

THERAPEUTIC PERSPECTIVES FOR TYPE 2 DIABETES MELLITUS: MOLECULAR AND CLINICAL INSIGHTS

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SUMMARY - Current antidiabetic agents do not suppress insulin resistance, do not reinstate physiological insulin secretion and fail to prevent the gradual loss of β -cell function. Thus, these molecules are unable to maintain long term euglycemia in all type 2 diabetic patients and there is a need for new antidiabetic drugs. Thiazolidinediones (TZD) are a new class of insulin sensitizers recently approved in Europe, in combination therapy with sulfonylureas or/and metformin, for the treatment of type 2 diabetes. TZD show beneficial effects on insulin action, glucose homeostasis and lipid metabolism despite a substantial weight gain. Their potential protective effect on β -cell function and on the development of macrovascular complication is of particular interest. Non TZD PPAR γ agonists are also under clinical trials. Other interesting therapeutic perspectives to treat insulin resistance lie in the development of inhibitors of protein tyrosine phosphatases and in the promotion of non insulin-dependent contraction-like muscle glucose uptake via stimulation of AMP protein kinase (AMPK). As to new insulin secretagogues, the phenylalanine derivative nateglinide is a first phase insulin secretion enhancer primarily intended at controlling post-prandial hyperglycemia. The most promising perspective to improve β -cell function lies in the development of glucagon-like peptide-1 (GLP-1) analogs. Clinical studies show beneficial effects on glucose homeostasis in type 2 diabetics and efficacy in sulfonylurea resistant patients without risk of hypoglycaemia. Animal studies predict beneficial effects on β -cell mass. Finally we will discuss the potential use of gene therapy to treat insulin resistance and β -cell dysfunction.

Key-words: type 2 diabetes, insulin resistance, treatment, thiazolidinediones, glucagon-like peptide-1.

RÉSUMÉ - Perspectives thérapeutiques dans le diabète de type 2. Aspects cliniques et moléculaires.

L'efficacité des agents antidiabétiques oraux actuellement disponibles pour traiter le diabète de type 2 est limitée pour plusieurs raisons: d'abord, ils ne suppriment pas l'insulinorésistance et ne restaurent pas une fonction normale à la cellule β . Par ailleurs, ils ne préviennent pas le déclin progressif de la fonction des cellules β . Ces agents sont donc incapables de maintenir l'euglycémie à long terme chez tous les patients et il devient nécessaire de découvrir de nouvelles molécules plus efficaces. Les thiazolidinediones (TZD) appartiennent à une nouvelles classe de molécules sensibilisant les tissus à l'insuline et ont récemment eu l'autorisation de mise sur le marché en Europe en combinaison avec la metformine ou les sulfamides hypoglycémisants. Les TZD améliorent la sensibilité à l'insuline, réduisent la glycémie et améliorent le bilan lipidique des diabétiques au prix d'une prise de poids substantielle. Leur potentiel effet protecteur à long terme sur le fonctionnement des cellules β et sur la progression de la macroangiopathie est particulièrement intéressant. Les agonistes de PPAR γ non TZD de même que les agonistes mixtes PPAR γ et PPAR α sont également en cours d'investigation. Pour traiter plus spécifiquement l'insulinorésistance, le développement d'inhibiteurs des protéines tyrosine phosphatases et la stimulation du captage musculaire de glucose par un mécanisme similaire à la contraction musculaire et impliquant la protéine kinase AMP-dépendante (AMPK) sont également des perspectives intéressantes en cours d'investigation chez l'animal. En ce qui concerne les nouveau insulinosécrétagogues, le nateglinide stimule l'insulinosécrétion en réponse au glucose d'une façon physiologique et entraîne un bon contrôle de la glycémie post-prandiale. La perspective la plus prometteuse pour améliorer la fonction des cellules β réside dans le développement des analogues du glucagon-like peptide-1 (GLP-1). Les études cliniques montrent un effet bénéfique sur le contrôle de la glycémie chez les diabétiques de type 2 et une efficacité chez les patients échappant aux sulfamides. Les études chez l'animal prédisent également un effet trophique sur la masse des cellules β . Finalement nous discutons des développements potentiels de la thérapie génique pour traiter l'insulinorésistance et les déficit insulinosécrétoires chez les diabétiques de type 2.

Mots-clés: diabète de type 2, insulinorésistance, traitement, thiazolidinediones, glucagon-like peptide-1.

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Type 2 diabetes mellitus (T2DM) is among the most common chronic disease, affecting about 5% of the population of Western countries. The prevalence of this disease has increased rapidly over the past fifty years, especially in the United States of America. Indeed, it has been estimated that the number of people affected with type 2 diabetes worldwide will increase from 135 million to over 300 million by 2025, with the most of this increase occurring in developing countries [1]. Although there has been long debate about the value of treatment of type 2 diabetes, the recently completed UKPDS has conclusively demonstrated that improved glucose control of type 2 diabetes reduces the incidence of long term complications and prolongs life [2].

Current antidiabetic agents offer a wide range of metabolic actions to reduce hyperglycaemia [3] but the efficacy of such drugs is compromised for several reasons. First they do not suppress insulin resistance and they do not restore normal β -cell function. Second, they do not reverse the deleterious effects of chronic hyperglycaemia and hyperlipidemia, i.e. glucose- and lipotoxicity. Finally and most importantly, they do not prevent the gradual loss of β -cell function and mass which is known to account for the gradual deterioration of glucose homeostasis [4]. Cellular and molecular defects underlying insulin resistance and β -cell dysfunction in type 2 diabetes are still not clearly defined. Whether these abnormalities are genetically determined, acquired or both, the disease involves multiple signalling defects in target tissues and multiple signalling defects of stimulus-secretion coupling within the β -cells. Thus, it is not possible to isolate a single drug target to reverse all aspects of the disease. Rather, it seems more promising to develop drugs aimed at correcting specific metabolic abnormalities or specific signalling defects. This review, which is not exhaustive, will focus on new antidiabetic drugs recently licensed for the treatment of type 2 diabetes as well as on molecules currently in final clinical trials and which will soon be released in Europe. We will also discuss promising therapeutic perspectives for type 2 diabetes based on molecular targets that are of particular importance, given the pathogenesis of this disease.

■ INSULIN SENSITISERS

The growing incidence of sedentary lifestyle and obesity, both leading to insulin resistance, is directly responsible for the threatening epidemics of type 2 diabetes around the world. Thus, insulin resistance with its associated lipid abnormalities has become a major challenge in the new millenium for the treatment of T2DM. This has prompted pharmaceutical companies to search for new insulin sensitising agents.

Thiazolidinediones

Before the introduction of thiazolidinediones (TZD) in 1997, metformin was considered as the only drug able to sensitise target tissues to insulin. TZD are a new class of antidiabetic agents and include three compounds that have come to clinical use: troglitazone (Rezulin®), pioglitazone (Actos®) and rosiglitazone (Avandia®). TZD were initially discovered by screening compounds for a hypoglycaemic action in the diabetic obese diabetic (ob/ob) mouse, and subsequently they were shown to improve insulin action in a variety of insulin resistant obese and diabetic animal models [5]. In all these models, TZD improve plasma glucose and insulin levels and correct some of the abnormalities in lipid metabolism. In the obese Zucker fatty (ZF) rat, an animal model of T2DM, the combined therapy of chronic exercise and TZD completely normalize insulin sensitivity [6]. TZD improve insulin action by binding to a nuclear receptor, the nuclear peroxisome proliferator activated receptor γ (PPAR- γ) which heterodimerize with retinoid X receptor and subsequently increases the transcription of a variety of insulin sensitive genes responsible for adipocytes differentiation, glucose and lipid metabolism [7] (*Fig. 1*). In adipose tissue, where PPAR γ is most strongly expressed, stimulation by TZD promotes the expression of genes encoding lipoprotein lipase, fatty acid transporter protein, fatty acid binding protein, fatty acid synthase and the insulin sensitive glucose transporter GLUT 4 [7] (*Fig. 1*). Expression of these genes promotes adipose glucose, free fatty acids (FFA) and triglyceride (TG) uptake. The resulting fall in circulating lipids prevents excessive accumulation of TG in muscle and liver, thereby improving muscle glucose uptake [8, 9] and limiting liver steatosis [10]. Thus, the hypoglycaemic effect of TZD result from a direct increase in fat glucose uptake and an indirect enhancement of glucose uptake in other insulin sensitive tissues (through improvement of the glucose-FFA/Randle cycle). Indeed, the hypoglycaemic effect of TZD is dependent on white adipose tissue (WAT) and is lost in transgenic mice totally lacking WAT [11]. In pancreatic β -cells, the reduced deposition of lipids limits lipotoxicity. Treatment of Zucker fatty rats with TZD reduces lipotoxicity-induced loss of pancreatic β -cell mass by reducing β -cells apoptosis [12, 13]. It is noticeable that TZD also have beneficial vascular effects. They inhibit mesangial cell proliferation, the generation of reactive oxygen species and improve fibrinolysis [14, 15]. Indeed, troglitazone inhibits formation of early atherosclerotic lesions in LDL receptor-deficient mice, an animal model of premature atherosclerosis [16]. TZD also upregulate the CD36 scavenger receptor and thereby limit the development of lipid-accumulating macrophages in mice coronary arteries. In addition, they induce cholesterol removal from human macrophage foam cells [17, 18]. Thus,

TZD might directly prevent the progression of macroangiopathic complications.

Consistent with animal studies, clinical studies have shown that treatment of type 2 diabetic patients with TZD decreases serum glucose and insulin levels and increases peripheral glucose uptake. Clamp studies comparing the effect of troglitazone to metformin in type 2 diabetics have shown that while both drugs have equal and additive effect on glycaemic control, metformin acts primarily by decreasing hepatic glucose production and troglitazone by increasing the rate of peripheral glucose disposal (represented by skeletal muscle in clamp studies) [8] (Fig. 2). TZD have relatively little effect on hepatic glucose output in humans [8, 19]. TZD also produced a sustained improvement in β -cell responsiveness and function in humans with type 2 diabetes as assessed by HOMA [20, 21]. These beneficial effects on glucose homeostasis results in an average 1.5% to 2.5% fall in HbA1c relative to placebo following long term treatment with TZD [3, 22-26]. Lipid metabolism is also profoundly affected during long term treatment with TZD. Patients with T2DM often have elevated TG and low HDL-cholesterol (HDL-C). Pioglitazone treatment reduces TG and increases HDL-C without changing LDL-C concentrations [23, 25-27]. However, treatment with rosiglitazone raises LDL-C and is without effect on TG [22, 24, 28] Long term follow up will tell if this has a negative impact on the progression of atherosclerosis. Interestingly, chronic treatment of obese patients with TZD induces a redistribution of fat from insulin resistant visceral adipose depots to insulin sensitive subcutaneous adipose depots [29]. Since visceral adiposity is linked with the syndrome of insulin

resistance and accelerated atherosclerosis, this is another mechanism by which TZD might reduce cardiovascular risk.

Troglitazone was first introduced in the USA in 1997 in the treatment of type 2 diabetes mellitus and was withdrawn in March 2000 when the FDA received report of 61 deaths from hepatic failure and seven liver transplant associated with the drug. Troglitazone has been replaced by two more potent agents in terms of activation of PPAR γ , pioglitazone and rosiglitazone. Rosiglitazone and pioglitazone were both launched in Europe and the UK in July and November 2000 respectively. Unlike in the USA were TZD are licensed for use as monotherapy, in Europe, these drugs have been granted limited indications in defined circumstances: In combination with metformin in obese type 2 diabetic patients with insufficient glycaemia control or in combination with sulfonylureas if metformin is either not tolerated or contraindicated. Clinical trials show that combination therapy using TZD with metformin or a sulfonylurea is particularly effective in lowering blood glucose [3, 22, 24-26]. Reassuringly, extensive use of pioglitazone and rosiglitazone in humans has produced no evidence of hepatic toxicity [22-26, 28]. Pioglitazone has the advantage over rosiglitazone to be a once-daily drug. Interestingly, these drugs provide effective glycemic control without evidence of hypoglycaemia [22-26, 28]. As a result of the adipogenic and lipogenic effect of these drugs, a substantial weight gain (5%) is an undesirable side effect. However, because of the recruitment of small, highly insulin sensitive adipocytes, and because of the lipid lowering effect (see above), the increased fat mass results in increased insulin sensitivity. Of note, there is no blood glucose response to these drugs in one quarter of patients.

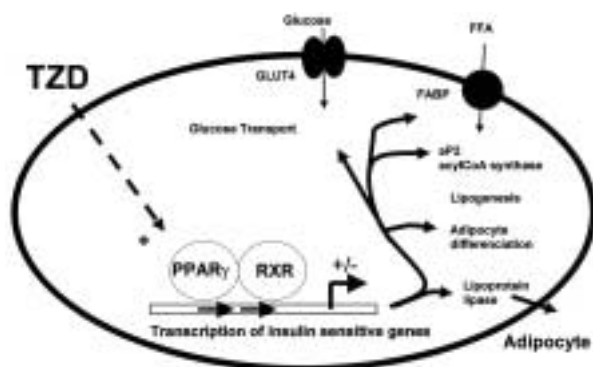


FIG. 1. Molecular mechanisms of TZD action in adipocytes. TZD improve insulin action by binding to the nuclear peroxisome proliferator activated receptor γ (PPAR- γ) which heterodimerize with the retinoid X receptor (RXR) and subsequently increases the transcription of a variety of insulin sensitive genes such as lipoprotein lipase, fatty acid transporter protein, fatty acid binding protein (FABP), fatty acid synthase and the insulin sensitive glucose transporter GLUT 4. Expression of these genes promotes glucose, free fatty acids (FFA) and triglyceride (TG) uptake.

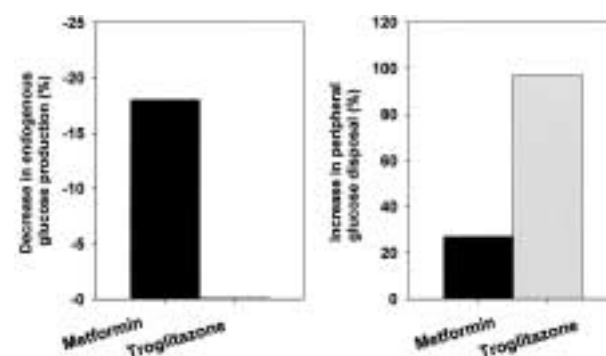


FIG. 2. Mechanisms responsible for the hypoglycaemic effect of troglitazone compared to metformin.

Changes in endogenous glucose production (left panel) and glucose disposal rate (right panel) under hyperinsulinemic clamp conditions after 3 months of therapy with metformin or troglitazone. Metformin acts primarily by decreasing hepatic glucose production and troglitazone by increasing the rate of peripheral glucose disposal (represented by muscle in clamp study). Troglitazone has relatively little effect on hepatic glucose output in humans. Adapted from [7].

These non responders are more obese, have a longer standing insulin resistance and have depleted pancreatic insulin reserves [30]. Although TZD have been associated with cardiac hypertrophy when used at high doses in animal studies, this has not been seen in clinical trials [30]. Nevertheless, TZD are contraindicated in patients with New York Heart Association class III or IV heart failure. Fluid overload is another potential side-effect, especially when TZD are associated with insulin and the association of rosiglitazone and insulin has led to episodes of cardiac failure in clinical trials. For these reasons the association TZD with insulin has not been licensed in Europe and the UK.

In addition to a growing list of novel TZD currently under investigation, various non-TZD PPAR- γ agonists are also under development [30, 31]. All of these compounds show the expected features of improved insulin action in cell models, insulin resistant animals and in type 2 diabetics. Indeed, the L-Tyrosine-based non-TZD PPAR γ agonist GI262570, currently in phase III trial for type 2 diabetes seems to be more potent used as a monotherapy than TZD in lowering blood glucose and controlling lipid abnormalities [32]. Interestingly, some new PPAR- γ agonists are also agonists of PPAR- α (like fenofibrate, the PPAR α agonist), conferring a lipid lowering dimension to these drugs via the activation of FFA oxidation in mitochondria [33, 34]. These drugs provide an interesting potential in the management of both hyperglycemia and dyslipidemia.

In conclusion, TZD and other related PPAR γ modulators seem to have a significant antidiabetic activity. However, prescribed as a monotherapy, TZD exhibit a less potent glucose lowering effect than metformin or sulfonylureas. Indeed, the most promising effect of these drugs seems to be related to their ability to limit lipotoxicity thereby preventing the gradual decrease in β -cell mass and function observed during the course of T2DM. In addition, TZD seem to have a beneficial cardiovascular effect. Prospective studies will answer the issue as to whether TZD can delay or prevent long term degradation of β -cell function as well as the progression of macrovascular complications.

Tyrosine phosphatase inhibitors

Targeting the common form of insulin resistance in type 2 diabetes is difficult since it involves multiple non-specific insulin signalling defects. Protein-tyrosine phosphatases (PTPases) act as physiological negative regulators of insulin signaling by dephosphorylating the activated insulin receptor (IR) thereby limiting the insulin signal. Increased expression and activation of PTPases have been observed in muscle and fat from obese and diabetic humans and rodents and it is believed that such activation could be involved in the pathogenesis of the common form of

insulin resistance [35, 36]. Thus, inhibition of PTPase activity seems an appealing strategy to improve insulin action in T2DM.

Vanadium is a non-specific inhibitor of tyrosine phosphatases which has been shown to mimic a number of metabolic actions of insulin. It has been successfully used as a therapeutic agent in rodent models of diabetes [37, 38] and several clinical studies in humans with type 2 diabetes have confirmed that vanadium salts decrease hepatic glucose output and improve insulin action in muscle thereby reducing hyperglycaemia [39, 40]. Interestingly, vanadate is able to stimulate glucose transport in muscle by a mechanism independent of the classical insulin-dependent IRS-1/PI3-kinase/Akt pathway [41].

Another interesting molecular target to more specifically improve insulin resistance and a more specific approach to decrease PTP activity lies in the inhibition of PTP-1B. PTP-1B is a cytosolic PTPase that dephosphorylates and thus inhibits the IR (*Fig. 3*). Disruption of the PTP-1B gene in mice (PTP-1B^{-/-}) improves glucose tolerance and enhances insulin sensitivity as a result of increased insulin action in liver and skeletal muscle without affecting insulin action in adipose tissue [42]. In addition, PTP-1B^{-/-} mice are astonishingly resistant to obesity when placed on a high fat diet [42, 43]. Improvement of insulin action and control of body weight being two hallmarks of the treatment of type 2 diabetes, the data collected in PTP-1B^{-/-} mice prompted an international search for pharmacological inhibitors of PTP-1B. Some of these molecules have already been synthesised and tested in animal models of obesity and T2DM [44, 46] in which they normalized plasma glucose and insulin levels. Further studies in mice and humans are needed in order to define the efficacy of these molecules in the treatment of T2DM and obesity-related disorders.

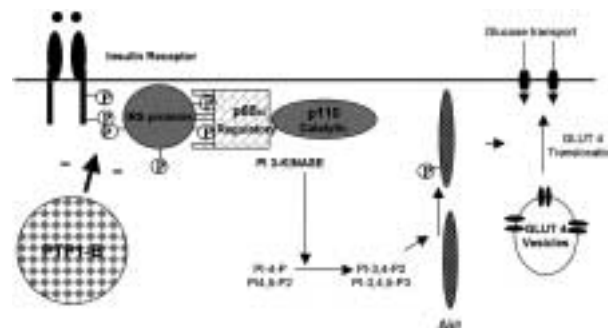


FIG. 3. PTP-1B: a new therapeutic target to treat insulin resistance. After insulin binds to its receptor, the activated receptor tyrosine kinase catalyses autophosphorylation of the receptor on tyrosine (Tyr) residues and subsequent Tyr-phosphorylation of the insulin receptor substrate (IRS) proteins. These proteins serve as docking platforms for phosphatidylinositol (PI) 3-kinase that mediate a variety of insulin's biological effects like glucose transport. PTP-1B dephosphorylates and thus inhibits the IR thereby limiting the insulin signal.

A more physiological approach to treat insulin resistance would be to promote glucose uptake and utilisation specifically in skeletal muscle by a mechanism similar to that which occurs during exercise without increasing glucose utilisation in adipose tissue (to prevent obesity). Insulin and exercise stimulate glucose uptake in skeletal muscle by inducing the translocation of GLUT4 to the cell surface [47]. These two physiological agonists appear to utilize different signalling pathways to mediate GLUT4 translocation [48]. While insulin promotes glucose transport after activation of insulin receptor substrate (IRS) proteins and the phosphatidylinositol (PI) 3-kinase pathway, exercise-stimulated glucose transport has been shown to involve the activation of AMP-activated protein kinase (AMPK) [49] (Fig. 4). AMPK can be pharmacologically activated by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) [50]. Studies in cell culture and animal models show that AICAR can promote glucose transport to the same extent that insulin and exercise do. Independent studies (reported in abstract form) demonstrated that AICAR administration in various rodent models of type 2 diabetes dramatically improves the diabetic phenotype mainly by increasing the expression of the glucose transporter GLUT-4 in skeletal muscle thereby enhancing glucose uptake [51-53]. Thus, AICAR carries an important potential for the treatment of hyperglycemia and insulin resistance.

Other perspectives in the field of insulin sensitising agents include a non-peptidyl fungal metabolite activator of the IR tyrosine kinase, currently in development. Analogues with high specificity for the IR show potent ability to activate the IR without inhibition of

phosphatases and exhibit insulin mimetic and insulin sensitising effects in cell models and animal models of type 2 diabetes [54, 55]. This molecule is highly specific of the IR and does not activate the IGF-1 receptor thereby limiting undesirable growth promoting side effects.

■ INSULIN SECRETAGOGUES

The deterioration of glucose homeostasis is linked to the progressive loss of β -cell function and enhancement of insulin secretion from pancreatic β -cells is a major goal for the treatment of type 2 diabetes. The observation that sulfonylureas could stimulate insulin secretion has provided a rationale for the use of these drugs in the treatment of the insulin secretory defects of type 2 diabetic patients. Unfortunately, the stimulation of insulin release by sulfonylureas is not strictly glucose-dependent, and hence hypoglycaemia is an undesirable side effect especially in elderly patients. Glucose is the main physiological stimulus for insulin biosynthesis (when glucose concentrations exceeds 2 mmol/l) and secretion (when glucose concentrations rise above 5 mmol/l). Thus, the ideal insulin secretagogue would stimulate insulin secretion: 1) in a physiological manner, that is rapidly after meal ingestion so that there is no delay between the rise in plasma glucose and the promotion of insulin release 2) in a strict glucose-dependent manner, that is not if plasma glucose falls below 60 mg/dl. In addition it should promote adequate β -cell mass growth and insulin biosynthesis according to the nutritional milieu.

Meglitinides

Repaglinide is the first representative of the meglitinides class and is the only non-sulfonylurea insulin secretagogue currently approved for the treatment of type 2 diabetes. Meglitinides, unlike sulfonylureas are almost ineffective in stimulating insulin release from glucose deprived islets thereby limiting the risk of hypoglycaemia [56]. The discovery that repaglinide could stimulate insulin secretion by binding to at least two and possibly three different receptors on β -cells, including the common sulfonylurea receptor, has brought promises of potential associations with sulfonylureas. However, to date, pharmacological studies have failed to elucidate whether the effect of repaglinide and sulfonylureas could be additive on insulin release [56]. Thus, so far there is no rationale to associate these drugs in patients with type 2 diabetes. Apart from repaglinide two other meglitinides are under investigation in humans and should be available in the near future. Among them, the KAD1229 also has extrapancreatic effects on hepatocytes resulting in inhibition of gluconeogenesis [56]. It is undergoing final clinical trials in Europe.

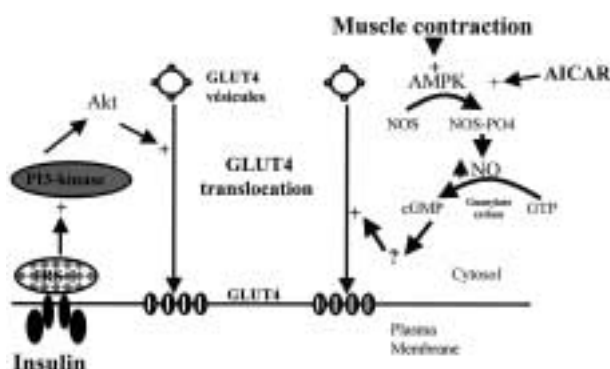


FIG. 4. AMPK: a potential therapeutic target to increase muscle glucose utilization.

Insulin and exercise stimulate glucose uptake in skeletal muscle by inducing the translocation of GLUT4 to the cell surface. These two physiological agonists utilize different signalling pathways to mediate GLUT4 translocation. Insulin promotes glucose transport after activation of IRS-1 and the phosphatidylinositol (PI) 3-kinase pathway. Exercise-stimulated glucose transport involves the activation of AMP-activated protein kinase (AMPK). AMPK can be pharmacologically activated by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR).

Nateglinide

The concept of developing amino acid analogues as insulin secretagogues resides in the possibility of using non-sulfonylurea drugs in sulfonylurea resistant patients. Nateglinide is a phenylalanine derivative, first phase insulin secretion enhancer, recently launched in Japan and approved for clinical use in other countries. It binds the common sulfonylurea receptor and exerts its insulinotropic effect via closure of the K-ATP channel [57]. Because of its transient interaction with SUR-1, nateglinide-induced insulin release is rapid and short lived [58]. Nateglinide does not stimulate insulin secretion from glucose deprived β -cells and a synergistic interaction occurs between nateglinide and elevated mealtime plasma glucose concentrations to stimulate insulin secretion [59]. Compared to glibenclamide and repaglinide, nateglinide causes an earlier post-meal plasma insulin peak and insulin levels return to normal more promptly [57, 60, 61]. This results in a more physiological insulin secretion and a lower frequency of hypoglycemic reactions [61]. Indeed, following a glucose challenge, the area under curve for glucose is better in patient treated by nateglinide compared to glibenclamide [60]. The fall in HbA1c relative to placebo during chronic treatment with nateglinide monotherapy is of 1.4% compared to a 1.5% relative reduction in the metformin group [62]. In large clinical studies with type 2 diabetics, combination therapy of nateglinide with metformin or troglitazone produced a significant better glycaemic control (-2.5% and -2.2% relative to placebo respectively) than either drug given as monotherapy [57, 62]. Mild hypoglycemia was reported in 1.3% of patients receiving nateglinide and weight gain was limited to 0.6 kg over a six month period [57]. In conclusion, nateglinide is an insulin secretagogue primarily intended at controlling postprandial hyperglycaemia. Prescribed in monotherapy or in combination with metformin and/or TZD, nateglinide appears as an interesting alternative to sulfonylureas.

Glucagon-like peptide (GLP)-1

The observation that glucose administered via the gastrointestinal tract was associated with a greater insulin release than that observed after a challenge with the same amount of glucose given intravenously prompted a search for the responsible incretin gut-derived factors that potentiate glucose-induced insulin secretion. Two incretins have been identified: gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). GIP is released from duodenal endocrine K cells after absorption of glucose or fat and further promotes glucose-induced insulin secretion from pancreatic β -cells. GLP-1 results from post-translational processing from proglucagon in the L cells of the lower intestinal tract. It is believed that

glucose and other nutrients directly or indirectly stimulate secretion of GLP-1 via release of GIP [63]. GLP-1 potentiates nutrient-induced insulin release via G-protein-coupled receptors expressed on islet β -cells which are functionally linked to adenylate cyclase [63]. The resulting rise in cytosolic cAMP potentiates insulin secretion initiated by other agents such as nutrients but also sulfonylureas. Other interesting effects of GLP-1 include stimulation of insulin biosynthesis, suppression of glucagon secretion, slowing of gastric emptying and induction of satiety [63] (Fig. 5). In addition, GLP-1 induces β -cell proliferation in pancreatic islets and increases β -cell mass [64, 66] (Fig. 5).

Clinical studies in type 2 diabetic patients have demonstrated the efficiency of GLP-1 to control blood glucose. When administered intravenously or subcutaneously injections, GLP-1 normalizes both fasting and post-prandial glycemia in poorly controlled diabetic patients predominantly by enhancing β -cell function, suppressing both glucagon secretion and gastric emptying [63]. In addition GLP-1 was shown to enhance the insulinotropic effect of glibenclamide in type 2 diabetic patients previously resistant to glibenclamide alone [63].

Because GLP-1 is a peptide which it is rapidly degraded into the circulation (plasma half-life < 5 min) by the enzyme dipeptidyl peptidase IV (DPP-IV) its therapeutic applications are limited by the need for infusions or repeated injections. To circumvent this problem, more stable GLP-1 analogues have been designed that are resistant to DPP-IV degradation *in vivo* (alterations at cleavage positions). The GLP-1 analogue LI307161 when administered subcutaneously three times a day, efficiently reduces postprandial blood glucose in type 2 diabetic patients without causing hypoglycaemia [67]. Exendin-4 is a long act-

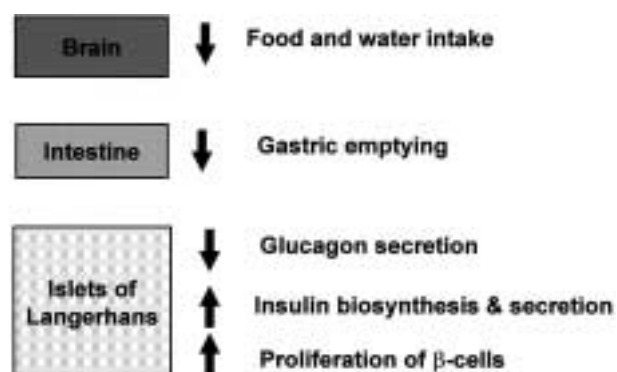


FIG. 5. Mechanisms for the hypoglycaemic effect of GLP-1. At the level of β -cells, GLP-1 potentiates insulin secretion initiated by other agents such as nutrients but also sulfonylureas, stimulates insulin biosynthesis, induces β -cell proliferation and increases β -cell mass. Other interesting effects of GLP-1 include suppression of glucagon secretion from α -cells, slowing of gastric emptying and induction of satiety.

ing analog of GLP-1 isolated from lizard venom, which binds to the GLP-1 receptor and shows potent insulinotropic effects *in vitro* and *in vivo*. Exendin-4 is a more potent insulinotropic agent when given intravenously to rats than is GLP-1 and causes a greater increase in cAMP concentrations in isolated islets [68]. Of even greater interest once daily injection of exendin-4 to diabetic mice achieved prolonged beneficial effects on blood glucose concentrations [68]. In addition, Bonner-Weir and co-workers recently reported that administration of exendin-4 to diabetic rats results in an increase in β -cell mass by enhanced replication and neogenesis of β -cells [69]. Subcutaneous exendin-4 administration also improves both basal and post-prandial glucose in subjects with type 2 diabetes [70].

DPP-IV inhibitors are also under investigation [71]. Preliminary data indicate that they improve glucose tolerance in both diabetic rodents and humans with the advantage to be given orally [72, 73].

In conclusion, GLP-1 analogues hold great promises as a novel therapy for type 2 diabetes: They act fast, enhance nutrient-induced insulin release without risk of hypoglycemia, they can stimulate β -cell growth in diabetic individuals with reduced β -cell mass and they can sensitise the β -cells to sulfonylureas.

Other insulin secretagogues under investigation include the rapid acting morpholinoguanide BTS 67582 [74], the phosphodiesterase inhibitor which enhances nutrient-induced insulin release [75] and the succinate ester derivatives, which improve β -cell function through enhancement of insulin biosynthesis and secretion [75].

■ PROSPECTS OF GENE THERAPY FOR TYPE 2 DIABETES

Since the gradual loss of β -cell function is the principal determinant of the degradation of glucose homeostasis, most of the research effort of gene therapy has been focused on optimising the insulin response of the body to glucose. This can be achieved in different ways. An important approach is the genetic engineering of β or non β -cells *ex vivo* to produce insulin followed by transplantation back into a diabetic patient [76]. Cheung *et al.* recently reported the generation of mice genetically engineered to produce human insulin from K cells derived from the duodenum. This discovery holds promise since K cells usually produce glucose dependent insulinotropic polypeptide (GIP), and so are already glucose responsive endocrine cells [77]. Another approach to enhance insulin release is the direct delivery of the insulin gene [78] or the GLP-1 gene [79] into tissues. This can be achieved by muscle injection of plasmid cDNA (muscle becomes the platform of ectopic hormone production) [78], by viral vector gene transfer to

the genome [80] or by encapsulated cells genetically engineered to produce the hormone [79]. Finally, a last approach is to promote regeneration of β -cells. For example, the pancreatic duodenum homeobox-1 (PDX-1) also called STF-1 is an important transcription factor for pancreas development and β -cells differentiation and neogenesis [81]. PDX-1 induces the expression of the insulin and the GLUT2 genes [81]. The discovery of a method to insert this gene in to β -cells of diabetic subjects could be of great interest to enhance β -cell mass.

Engineering non-pancreatic cells to express molecules that sensitise tissues to insulin or enhance glucose transport may also one day be a successful approach in the treatment of type 2 diabetes. For example, engineering endogenous skeletal muscle cells to locally produce the GLUT4 gene would certainly be effective to improve insulin action and to lower glycaemia [82, 83]. In addition, the possibility of controlling hepatic glucose production, which is an essential determinant of fasting and post-prandial hyperglycaemia, by transferring the glucokinase gene in liver could also be an interesting alternative and is currently under investigation in animal models of type 2 diabetes [84, 85].

■ CONCLUSION

Type 2 diabetes is a polygenic disease involving multiple defects in insulin action and insulin secretion resulting in a polymorph phenotype of abnormalities in carbohydrates and lipid metabolism. Current antidiabetic agents are unable to maintain long-term euglycaemia in all type 2 diabetic patients and there is a need for new antidiabetic drugs. Looking at the data presented here, it seems likely that at least five different insulin secretagogues will be available to treat insulin secretory defects within the next five years (*Fig. 6*). This brings the perspective of combination of different drugs acting on various receptors on β -cells

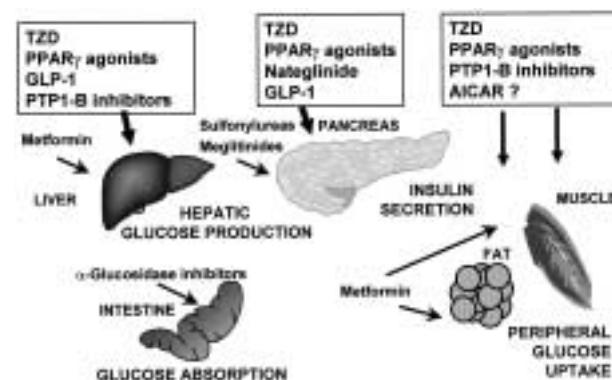


FIG. 6. Present and future therapies for type 2 diabetes.

to synergistically potentiate insulin release. Great hope lies in the development of GLP-1 analogs given the possibility of utilisation in sulfonylurea resistant patients and given their predicted beneficial effect on β -cell mass. In addition to insulin secretagogues, three different insulin sensitizers will probably be available to restore insulin sensitivity and to promote glucose uptake (Fig. 6). Indeed, extensive use of TZD will tell if they can prevent lipotoxicity and thereby delay the progressive decline in β -cell function but also if they can slow down the progression of macrovascular complications.

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