Finally, SUR use has been associated with weight gain[11]

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and, given that a large proportion of individuals with type 2 diabetes are overweight or obese, their use should ideally be restricted to patients within the normal weight range.

Meglitinide Analogues

Meglitinide analogues, such as repaglinide and nateglinide, were developed to minimise glucose excursions after food intake, and they are sometimes referred to as 'prandial insulin releasers'. They share the benzamido moiety of the second-generation SUR glibenclamide, but lack its SUR group. Similar to SURs, meglitinide analogues are taken orally and they stimulate insulin secretion by depolarising β cells

Figure 1: Regulation of Insulin Secretion by Beta-cell-acting Therapies for Type 2 Diabetes
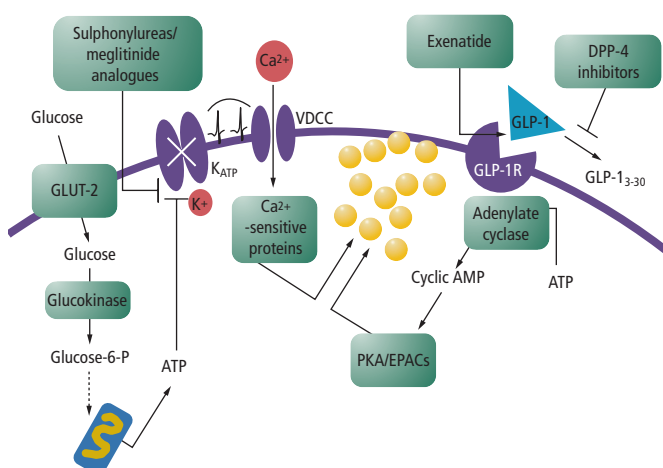
The figure shows the signalling pathways in a β cell that lead to exocytotic release of insulin-secretory granules in response to an elevation in circulating glucose concentrations, and indicates the sites of action of established β-cell acting therapies for type 2 diabetes. Glucose is transported into β cells by the glucose transporter (GLUT)-2 carrier and its metabolism leads to generation of adenosine triphosphate (ATP), which depolarises β cells by blocking ATP-sensitive potassium (K^+) channels (K_{ATP}) in the plasma membrane. Sulphonylureas and meglitinide analogues also block K^+ efflux through K_{ATP} channels, independently of glucose metabolism. Depolarisation leads to calcium (Ca^{2+}) ion influx through voltage-dependent Ca^{2+} channels (VDCC) and its interaction with Ca^{2+}-sensitive proteins leads to a fusion of insulin secretory granules with the plasma membrane and release of insulin into the circulation. Exenatide binds to glucagon-like peptide-1 (GLP-1) receptors (GLP-1R) to activate protein kinase A (PKA) and exchange proteins activated by cyclic adenosine monophosphate (cAMP) (EPACs) through adenylate cyclase-mediated generation of cAMP from ATP, and this results in enhanced release of insulin secretory granules in a glucose-dependent manner. Dipeptidyl peptidase-4 (DPP-4) inhibitors extend the half-life of endogenous GLP-1 by blocking its cleavage to the truncated, antagonistic GLP-1$_{3-30}$ peptide, allowing native GLP-1 to activate the signalling cascade described above for exenatide. DPP-4 inhibitors also inhibit cleavage of the insulinotropic peptides gastric inhibitory polypeptide (GIP) and pituitary adenylate cyclase-activating peptide to GIP$_{3-42}$ and PACAP$_{3-38}$, respectively (not shown).

through direct closure of ATP-sensitive K^+ channels. They exert their effects by binding to the benzamido site of the SUR1 complex, whereas SURs may bind to both benzamido and SUR1 domains.

The depolarising mode of action of glinides means that they too may cause hypoglycaemia by stimulating insulin exocytosis in the absence of a glucose stimulus. This is minimised by users taking their medication immediately before food intake and by the rapid hepatic metabolism of glinides to inactive metabolites. Weight gain is not considered to be a major problem with use of repaglinide and nateglinide, and there have been no reports to date on the effects of these agents on β-cell mass.

Incretin-based Therapies

Exenatide

It has been known for some time that an injection of the gut hormone GLP-1 into individuals with type 2 diabetes before a meal improves insulin and C-peptide responses and reduces the post-prandial increase in plasma glucose levels, but the short half-life of GLP-1 precludes its use as a diabetes therapy. Exenatide (Byetta) is a synthetic version of exendin-4, a GLP-1 analogue that is naturally secreted in the saliva of the Gila monster lizard. It has ~50% amino acid homology with GLP-1, binds to GLP-1 receptors and has a longer half-life than native GLP-1 *in vivo* as it is resistant to degradation by dipeptidyl peptidase-4 (DPP-4). Exenatide is a recent addition to the type 2

Table 1: Advantages and Disadvantages of Beta-cell Therapies

	Insulin Secretion	Beta-cell Mass	Weight Control	Other Potential Benefits	Potential Side Effects/ Disadvantages
Sulphonylureas	Glucose-independent	Decreased mass (induces β-cell apoptosis *in vitro*)	Weight gain	Have been in use for over 50 years, so long-term effects have been established; relatively cheap	Hypoglycaemia
Meglitinide analogues	Glucose-independent	Unknown	Weight-neutral		Hypoglycaemia
Exenatide	Glucose-dependent	Increased mass (stimulates β-cell replication and neogenesis in rats)	Weight loss	Inhibition of glucagon secretion; delayed gastric emptying	Link to acute pancreatitis; administration by subcutaneous injection
Dipeptidyl peptidase-4 inhibitors	Glucose-dependent	Increased mass (stimulates β-cell replication and neogenesis; inhibits apoptosis in rats)	Weight-neutral	Inhibition of glucagon secretion	Harmful effects of stabilisation of non-insulinotropic peptides? Insufficient data on long-term safety; relatively expensive
Thiazolidinediones	No direct effect?	Increased mass (reduces β-cell death in rats)	Weight gain	Exert insulin sensitising effects as well as improving β-cell function	Increased incidence of bone fractures and risk of heart attack

2 diabetes therapeutic armoury, having been introduced for clinical use in the US in 2005 and in the UK in 2007. As outlined above, GLP-1 receptors are coupled to increases in cAMP in β cells, and it is likely that this is the mechanism through which GLP-1 stimulates insulin secretion, although it has been reported to close ATP-sensitive K^+ channels in rat β cells in a cAMP-independent manner.[12]

Direct stimulation by exenatide of insulin secretion from glucose-unresponsive islets isolated from type 2 diabetic donor pancreases has recently been demonstrated, indicating its beneficial effects in increasing β-cell glucose sensitivity.[13] The requirement for elevated glucose levels for enhanced secretory output in response to incretins is one of the major therapeutic advantages over the β-cell depolarising

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agents described above, and means that exenatide therapy is not associated with the hypoglycaemic episodes that may occur with the use of SURs or meglitinide analogues. Exenatide has the additional advantage, at least in rodents, of increasing β-cell mass by stimulating replication of existing β cells and generation of new β cells,[14] and its use is also accompanied by weight reduction,[15] again providing a distinct advantage over SUR therapy. Furthermore, exenatide is reported to delay gastric emptying and decrease glucagon secretion from islet β cells,[16] both of which will be beneficial in maintaining normoglycaemia.

Exenatide certainly appears to be a model therapy in terms of its insulin secretagogue and β-cell-mass-enhancing effects. However, similar to insulin, exenatide is a peptide that would be degraded in the gastrointestinal tract if taken orally, so it is administered subcutaneously

twice daily. This route of administration may make it less attractive, but a once-weekly long-acting formulation (Byetta LAR) is undergoing a clinical trial with promising data, including a 1.7% reduction in glycated haemoglobin (HbA$_{1c}$) levels and a 3.8kg weight loss following use after 15 weeks.[17] One potentially serious drawback of exenatide therapy has been its recent link to acute pancreatitis in a small proportion of users, and the US Federal Drug Administration (FDA) issued a safety alert regarding its use in October 2007.

Dipeptidyl Peptidase-4 Inhibitors

Observations of the beneficial effects of exenatide on β cells and weight loss were paralleled by the development of therapeutic agents to extend the half-life of endogenous GLP-1 and thus circumvent the requirement for an injectable peptide therapy. This resulted in the introduction of a new class of oral β-cell-acting type 2 diabetes therapeutics such as sitagliptin (Januvia) and vildagliptin (Galvus), collectively known as DPP-4 inhibitors. Sitagliptin was first marketed in the US in 2005, vildagliptin was approved for use in Europe in February 2008 and alogliptin and saxagliptin are currently undergoing phase III clinical trials.

Under normal circumstances, circulating DPP-4 rapidly cleaves two amino acids from the N-terminus of insulin secretagogue incretins such as GLP-1, GIP and pituitary adenylate cyclase-activating polypeptide (PACAP), and the truncated peptides antagonise the effects of the native incretins. DPP-4 inhibitors maintain incretin levels and decrease production of truncated peptides, and their use is clinically associated with increased insulin and decreased glucagon,[18] as would be expected from elevated levels of active GLP-1. Also consistent with an elevation in active GLP-1, DPP-4 inhibition is reported to enhance β-cell mass in diabetic rats by stimulating β-cell replication and neogenesis and inhibiting apoptosis.[19] However, there have been no reports of weight loss in individuals prescribed DPP-4 inhibitor monotherapy, perhaps because endogenous GLP-1 levels obtained with these agents do not reach the relatively high levels required to enhance satiety and suppress food intake.[20]

DPP-4 inhibitors are proving to be effective and well-tolerated diabetes therapies with a capacity to both stimulate insulin secretion in a glucose-dependent manner and increase β-cell mass, at least in

animals. In addition, their once-daily oral administration makes them an attractive alternative to injectable GLP-1 analogues, although they do not show the beneficial effects on weight loss that exenatide does. However, it should be borne in mind that DPP-4 is responsible for degradation not only of insulinotropic incretins but also of a wide range of other regulatory peptides, including neuropeptide Y, peptide YY, GLP-2, growth-hormone-releasing hormone and macrophage-derived chemokine.[21] This raises the possibility that the inhibitors may exert effects unrelated to glucose homeostasis regulation, and elevated GLP-2 levels with DPP-4 inhibitors have been shown to increase the risk of tumour growth and metastasis *in vitro*.[22] In addition, although vildagliptin has been approved for European use, approval by the FDA has been delayed pending demonstration that the reduced kidney function seen in animal studies does not occur in human trials of individuals with renal impairment.[23]

Other Possible Beta-cell Therapies

Thiazolidinediones

Thiazolidiniediones (TZDs), such as rosiglitazone and pioglitazone, are a relatively new class of antidiabetic drug that have been used clinically for less than a decade. They were introduced to improve insulin sensitivity in patients with type 2 diabetes, and TZDs are effective by binding to the nuclear peroxisome proliferator-activated receptor-γ (PPARγ) to stimulate transcription of anabolic genes involved in insulin action. TZDs are thought to primarily act in adipose tissue, skeletal muscle and the liver, but human β cells also express PPARγ receptors,[24] and two separate clinical studies have suggested that TZDs improve β-cell function in individuals with type 2 diabetes.[25,26] Thus, it is possible that TZDs act not only as insulin sensitisers but also as stimulators of insulin secretion, either through direct interaction with β-cell PPARγ receptors or through the release of adipocytokines such as adiponectin, which can potentiate glucose-stimulated insulin

secretion in insulin-resistant mice.[27] Furthermore, rosiglitazone is reported to induce significant decreases in β-cell death and increases in β-cell function in Zucker diabetic fatty rats,[28] suggesting that it may also have a β-cell-mass-protecting effect in humans. However, although these data are promising, these potential advantages need to be considered in the context of safety concerns about rosiglitazone use. In February 2007, the FDA issued an alert following observations of an increased incidence of bone fractures in women, and the FDA has been monitoring heart-related adverse events in patients prescribed rosiglitazone.

Summary

Clinicians are faced with difficult choices when deciding the most appropriate therapy for individuals with type 2 diabetes who do not respond to lifestyle intervention with reductions in HbA_{1c} levels. As recently as 2005, the International Diabetes Federation (IDF) Global Guideline for type 2 diabetes recommended SURs as the first-choice β-cell-acting therapy (www.idf.org). However, the recent introduction of incretin-based therapies suggests that either GLP-1 analogues or DPP-4 inhibitors may be a preferred choice to SURs in that they stimulate insulin secretion only in the presence of elevated blood glucose levels and they may also protect against β-cell-mass reduction. Furthermore, observations of beneficial effects of TZDs on β cells may mean that prescribing TZDs results in an improvement of both insulin sensitivity and β-cell function. Ultimately, however, when considering which β-cell acting therapy to prescribe, the decision is likely to take into account the relatively cheap cost of SURs, their long history of successful use and the potentially serious safety concerns of newly introduced therapies. Of course, this article has focused solely on the use of β-cell therapeutics as monotherapies, but β-cell-depolarising agents and incretin-based therapies are even more effective when used in combination with a therapy that combats insulin resistance, such as metformin. ■

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