New Emerging Targets for Type-2 Diabetes

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Abstract: The development pipeline for new oral therapeutic agents for type 2 diabetes is encouraging and continues to expand. These intensive research and development efforts are in response to the increasing prevalence of the disease and related co-morbidities, realization by caregivers that successful glycaemic control will likely require combination therapy, a growing understanding of the pathophysiology of the disease, and the identification and validation of new pharmacological targets. These targets include receptors and enzymes that enhance glucose-stimulated insulin secretion, suppress hepatic glucose production, increase skeletal muscle glucose transport and utilization, increase insulin sensitivity and intracellular insulin signaling, and reduce circulating and intracellular lipids. Due to their promise for future clinical success and because they exhibit mechanisms of action distinct from current therapies, some of the emerging approaches have been highlighted here. Keywords: Diabetes, Hyperglycemia, Mechanism, PPARs, PTP-1B.

Introduction:

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, glycosuria, negative nitrogen balance and sometimes ketonaemia. It causes a number of complications like retinopathy, neuropathy and peripheral vascular insufficiencies. Diabetes mellitus, long considered disease of minor significance to world health, is now taking its places as one of the main threads to human wealth in 21st century. The incidence of the disease currently estimated is to reach 210 million by the year 2010 and 300 million by the year 2025. The rising prevalence of type 2 diabetes and obesity in the worldwide population has fueled an intensified search for new therapeutic treatment options. Current pharmacological approaches are unsatisfactory in improving the consequences of insulin resistance. There is no single approach to treat this disease and usually a combination therapy is adopted to treat the disease.

Peroxisome Proliferator Activated Receptors (PPARs):

Peroxisome proliferator activated receptors are transducer proteins belonging to nuclear receptor superfamily. These receptors were identified in the 1990s in rodents and named after their property of peroxisome proliferation. They are ubiquitously expressed throughout the body. Prostaglandins, fatty acids, fibrates class of hypolipidemic drugs and thiazolidinediones are known activators of PPARs. On activation by these ligands they initiate transcription of an array of genes that are involved in energy homeostasis. PPARs have been assigned to a subfamily of nuclear receptors that includes the retinoic acid receptors (RARs), the thyroid hormone receptors (TRs) and the steroid receptors. To date,
three major types of PPAR, encoded by separate genes, have been identified; they are PPARa, PPAR(3/5 and PPARy). In the last decade, research on PPARs has unveiled new mechanisms for the regulation of lipid metabolism and possible molecular determinants of metabolic disorders, including type 2 diabetes.

### Molecular Mechanism

 Peroxisome-proliferator-activated receptor gamma (PPARY) is a transcription factor activated by thiazolidinediones (TZDs). In transactivation, which is DNA-dependent, PPARy forms a heterodimer with the retinoid X receptor (RXR) and recognizes specific DNA response elements called PPAR response elements (PPRE) in the promoter region of target genes. This results ultimately in transcription of PPARy target genes. After ligand binding, PPARs undergo conformational changes, which lead to recruitment of cofactor proteins and coactivators. The coactivators interact with nuclear receptors in a ligand-dependent way and influence the set of genes transcribed. In transrepression, PPARs can repress gene transcription by negatively interfering with other signal-transduction pathways, such as the nuclear factor-κB (NFκB) signaling pathway, in a DNA-binding-independent manner. STAT denotes signal transducers and activators of transcription, ISGF-RE interferon-stimulated gene factor responsive element, and TRE TPA responsive element, where TPA is a phorbol ester.

### Biological Roles of PPAR Receptors:

**PPAR-a:** PPARa is expressed in numerous tissues in rodents and humans including liver, kidney, heart, skeletal muscle and brown fat and it is also expressed in a range of vascular cells such as endothelial cells, VSMCs and monocytes/macrophages. The endogenous ligands of PPARa are palmitic acid, stearic acid, arachidonic acid etc. and exogenous ligands are fibrates class of hypolipidemic drugs, gemfibrozil and Nafenopin. PPARa exerts its multiple effects in the liver, heart and vessel wall by such as lipid metabolism, inflammation and dyslipidemia/atherosclerosis by regulating expression of corresponding genes.

**PPAR-p/5:** Despite vigorous research on PPAR-a and PPAR-y, the functional identity of PPAR-(3/5 remains unclear. PPAR-p/5 is expressed in a wide range of tissues and cells, with relatively higher levels in the brain, adipose tissue and skin. PPAR-p are found to be implicated in dyslipidemia, insulin resistance, hyperlipidemia, inflammation, atherosclerosis, obesity, infertility, cancer, and nervous system. The PPAR- gene contains three promoters that yield three mRNA isoforms, namely, PPAR-y 1, PPAR-y2 and PPAR-y3. PPAR-y 1 and y3 RNA transcripts translate into the identical PPAR-y 1 protein. PPAR expression is tissue dependent. PPAR-y 1 is found in a broad range of tissues, whereas PPAR-y2 is restricted to adipose tissue. PPAR-y3 is abundant in macrophages, the large intestine and white adipose tissue. The endogenous ligands of PPARy are linoleic acid, arachidonic acid, 9-HODE, 13-HODE, 15-HODE and 15d-PGJ2, while the exogenous ligands are TZDs and JTT-501 (isoxazolidinedione). Adipogenesis, glucose homeostasis and lipid metabolism are the major mechanisms of PPARy involved in the improvement of insulin resistance.

### Role of PPAR-y in Insulin Sensitivity:

PPAR-y has also been associated with several genes that affect insulin action. Tissue necrosis factor alpha (TNF-a), a pro-inflammatory cytokine that is expressed by adipocytes, has been linked to insulin resistance. In vivo investigations showed that PPAR-y agonists improve insulin resistance by opposing the effect of TNF-a in adipocytes. Expression of the glucose transporter protein GLUT4 by PPAR-y agonists in adipocytes is also pivotal in the process of glucose uptake. On the other hand, resistin, a hormone secreted by adipocytes that elevates blood glucose levels, was inhibited by TZDs.

### PPAR a/y Dual Agonist:

These agents are shown to ameliorate the hyperglycemia and hyperlipidemia associated with type 2 diabetes. In addition to their benefit on lipids the activation of PPARa may mitigate the weight gain induced by PPARy activation. So this dual agonist is supposed to provide additive and possibly synergistic. First literature report of a balanced PPAR a/y dual agonist was KRP-297 (MK-767), a TZD derivative that was reported to bind PPARa and PPARy with an affinity of approx.0.230 and 0.33 uM respectively and to trans activate PPARa and PPARy with potencies of 1.0 and 0.8 uM followed by phenylpropionic acid based PPAR a/y dual agonists Tesaglitazar (AZ-242) by Astra Zeneca, reportedly in phase III clinical trial, Ragaglitazar (DRF-2725) by Dr. Reddy’s Research foundation, reportedly completed phase II clinical trial but development being terminated due to an incidence of bladder tumors in rodents. LY-510925 is a result of collaborative effort of Ellily Lilly and Ligand pharmaceuticals, Muraglitazar (BMS -298585) is disclosed by Cheng.

### Glucagon Like Peptide-1 (GLP-1) Hormone:

It is the incretin hormone acting via GLP-1 receptor (a G-protein coupled receptor). When blood glucose
levels are high this hormone stimulates insulin secretion and biosynthesis and inhibits glucagon release leading to reduce hepatic glucose output. In addition it serves as an “ileal brake”, slowing gastric emptying and reducing appetite. GLP-1 has a number of effects on regulation of P-cell mass: stimulation of replication and growth and inhibition of apoptosis of existing β-cells and neogenesis of new β-cells from precursors. Thus, GLP-1 therapy for the treatment of type 2 diabetes is an area of active research. There are two sub-classes of GLP-1 in clinical development. One is natural GLP-1 and the other is exendin-4, a peptide agonist isolated from the venom of lizard and is more potent than natural GLP-1. Exenatide (AC2993) is a peptide consist of 39 amino acid approved recently developed by Lilly and Amylin & used for treatment of diabetes.  

β3-Adrenoreceptor Agonist: These shows marked selectivity for stimulation of lipolysis and hence oxygen and energy consumption in skeletal muscle and adipose tissue. An initial compound, which displayed excellent activity in rodents failed in human trials due to the difference in β1 receptor isofoms in different species. Recent cloning of human β3 receptor has enabled companies to develop compounds selective for β3 receptor. SR-58611 (Sanofi-Synthelabo) and TAK-677 (Takeda) are some of the compounds in this series undergoing phase II clinical trials. In certain types of fat cells, the beta3 adrenergic receptor (b3AR), which belongs to the superfamily of G-protein coupled receptors (GPCRs), functions in a manner contrary to the general adrenergic system in that activation of b3AR actually induces the wasting of metabolic energy. Agonists of this receptor activate the uncoupling protein (UCP) which causes the expenditure of metabolic calories as heat. Suggesting that b3AR could be used to dissipate metabolic energy as heat in diabetic and morbidly obese patients instead of storage of this energy as fat. These ligands has been recently reviewed by Sawa and Harada.  

α-Lipoic Acid: α-Lipoic acid (LA) is an eight-carbon fatty acid that is synthesized in trare quantities in organisms ranging from bacteria to man. LA functions naturally as a cofactor in several mitochondrial enzyme complexes responsible for oxidative glucose metabolism and cellular energy production. LA has been prescribed in Germany for over thirty years for the treatment of diabetes-induced neuropathy. Results from several recent controlled clinical studies indicate that this compound is safe, well tolerated, and efficacious. Although the exact mechanism of action of LA is unknown, in vitro data from the laboratories of Rudich and others have indicated that LA pretreatment maintains the intracellular level of reduced glutathione (the major intracellular antioxidant) in the presence of oxidative stress, and blocks the activation of serine kinases that could potentially mediate insulin resistance. Thus, one potential explanation for the protective effects of LA might be related to its ability to preserve the intracellular redox balance, thereby blocking the activation of inhibitory stress-sensitive serine kinases including IKKbeta. This stress-sensitive kinase is a crucial regulator of the transcription factor-necrosis factor-kappaB (NF-kappaB), a major target of hyperglycemia, cytokines, reactive oxygen species, and oxidative stress. The aberrant regulation of NF-kappaB is associated with a number of chronic diseases including diabetes and atherosclerosis. Furthermore, LA and other agents that interfere with the persistent activation of the NF-kappaB pathway appear to be promising approaches to increase insulin sensitivity, and perhaps even as treatments for complications of diabetes in which NF-kappa B activation has been implicated.  

Liver Selective Glucocorticoid Antagonists: Glucocorticoids raise blood glucose levels by functionally antagonizing the action of insulin, thereby inhibiting glucose disposal and promoting hepatic glucose production and output. So the approaches towards liver selective glucocorticoid antagonist have potential role in the management of type II diabetes mellitus. Mifepristone has shown glucocorticoid antagonist action and few other similar compounds have been tested in which A-348441 showed reduction in glucose levels and improved lipid profiles in an animal model of diabetes.  

Dipeptidyl Peptidase IV Inhibitors: Dipeptidyl peptidase (DPP-IV) is a protein that has multiple functions in the body. It is known under different names depending upon location such as Dipeptidylpeptidase IV (DPP-IV), CD26, adenosine deaminase complexing protein 2 (ADCP2), T-cell activation TP103 antigen. DPP-IV is found on and imbedded on the epithelial brush border mucosal membrane of the intestinal tract lining. DPP-IV has primary function in breaking down casein and side chain activity in breaking down gluten. DPP-IV inhibitors stabilize endogenous GLP-1 and induce insulin secretion in a glucose-dependent manner in contrast to insulin tropic agents which release insulin in glucose independent manners which manifest the hypoglycemic as residual effect. DPP-IV inhibitors are orally active and stable compound. They also give synergistic action with insulin tropic agents like
sulphonamides, biguanides e.g. metformin and phenformin. DPP-IV inhibitors should prolong the action of incretins, like GLP-1 which is released postprandially from L-cells in the gut and increase insulin secretion. It is known that T2DM has a low level of secretion of incretins in response to meals. Dipeptidyl peptidase IV inhibition reduces the degradation and clearance of GIP and GLP-1. Thus the use of DPP-IV inhibitors increases the circulating levels of endogenous GLP-1 leading to increase insulin secretion, biosynthesis and inhibits glucagon release.

Merck has presented new data at the American Diabetes Association meeting, on Januvia™ (sitagliptin phosphate), its oral, once-daily treatment for type 2 diabetes drug. Merck also presented data reinforcing the hypothesis that the drug improved the function of pancreatic beta cells. Januvia was accepted for US regulatory review earlier this year, and if approved, would be the first in a new class of oral drugs, known as the Dipeptidyl peptidase-IV inhibitors.

Sitagliptin phosphate (januvia) has been recently approved as an antidiabetic drug for oral administration. It has been used alone or in combination with metformin or thiazolidinediones. The main mechanism of this compound is the inhibition of DPP-IV. Weight gain and low blood sugar are not side effect of januvia. Saxagliptin (Onglyza) has been recently approved by FDA as antidiabetic drug for oral administration. It has been used single agent (monotherapy) or in combination with metformin, thiazolidinediones, and sulfonylureas. The main mechanism of this compound is the inhibition of DPP-IV. Preliminary results have been disclosed on three DPP-IV inhibitors, Isoleucylthiazolidide (P32/98), DPP-728 and LAF-237. Novartis has recently developed (NBP-LAF-237, LAF-237) as vildagliptin, a potent, selective, stable orally active DPP-IV inhibitor with antihyperglycemic properties, currently in phase III clinical trials as potential new treatment for type II diabetes. The lead agent in a new class of drugs for the treatment of diabetes Novartis’ LAF-237 seems to improve glucose control without the weight gain associated with other orally-active drugs for this common disease, reports Phil Taylor. The promise of this treatment remains to be realized as a potent and selective DPP-IV inhibitors progress through clinical studies\(^\text{12}\).

Protein Tyrosine Phosphatase-1b (PTP-1b):
PTB-1B a founding member of PTPase with 435 amino acid residues was first purified from human placental tissue in 1988 and first crystallized in 1994. PTP-1B belongs to non transmembrane class of enzymes. PTP-1B is an abundant enzyme expressed in nearly all tissues where it is localized primarily on intracellular membranes by a C-terminal sequence. PTP-1B acts as negative regulator of insulin signalling. It acts by causing dephosphorylation of insulin receptor. It also causes negative regulation of insulin signaling. It is involved in type-2 diabetes & obesity. It has been shown mice lacking PTP-1B show enhance insulin activity, resistant to development of obesity. In vitro, it is a non-specific PTP and dephosphorylates a wide variety of substrates. In vivo, it is involved in down regulation of insulin signalling by dephosphorylation of specific phosphotyrosine residues on the insulin receptor. Administration of PTP-1B antisense oligonucleotides to diabetic obese mice reduces plasma glucose and brings insulin level to normal. PTP-1B knockout mice have shown increased insulin sensitivity and decreased weight gain after a high fat diet. All these evidences help to validate PTP-1B as a keynegative regulator of insulin signal transduction and a potential therapeutic target in the treatment of NIDDM and obesity.

Role of PTP-1B in Insulin & Leptin Signaling:
Binding of insulin to insulin receptor α-subunit induces conformational change in β-subunit which in turns activates insulin receptor tyrosine kinase which causes phosphorylation of insulin receptor substrate which is responsible for down stream signaling through recruitment of appropriate signal transducers which is responsible for various effect exerted by insulin.

It has been shown recently that PTP-1B negatively regulates leptin receptor signaling in a murine neuronal subline. PTP-1B acts to block leptin signaling by dephosphorylating jleptinanus kinase (jak)-2. Leptin is a key adipokine regulating food intake & energy expenditure. It is likely that the resistance to diet induced obesity is due, atleast in part, to increased leptin sensitivity in the PTP-1B knockout mice.

PTP-1B Inhibitors:
Phosphatase LAR, CD45, SHP-2, cdc25c and T-cell PTP (TCPTP) share 50–80% homology in the catalytic domain with PTP-1B, which presents a challenging task of achieving selectivity, especially over TCPTP. Thus it was necessary for the inhibitors to interact with the regions outside the catalytic site in order to be selective. A non-catalytic phosphotyrosine-binding site was identified, which seems to be ideal since it is close to the catalytic site and is less homologous between the PTP-1B and TCPTP when the amino acid sequences were compared. Hence targeting both the sites simultaneously may show good activity and
selectivity against PTP-1B\textsuperscript{16, 17}.

PTPase have been inhibited experimentally using a variety of mechanisms and chemical entities. PTPase can be inhibited by chemical inactivation of the active site cysteine residue common to all members of the family. This inactivation may occur via an oxidative mechanism initiated by reactive species such as peroxynate and peroxides e.g. Most of early PTP-1B inhibitors are phosphate-based and the most studied phosphate-based PTP-1B inhibitors are difluorophosphonates. This difluorophosphonate group was introduced as a nonhydrolyzable phosphotyrosine mimetic in 1992 by Burke and coworkers. 2-(Oxalylamino)-benzoic acid (OBA) was identified as a general, reversible and competitive inhibitor of several PTPase using a scintillation proximity-based high throughput screening by workers at Novo Nordisk.

High-throughput screening has allowed the identification of several more PTP-1B inhibitor classes having various mechanisms of action. Pyridazine derivatives such as were identified at Biovitrum potencies in a low micromolar range (5.6\mu M) and over 20 fold selectivity over TC-PTP. Hydroxyp phenylazole derivatives such as with IC\textsubscript{50} value in the micromolar range, were claimed by Japan Tobacco. A series of azolidinediones and phenoxyacetic acid based PTP1B inhibitors have been reported by American Home Products. More recently a group at Hoffmann-LaRoche described novel pyridotriazinepiperidine analogues with oral glucose lowering effect in ob/ob mice. The inhibition of PTP1B by this class of compounds presumably involves the oxidation of the active site e.

Alpha-bromoaceto phenone derivatives act as potent PTP inhibitors by covalently alkylationing the conserved catalytic cysteine in the PTP active site. Derivatization of the phenyl ring with a tripeptide Gly–Glu–Glu resulted in potent, selective inhibitors against PTP-1B cysteine of PTP1B to the corresponding sulfenic acid.

Despite good biological target validation, designing PTP-1B inhibitors as oral agent is challenging because of the highly charged nature of the catalytic domain of the target. Furthermore the development of selective, potent and bioavailable inhibitors of PTP-1B will be a formidable challenge although some of the groundwork has now been laid out.

Glycogen synthase kinase-3:

GSK-3, initially described as a key enzyme involved in glycogen metabolism, is now known to regulate a wide range of cell functions\textsuperscript{18}. GSK-3 is a serine/threonyne kinase originally discovered because of its ability to phosphorylate and inhibit glycogen synthase (GS)\textsuperscript{22}. Human GSK-3 exists as two isoforms, \(\alpha\) and \(\beta\). GS activity is regulated by allosteric and covalent (phosphorylation/dephosphorylation) mechanism\textsuperscript{19, 20} whereas a number of kinases and phosphates can act on GS, GSK-\(\alpha\) plays an important role\textsuperscript{22, 23, 24}. GSK-3 phosphorylates GS on three specific residues. This phosphorylation causes deactivation of GS and decrease its affinity to allosteric activation by glucose-6-phosphate. In addition, it has been reported that GSK-3 can also phosphorylated insulin receptor substrate (IRS-1). A key early molecule in insulin-signaling cascades, suggesting the potential for involvement of GSK-\(\alpha\) in multiple stages of insulin action\textsuperscript{25} Lithium ion (Li) has been found to casue relatively specific inhibition of GSK-\(\alpha\) and has been reported to have insulin like effects on glucose metabolism, including increase uptake, activation of GS activity, and stimulation of glycogen synthesis in skin, muscle and fat cells\textsuperscript{26}.

**AMP-Activated Protein Kinase:**

Skeletal muscle is the major site of insulin-stimulated glucose disposal and insulin resistance in this tissue is one of the earliest contributing factor to the pathogenesis of type 2 diabetes. One important finding is that activation of muscular AMPK by physical activity (one of the most potent physiological AMPK activator) or by a pharmacological compound called AICAR (5-amino-imidazole-4-carboxamide ribonucleo-side, metabolized to ZMP which is an analogy of AMP) increases muscle glucose uptake concomitantly with glucose transporter 4 (GLUT4) translocation to the plasma membrane\textsuperscript{27, 28}. Interestingly, AMPK-induced glucose transport occurs through a mechanism distinct Tmoth that used by the classical insulin-signalling pathway. In consequence, AMPK enhanced glucose transport in skeletal muscle is observed both in rodents or in humans even if insulin resistance is present suggesting that muscular AMPK could be a therapeutic target for the management of insulin resistance\textsuperscript{29}.

Increased hepatic glucose production is a key role factor in type-2 diabetes. Since AMPK is usually considered as part of a mechanism involved in energy sparing, a potential role for AMPK in the regulation of the energy-consuming process of hepatic gluconeogenesis. Results obtained with pharmacological compounds and adenovirus-mediated AMPK activation/ inactivation strategies have demonstrated that AMPK plays a role in the control of glucose production by the liver. Thus liver–specific mice AMPK\(\alpha\)2 isoform is essential to suppress hepatic glucose production and maintain fasting blood glucose level in the physiological range\textsuperscript{30}.
Fructose-1, 6-bisphosphatase as a therapeutic target for type 2 diabetes:
Overproduction of glucose by gluconeogenesis is a primary determinant of hyperglycemia in patients with type 2 diabetes. Current drugs neither inhibit glucose production directly nor provide adequate glycemic control. The recent discovery and characterization of potent and selective inhibitors of fructose-1,6-bisphosphatase (FBPase) has provided new insights into the therapeutic utility of gluconeogenesis inhibitors and the potential of FBPase inhibitors as a new class of antidiabetic drugs and increased endogenous glucose production (EGP). These abnormalities underline the high circulating glucose levels (hyperglycemia) associated with the disease. Because hyperglycemia leads to severe micro vascular complications (e.g. loss of vision) and macro vascular complications (e.g. heart disease), the primary treatment goal is to reduce glucose levels. Metformin is the only prescribed drug whose primary mechanism of action in humans is the reduction, albeit indirect, of EGP. Inhibitors of fructose-1,6-bisphosphatase (FBPase) represent a new strategy for direct inhibition of EGP.

Mechanism and Significance of Elevated EGP in Type 2 Diabetes:
The liver is the main organ responsible for EGP. Glucose is produced by the liver by two pathways: gluconeogenesis (the de novo synthesis of glucose from lactate, alanine and glycerol) and glycogenolysis (the breakdown of glycogen stored in the liver). In healthy individuals, gluconeogenesis accounts for 50% of EGP after an overnight fast and increases progressively to account for over 90% of EGP following 40 h of fasting. The contribution of gluconeogenesis to EGP declines reciprocally during fasting periods reaching a negligible contribution by 96 h. During the postprandial period in healthy individuals, EGP is suppressed by a rapid and near complete inhibition of gluconeogenesis and slower and more modest inhibition (30–50% within 4 h) of gluconeogenesis. In patients with type 2 diabetes, the rate of gluconeogenesis is increased during fasting, whereas the rate of glycogenolysis is either unchanged or reduced. The percent contribution of gluconeogenesis to overall EGP is thereby increased. During the postprandial period, reduced suppression of both glycogenolysis and gluconeogenesis is observed in patients with type 2 diabetes. Thus, in type 2 diabetes, increased gluconeogenesis is primarily responsible for increased EGP during fasting, whereas inappropriate rates of both glycogenolysis and gluconeogenesis account for increased postprandial EGP.

Elevated EGP in type 2 diabetes is a consequence of pancreatic dysfunction and insulin resistance. In healthy individuals, a balance between insulin and glucagon secretion by the pancreas ensures an appropriate rate of EGP. Insulin inhibits the activity of glycogen phosphorylase, the enzyme responsible for glycogenolysis, and decreases expression and activity of gluconeogenic enzymes. Glucagon opposes the aforementioned actions. In patients with type 2 diabetes, insulin deficiency, hepatic insulin resistance, and relative glucagon excess increase the activity of gluconeogenic and glycogenolytic enzymes. Increased gluconeogenesis is exacerbated by insulin deficiency and insulin resistance in extrahepatic tissues. The significance of elevated EGP in type 2 diabetes is clear from the strong correlation between the rate of EGP and fasting hyperglycemia in patients: for each incremental increase in EGP there is a corresponding increase in fasting glucose levels. Because direct inhibition of EGP could potentially provide effective glycemic control across a broad patient population and none of the current antidiabetic drugs reduce EGP by direct mechanisms, the discovery of direct EGP inhibitors has been of considerable interest to the pharmaceutical industry for many years.

Natural and novel inhibitors of fructose-1,6-bisphosphatase:
Human liver FBPase is a cytosolic enzyme that consists of four identical 36.7 kDa subunits each containing a substrate site and an allosteric site. The intracellular activity of FBPase is regulated synergistically by fructose-2,6-bisphosphate (F2,6BP), an inhibitor that binds to the substrate site, and adenosine monophosphate (AMP), an inhibitor that binds to the allosteric site. Intracellular F2,6BP levels are controlled by a glucagon-sensitive enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, in such a way that F2,6BP levels are decreased and FBPase activity increased during times of glucose demand. Relative hyperglucagonemia in type 2 diabetes, in addition to increasing expression of FBPase, is expected to reduce intracellular levels of F2,6BP and further augment the activity of FBPase. Both the substrate site and the AMP site of FBPase have been the target of drug discovery efforts. Early efforts at the substrate site were unsuccessful as were high-throughput screening efforts to identify noncompetitive AMP site inhibitors. However, a structure-guided approach using AMP as a starting point (Metabasis Therapeutics) yielded a lead compound, MB05032, that inhibits human liver FBPase noncompetitively with an IC50 of 16 nm and shows excellent selectivity for FBPase relative to other AMP-binding enzymes. CS-917, a prodrug form of...
MB05032 designed to overcome its cellular penetration and pharmacokinetic limitations, is currently undergoing clinical evaluation. More recently, clinical development of a second-generation drug in the FBPase inhibitor class was reported: MB07803. Overall, FBPase inhibition is safe and well tolerated in animal models, with profound glucose lowering as the main metabolic ramification.

**Clinical Trials of CS-91:**
Initial single and multiple dose studies of CS-917 in overnight-fasted healthy volunteers revealed encouraging tolerability and safety profiles and showed no evidence of hypoglycemia. In a subsequent 14-day Phase 2a trial in patients with type 2 diabetes, CS-917 treatment was also safe and well tolerated. Furthermore, treatment at 50, 200 and 400 mg QD resulted in statistically significant lowering of mean fasting plasma glucose relative to placebo (30–35 mg/dL), while mean plasma lactate levels remained within normal limits. Analysis of a recently completed 12-week Phase 2b trial is in progress.

**Estrogen Receptors: New Players in Diabetes Mellitus:**
Diabetes mellitus type 2 is a systemic disease characterized by imbalance of energy metabolism, which is mainly caused by inadequate insulin action. Recent data have revealed a surprising role for estradiol in regulating energy metabolism and opened new insights into the role of the two estrogen receptors, ERα to ERβ in this context. New findings on gene modulation by ERα to ERβ of insulin-sensitive tissues indicate that estradiol participates in glucose homeostasis by modulating the expression of genes that are involved in insulin sensitivity and glucose uptake.

Drugs that can selectively modulate the activity of either ERα or ERβ in their interactions with target genes represent a promising frontier in diabetes mellitus co-adjuvant therapy. The use of E2 in postmenopausal women to prevent chronic diseases has been available for decades, but the consequences of estrogen replacement are still controversial. For many years, it has been assumed that E2 decreased vasomotor symptoms, vaginal atrophy, and osteoporosis and coronary heart disease (CHD) and increased the incidence of breast cancer. However, recent research has indicated that E2 in postmenopausal women does not affect the incidence of CHD or breast cancer. Moreover, it increases triglyceride levels and the risk of stroke. The existence of conflicting data about E2 actions and the possibility that it might be related to glucose homeostasis and insulin resistance have put E2 replacement therapy under intense investigation. E2 is involved in gene regulation and has an important role in several physiological and pathological states in both men and women, including glucose homeostasis and insulin resistance. Model summarizing the interaction of insulin and glucose uptake in insulin sensitivity tissues. Insulin receptors are located on the cell membrane and their activation by the hormone cause the phosphorylation of the transmembrane subunit β. Once activated, several cytoplasmatic protein are phosphorylated, including IRS-1 and PI3.K, which are essential for insulin signaling. The end result of this phosphorylation cascade is the translocation of vesicles that contain GLUT4 to the cell membrane, where the protein anchors and enables the uptake of glucose by facilitated diffusion. Any alteration in insulin signaling 1) GLUT4 expression 2) translocation or anchorage 3) cause insulin resistance. Abbreviation: GLUT4, glucose transporter 4, IRS-1, insulin receptor substrate, PI3-K, phosphatidylinositol 3-kinase.

In conclusion, a full understanding of modulation of GLUT4 by ERs and development of drugs that can selectively change the ERα to ERβ expression ratio or actions in tissues involved in glucose homeostasis might lead to new adjuvant therapies for DM. Although the present knowledge is not enough to indicate whether this modulation would result in improved glucose uptake and homeostasis in humans, the discovery of ERβ and its participation in the regulation of GLUT4 expression open up a new horizon for studies of glucose homeostasis and related diseases.

**Conclusion:**
These studies have provided evidence that these enzymes and receptors are potential targets for antihyperglycemic therapy. The development of these two targets PTP1B and nuclear receptor super family PPARs offer significant contribution relating to glycemic control. Development of therapeutic agents targeting these receptors or screen a new and safer Ant diabetic drugs for future.
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