Novel Pharmacological Approaches to the Treatment of Type 2 Diabetes

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Abstract—The huge increase in type 2 diabetes is a burden worldwide. Many marketed compounds do not address relevant aspects of the disease; they may already compensate for defects in insulin secretion and insulin action, but loss of secreting cells (β-cell destruction), hyperglycagonemia, gastric emptying, enzyme activation/inhibition in insulin-sensitive cells, substitution or antagonizing of physiological hormones and pathways, finally leading to secondary complications of diabetes, are not sufficiently addressed. In addition, side effects for established therapies such as hypoglycemics and weight gain have to be diminished. At present, nearly 1000 compounds have been described, and approximately 180 of these are going to be developed (already in clinical studies), some of them directly influencing enzyme activity, influencing pathophysiological pathways, and some using G-protein-coupled receptors. In addition, immunological approaches and antisense strategies are going to be developed. Many compounds are derived from physiological compounds (hormones) aiming at improving their kinetics and selectivity, and others are chemical compounds that were obtained by screening for a newly identified target in the physiological or pathophysiologic machinery. In some areas, great progress is observed (e.g., incretin area); in others, no great progress is obvious (e.g., glucokinase activators), and other areas are not recommended for further research. For all scientific areas, conclusions with respect to their impact on diabetes are given. Potential targets for which no chemical compound has yet been identified are also described.

I. Introduction

The number of people with diabetes has increased to double to ~300 million within 20 years (in 2030), of which 90% will have type 2 diabetes (non-insulin-dependent diabetes mellitus (Zimmet et al., 2001). Type 2 diabetes mellitus is characterized by defects in insulin action in tissues (insulin resistance) and/or defects in pancreatic insulin secretion (β-cell dysfunction), which eventually includes loss of pancreatic insulin-secreting cells. The associated complications of diabetes, such as cardiovascular disease, peripheral vascular disease, stroke, diabetic neuropathy, diabetic retinopathy (eventually blindness) result in increasing disability, reduced life expectancy, and enormous health costs.

Current therapies for type 2 diabetes mellitus have mainly centered on elevating plasma insulin levels (direct insulin administration or oral agents that promote insulin secretion), improving insulin sensitivity of tissues, and eventually reducing the rate of carbohydrate absorption from the gastrointestinal tract. The established drugs [sulfonylureas, glinides, glucagon-like peptide 1 (GLP-1)] receptor agonists, metformin, thiazolidinediones and pathways, finally leading to secondary complications of diabetes, are not sufficiently addressed. At present, nearly 1000 compounds have been described, and approximately 180 of these are going to be developed (already in clinical studies), some of them directly influencing enzyme activity, influencing pathophysiological pathways, and some using G-protein-coupled receptors. In addition, immunological approaches and antisense strategies are going to be developed. Many compounds are derived from physiological compounds (hormones) aiming at improving their kinetics and selectivity, and others are chemical compounds that were obtained by screening for a newly identified target in the physiological or pathophysiologic machinery. In some areas, great progress is observed (e.g., incretin area); in others, no great progress is obvious (e.g., glucokinase activators), and other areas are not recommended for further research. For all scientific areas, conclusions with respect to their impact on diabetes are given. Potential targets for which no chemical compound has yet been identified are also described.
lidanidiones, and α-glucosidase inhibitors] generally target only insulin resistance or β-cell dysfunction by increasing insulin secretion or tissue sensitivity to insulin.

Drugs addressing other aspects of the disease including promising emerging biological/molecular targets are under investigation. Many unsolved problems exist: 1) reduced β-cell sensitivity to glucose (“sensor defect”); 2) loss of β-cell number/function (no halting of diabetes progression); 3) loss of oscillations of insulin secretion; 4) loss of first-phase insulin response to a glucose challenge; 5) elevated proinsulin/insulin ratio; 6) abnormally high secretion of amylin; and 7) increased glucagon secretion (gluconeogenesis, glucose production). Normal physiological functions such as gastric emptying (slowing) or renal glucose reabsorption (blocking to increase glucose loss) could also be potential targets for future therapies.

In addition, many marketed drugs have major drawbacks that hamper therapy, and modifications in dosing and or new compounds should be developed to overcome these issues. Among others, the following problems continue to plague current therapy: 1) hypoglycemia (especially when initiating therapy; severe hypoglycemia are known to lead to myocardial infarction and to the development of dementia); 2) weight gain (a leading factor driving the epidemic of diabetes); 3) increase in insulin resistance; and 4) β-cell destruction.

In the United States, ~180 compounds for type 2 diabetes are in development (many are reviewed by Wagman and Nuss, 2001; Vats et al., 2005; Waknine, 2009; Mahajan and Gupta, 2010). In this review, compounds that do not focus primarily on diabetes but more or less are being developed to treat obesity (although related to diabetes) will not be included. In addition, the unexpected benefit of bariatric surgery for diabetes (independent of reducing fat) and the underlying signaling (Ferrannini and Mingrone, 2009) are not discussed. Also not discussed are anti-inflammatory effects of IgG2 antibodies or new technical applications and or formulations of known compounds [e.g., micropump metformin, MetControl (metformin chewing gum)].

II. Incretin Mimetics and Incretin Analogs

A new perspective, even a paradigm change, has recently been brought forward by two new classes called incretin hormones and incretin enhancers. Incretins are defined as being responsible for a higher insulin response to oral intake of glucose compared with an equal intravenous glucose load (i.e., reaching equivalent plasma glucose levels) (Elrick et al., 1964; Creutzfeldt, 1979, 2005; Nauck et al., 1986). The effects of GLP-1, the incretin of major importance, are summarized (Fig. 1), especially with respect to the pathophysiological aspects of type 2 diabetes (Table 1). GLP-1 receptor function and subsequent signaling has been recently reviewed (Verspohl, 2009).

There is no general agreement on a defect in GLP-1 response to nutrients in patients with type 2 diabetes (Nauck et al., 2011). Any impairment seems to be secondary to increased blood glucose and insulin resistance (i.e., not the cause of diabetes). The major advantage of new compounds in this field is the glucose dependence of insulin release (one major advantage over sulfonylureas), which may depend on the $K_{ATP}$ channels of the β-cell that close when activated by a rise in the intracellular ATP concentration, making them sensitive to the intracellular glucose metabolism and therefore the plasma glucose concentration. It is noteworthy that metformin has been shown to influence GLP-1 secretion (Cho and Kieffer, 2011; Maida et al., 2011), which previously was not a known action.

Two compounds, a GLP-1 analog (extremely high structure homology) and a GLP-1 mimetic (only 53% structure homology) have been developed. Developing new compounds based on GLP-1 must focus on improving the pharmacokinetics (longer effect) and the nonproduction of antibodies (though not a major clinical problem for marketed compounds until now) relative to the native incretin. While retaining proper receptor interaction and biological effects, these compounds are anticipated to have fewer side effects and decreased toxicity although nausea still occurs in ~50% of treated patients (Mikhal, 2008). Antibody development is not limited to mimetics (e.g., 53% homology) since also the development of the analog tasoglutide (95% GLP-1 homology) was stopped in 2011 because of allergic skin problems. Efforts focus on GLP-1 receptor agonists with better pharmacokinetics.

A. Exenatide Long-Acting Release (Bydureon)

Exenatide Long-Acting Release (LAR), which was scheduled to be marketed in Europe by the end of 2011, is based on the marketed exenatide (its effect lasts for only 6–8 h) and will enable once-weekly dosing (Drucker et al., 2008; Trautmann et al., 2008). It contains a polyac-
in diabetic rats for up to 28 days after only one subcutaneous injection (Gedulin et al., 2005). The time point of injection is not important. Exenatide levels in the periphery probably will be kept a little bit lower using this long-acting preparation to avoid peak values mainly responsible for the above-mentioned concentration-dependent GI side effects. There is no tachyphylaxis as sometimes recorded after continuous application of other hormones.

Exenatide LAR induces a much greater reduction (15-week treatment) in fasting glucose concentrations and HbA1c (to 6.8% from a baseline value of 8.5%) and weight loss (by roughly 4 kg) compared with normal exenatide administered twice daily (Kim et al., 2007). The side effects are not different compared with normal exenatide (DURATION-5 study; Blevins et al., 2011). The U.S. FDA wants additional proof of noncardiotoxicity. A drawback also is the big 23-gauge needle and the need for reconstitution before use. Another possibility to provide continuous and consistent delivery of exenatide is the DUROS technology, a subcutaneous osmotic delivery system acting for 12 months (ITCA 650; Intarcia Therapeutics, Inc., Hayward, CA) (Henry et al., 2010). Another possibility is to use a fusion protein containing exenatide and a long hydrophilic tail of natural amino acids for once-monthly injection (Cleland et al., 2011).

B. Albiglutide (Albugon, Syncria)

The clinical properties of the GLP-1 receptor agonist albiglutide (phase III; structural details in Fig. 2) have been reviewed (Rosenstock et al., 2009). Its long plasma half-life of 5 days (Rosenstock et al., 2009) (improved pharmacokinetics) enables once-weekly dosing, as a result of covalent binding of albumin. The tandem repeat structure (Fig. 2) improves the potency observed when only one GLP-1 moiety was covalently linked to albumin (note: a bulky carrier molecule). Covalently linked serum albumin is a well studied nonimmunogenic protein carrier that has been used to improve delivery and pharmacokinetic properties of other peptide-based drugs (Kratz, 2008). Albiglutide mimics the full range of GLP-1 actions, second messengers, and mechanisms (they are outlined at the beginning of section II) (Baggio et al., 2004; Matthews et al., 2008), albeit at higher concentrations than seen for in vitro insulin secretion ($EC_{50}$, 0.606 versus 0.019 nM for albiglutide and GLP-1, respectively). In vitro, no cleavage of the N-terminal sequence is observed up to 60 min after incubation with the degradation enzyme. In contrast, more than 80% degradation of the native hormone was observed after 60 min. A comprehensive phase III clinical research program (HARMONY) investigated the efficacy, safety, and long-term durability of albiglutide in comparison with monotherapy and combined therapies [e.g., metformin, metformin/thiazolidinedione, or metformin/sulfonylurea, combination with insulin and comparisons with established therapies including dipeptidyl-peptidase 4 (DPP-4; EC 3.4.14.5) inhibitors (http://clinicaltrials.gov/ct2/show/NCT00839527). Albiglutide improves glycemic control across a variety of doses and dosing schedules (i.e., 30 mg weekly, 50 mg
biweekly, and 100 mg monthly), HbA1c reductions are similar in weekly, biweekly, and monthly treatment groups, but variability in fasting plasma glucose levels and increased GI events were more likely to occur with higher doses of albiglutide administered monthly compared with weekly or biweekly dosing (Rosenstock et al., 2009).

Decreases in both systolic and diastolic blood pressure (−5.8 and −1.9 mm Hg, respectively) were also seen with 30 mg weekly albiglutide (Rosenstock et al., 2009). Albiglutide has less potent anorectic effects in animal studies compared with marketed exenatide and liraglutide; it is not clear whether this disparity is due to albiglutide itself or secondary to the impaired permeability of the blood-brain barrier as a result of the enlarged (albumin) molecule (Baggio et al., 2004).

The risk of side effects is low: hypoglycemias (0–3.1% in the range of placebo), nausea and/or vomiting (Rosenstock et al., 2009), and anti-albiglutide antibodies (2.5%), which in some patients appeared only transiently. The antibodies are non-neutralizing, have a low titer, and generally show cross-reactivity with GLP-1.

C. Taspoglutide (Ro 1583/BIM51077)

Taspoglutide is a matrix-free sustained-release formulation usable as a water-soluble application once a

| TABLE 1 | Comparison of defects in type 2 diabetes (pathophysiological state) and biological actions of incretin mimetics/analogues, and DPP-4 inhibitors |
| --- | --- | --- | --- |
| Defect/Feature in/of Type-2 Diabetes | Biological Action of Compounds (Pharmacological Groups) | Incretin Mimetics/Analogues | DPP-4 Inhibitors (Gliptins) |
| ↓ Insulin secretion | ↓ Insulin secretion (glucose-dependent; effectiveness preserved in type 2 diabetes) | Yes | Yes |
| Lack of first phase insulin secretion | Restoration of first phase of insulin secretion | Yes\(^a\) | Not known |
| Delayed insulin response (lacking first phase insulin release) (defect of incretin involved) | Reversed | Yes | |
| Disturbed pulsatility of insulin release | Improved | Replacement of incretin effect | Yes | |
| ↓ Incretin effect\(^b\) | ↓ Insulin biosynthesis | Improved | Yes | Yes |
| ↑ Glucagon secretion | ↑ Glucagon secretion (possibly indirectly) | Yes | Yes |
| Hyperglucagonemia (deficit of incretin involved) | | | |
| Hypoglycemia counter-regulation | ↑ Glucagon secretion at low glucose | | Yes |
| Postprandial hyperglycemia (defect of incretin involved) | Delay in gastric emptying, increase in glucose-induced insulin secretion | Yes | |
| ↑ ↓ ↓ Gastric emptying | | | |
| ↓ B-cell insulin content | ↑ Proinsulin biosynthesis | Yes | |
| ↓ B-cell mass (defect of incretin involved), preservation of B-cell mass | ↑ Proliferation of B-cells, ↑ B-cell replication (humans?) (see below) | Yes\(^c\) | Needs more investigation (Pospisilik et al., 2003; Deacon, 2004; Mu et al., 2006) |
| ↑ B-cell apoptosis | Increase in B-cell neogenesis | Yes\(^d\) | |
| No pathophysiological equivalent | ↑ Velocity of gastric emptying | Yes | |
| Energy/food uptake | ↑ Energy/food uptake (satiety effect)\(^e\) | Yes | No\(^d\) |
| Hunger | ↑ Central satiety signals (Flint et al., 1998) (although GLP-1 synthesis was demonstrated in the brain, peripheral GLP-1 may bind to brain areas) | Yes | |
| Weight problem | | | |
| Obesity (weight gain) (peripheral insulin induces weight gain, but not in case of incretina) | ↑ Weight gain or even weight loss | Yes\(^f\) | No\(^e\) |
| ↓ Food intake, ↓ body weight (Flint et al., 1998; Näslund et al., 1999a; Verdich et al., 2001; Zander et al., 2002) | ↓ Triglycerides, ↓ free fatty acids | Yes | |
| Hyperlipidemia | No immediate effect [↑ Insulin sensitivity (probably)] | Yes | |
| Insulin resistance | Improved | Yes | |
| Genetic disturbance of GLP-1 effect (polymorphism of TCF7L2 gene with increased type 2 diabetes risk)\(^f\) | | | |

\(^a\) Increase; ↓ decrease; ↓, no change.
\(^b\) Not known for liraglutide.
\(^c\) It is still a matter of debate whether type 2 diabetes is generally associated with defect in GLP-1 secretion (Näslund et al., 2011).
\(^d\) Only animal experiments; no clinical data yet available.
\(^e\) Incretin enhancers do not pass the blood-brain barrier, which may be the reason.
\(^f\) Weight loss of approximately 2 kg after 30 weeks is a common outcome of therapy with native GLP-1 (Zander et al., 2002), exenatide (Buse et al., 2004; DeFronzo et al., 1998; Näslund et al., 1999a; Verdich et al., 1993; Nauck et al., 1997; Näslund et al., 1999b) using cholinergic pathways (Schirra et al., 2009).
\(^g\) Decrease.
\(^h\) Not known for liraglutide.
\(^i\) Needs more investigation (Pospisilik et al., 2003; Deacon, 2004; Mu et al., 2006).
\(^j\) Needs more investigation (Pospisilik et al., 2003; Deacon, 2004; Mu et al., 2006).
\(^k\) Needs more investigation (Pospisilik et al., 2003; Deacon, 2004; Mu et al., 2006).
\(^l\) It is still a matter of debate whether type 2 diabetes is generally associated with defect in GLP-1 secretion (Näslund et al., 2011).
\(^m\) Only animal experiments; no clinical data yet available.
\(^n\) Incretin enhancers do not pass the blood-brain barrier, which may be the reason.
\(^o\) Weight loss of approximately 2 kg after 30 weeks is a common outcome of therapy with native GLP-1 (Zander et al., 2002), exenatide (Buse et al., 2004; DeFronzo et al., 1998; Näslund et al., 1999a; Verdich et al., 1993; Nauck et al., 1997; Näslund et al., 1999b) using cholinergic pathways (Schirra et al., 2009).
\(^p\) Decrease.
\(^q\) Not known for liraglutide.
\(^r\) It is still a matter of debate whether type 2 diabetes is generally associated with defect in GLP-1 secretion (Näslund et al., 2011).
\(^s\) Only animal experiments; no clinical data yet available.
\(^t\) Incretin enhancers do not pass the blood-brain barrier, which may be the reason.
\(^u\) Weight loss of approximately 2 kg after 30 weeks is a common outcome of therapy with native GLP-1 (Zander et al., 2002), exenatide (Buse et al., 2004; DeFronzo et al., 1998; Näslund et al., 1999a; Verdich et al., 1993; Nauck et al., 1997; Näslund et al., 1999b) using cholinergic pathways (Schirra et al., 2009).
\(^v\) Decrease.
\(^w\) Not known for liraglutide.
\(^x\) It is still a matter of debate whether type 2 diabetes is generally associated with defect in GLP-1 secretion (Näslund et al., 2011).
\(^y\) Only animal experiments; no clinical data yet available.
\(^z\) Incretin enhancers do not pass the blood-brain barrier, which may be the reason.
\(^{10}\) Weight loss of approximately 2 kg after 30 weeks is a common outcome of therapy with native GLP-1 (Zander et al., 2002), exenatide (Buse et al., 2004; DeFronzo et al., 1998; Näslund et al., 1999a; Verdich et al., 1993; Nauck et al., 1997; Näslund et al., 1999b) using cholinergic pathways (Schirra et al., 2009).
week. Development was suspended in phase III because of allergic hypersensitivity (e.g., skin).

D. Other Glucagon-Like Peptide 1 Receptor Agonists

Agonists to be developed are given in Table 2. The various future strategies are summarized in the remainder of this section.

1. Slower Degradation. N-terminal modification will prevent degradation by DPP-4; this is done by amide nitrogen substituents or by substituting with non-natural amino acids at the N terminus of the peptide. Site-specific PEGylated GLP-1 at the Lys34 position profoundly improves enzymatic stability and retains or even enhances biological activities (Lee et al., 2005, 2006).

2. Reduction of Rapid Renal Filtration. This is done by C-terminal modification (e.g., either by attaching fatty acids or PEG moieties to facilitate binding to blood proteins (albumin) or by direct fusion with a blood protein such as albumin or transferrin).

3. Oral Application. To improve the naturally limited intestinal absorption of a peptide, a series of GLP-1 analogs has been developed via site-specific conjugation to biotin-N-hydroxysuccinimide and/or to biotin-PEG-N-hydroxysuccinimide at Lys26 and Lys34 of GLP-1(7–36), respectively. The resultant GLP-1 analogs are Lys26,34-DiBiotin-GLP-1 and Lys26-Biotin-Lys34-(Biotin-PEG)-GLP-1. Both show enhanced intestinal bioavailability; their plasma concentration is rapidly increased 30 min after oral administration to rats. Both had a markedly better proteolytic stability than native GLP-1 and preserve their pharmacological activities (Chae et al., 2008). Many other types of peptide bioconjugation (e.g., using vitamins, fatty acids, and bile acids) have been used to develop orally active peptide agents (reviewed by Chae et al., 2008).

For oral application of GLP-1, the Eligen technology is being investigated (Beglinger et al., 2008). Eligen is a drug delivery agent forming a conformational complex with the peptide that is protected against degradation and helps the peptide to be absorbed. In high-throughput screening for an oral GLP-1 mimetic, Boc5 (i.e., butyloxycarbonyl as a protective group, a substituted cyclobutane) conjugate has been identified (Chen et al., 2007). It is a general-purpose technique that enables peptides (e.g., insulin) and even negatively charged heparin to be absorbed [companies Emisphere (Cedar Knolls, NJ) and Nobex (Innovaro Pharmalicensing, York, UK)].

A nonpeptide GLP-1 receptor agonist has been developed (6,7-dichloro-2-methylsulfonyl-3-N-tert-butylaminoquinoxaline) that is also an allosteric modulator of GLP-1 binding; however, it potentially damages cells (Coopman et al., 2010).
4. Intranasal and Pulmonary Application. Intranasal administration of exenatide and pulmonary administration of GLP-1 as Technosphere powders (Mannkind Corp., Valencia, CA) being investigated (Cassidy et al., 2008; Leone-Bay et al., 2009), a GLP-1(7-36)amide absorbed onto Technosphere microparticles, can be used for inhalation.

5. Transdermal Application. A transdermal application of exenatide (once a day) is under investigation by Eli Lilly & Co. (Indianapolis, IN) and Amylin (San Diego, CA).

E. General Comments

Better pharmacokinetics with no increase in side effects offer the potential to further improve the benefits already known for GLP-1 receptor agonists. Even compounds with once-a-month administration should be without problems because the insulinitropic effect is glucose-dependent; hypoglycemics, therefore, are not expected. It has to be noted that it takes approximately 6 weeks to reach optimum steady state blood levels, which will make it difficult to switch from twice-daily to weekly doses.

Possible side effects that have to be looked at during the development of new compounds: a few cases of pancreatitis have been reported since 2006 (Denker and Dimarco, 2006). As a result, the FDA has issued a statutory warning for exenatide, although the risk may be as low as 1.8 per 1000 patient years. It is unknown whether this side effect holds for the whole class of compounds or is an overestimate. Induction of pancreatitis by GLP-1 receptor agonists is doubtful (Bloomgren et al., 2010; Butler et al., 2010; Mølck et al., 2010) because 1) there is no plausible mechanism and 2) it is “typical” for type 2 diabetes; this means it might not be a therapeutic side effect.

In 2009, the FDA advisory panel expressed serious concern that liraglutide causes C-cell tumors (benign and malignant), which makes it mandatory to investigate future products in this class (Neumiller et al., 2010; News in Brief, 2010). In particular, the question of whether these rodent data are relevant to humans is unresolved.

Several therapeutic implications need clinical confirmation: the durability of the weight loss, the ability to preserve functional β-cell mass (β-cell regeneration; see section II.E.1) and the applicability to patients other than those with type 2 diabetes (glucagon suppression may be important for those with type 1 diabetes as well) and obese patients lacking diabetes (influence on satiety and gastrointestinal emptying). In addition, long-term studies concerning clear cardiovascular end-points are needed; there exist many GLP-1 effects on the heart that are either receptor dependent or -independent (Ban et
GLP-1 receptors are expressed in the proximal tubules: effects are increased diuresis, ion loss, and decreased excretion of $H^+$. 

1. β-Cell Regeneration. Data obtained from rodents indicate that adult β cells replicate primarily from pre-existing β cells rather than from non-β-cell precursors (Chen, 2009). This in vivo regeneration provides a novel therapeutic approach to replace β cells lost by autoimmune destruction (type 1 diabetes) or to restore β-cell mass damaged by the failure of compensation (type 2 diabetes). A new aspect is the switching of glucagon-producing A-cells to provision of GLP-1 as a local factor in combination with SDF-1 (stroma cell-derived factor-1) thus promoting growth, survival, and viability of β cells (Liu et al., 2011).

Regeneration is a promising effect of GLP-1 because of its ability to increase β-cell mass by stimulating neogenesis and reducing apoptosis in rodents (as discussed before). This goal is also addressed by other compounds: DG770 (Austen and Burk, 2011), with its possible proliferative effect, and paullone class compounds, with their additional apoptotic effect (Lahusen et al., 2003). NBI 6024 (altered peptide ligand corresponding to the 9-23 amino acid region of the insulin B chain) was designed to inhibit autoreactive T cells but failed in improving or maintaining β-cell function (Walter et al., 2009). Gastrin is thought to play a key role in β-cell differentiation and regeneration; TT-223 E1 I.N.T. for injection, phase IIa is composed of a gastrin analog and an epidermal growth factor analog that together reduce insulin requirement (von Herrath, 2005).

It has to be added that other manipulations leading to an increase of GLP-1 [and glucose-dependent insulinotropic polypeptide (GIP); e.g., by reducing DPP-4 action (section III)] are effective as well (Pospisilik et al., 2002; Conarello et al., 2003). There is no means for testing directly the changes of β-cell mass in patients with diabetes. Nevertheless, a preliminary report on protection of human β-cell function has been published (Foley et al., 2011).

2. Gene Therapy with Respect to Glucagon-Like Peptide 1. Long-term effects of a single administration of recombinant adenoviral vector expressing GLP-1 via the tail vein into diabetic (streptozotocin) nonobese and immunodeficient mice (NOD/SCID mice) resulted in remission of diabetes within 10 days and in sustained normoglycaemia (Liu et al., 2007). Intramuscular gene transfer of a plasmid coding for the GLP-1/Fc peptide in db/db mice enhanced insulin secretion and suppressed glucagon release (Kumar et al., 2007). Hence, gene therapy leading to overexpression of GLP-1 related peptides may have therapeutic potential.

3. Glucose-Dependent Insulinotropic Polypeptide (Another Incretin). GIP (originally referred to as gastric inhibitory peptide) is another incretin (Holst, 2004). It is difficult to state which incretin, GLP-1 or GIP, is more important; GIP may be more effective because its post-prandial concentrations are much higher than minimum insulinotropic concentrations, which is in contrast to GLP-1 (for review, see Creutzfeldt and Nauck, 1992). On the other hand, GLP-1 actions remain relatively preserved in patients with type 2 diabetes, which is in contrast to GIP (Nauck et al., 1993a). Clinical interest has been hampered by a report of resistance to its insulinotropic action in patients with type 2 diabetes (Nauck et al., 1993). However, this may not be true, according to a conflicting report that used therapeutic concentrations (Deacon et al., 2000). GIP is also disappointing with respect to its lack of gastric emptying activity and absence of weight reduction when used. Recent data show a bifunctional regulation of glucagon secretion: increase during fasting and hypoglycaemia conditions, but no effect during hyperglycaemia (Christensen et al., 2011).

The effectiveness of GIP receptor agonists may be underestimated when other beneficial effects are not taken into account [e.g., their role in lipid metabolism and fat deposition (Irwin and Flatt, 2009), stimulation of growth, differentiation, proliferation, and survival of β cells (Trümper et al., 2001, 2002; Ehses et al., 2002)]. A lowered GIP receptor expression and resulting GIP resistance in type 2 diabetes was observed as well as an impaired GIP secretion (Skrha et al., 2010). Long-acting GIP analogs are being investigated more extensively (Irwin and Flatt, 2009), including analogs that did not make it to market.

III. Dipeptidyl-Peptidase 4 Inhibitors

The incretin concept allows for two different therapeutic approaches, the first having been discussed in section II. The second approach is to enhance endogenous incretin concentrations by inhibiting/delaying their degradation mediated by the enzyme DPP-4 (also called incretin enhancers). More than 40 years ago, a serine protease was purified, later called DPP-4 (Hopps-Havu and Glenner, 1966; Pattzi et al., 2010), that cleaves N-terminal dipeptides with a proline or alanine residue and thus also inactivates both incretins, GLP-1 and GIP.

A. Sitagliptin, Vildagliptin, and Saxagliptin

Three DPP-4 inhibitors have been approved in Europe: sitagliptin, vildagliptin, and saxagliptin. Their differences from GLP-1 receptor agonists include 1) oral bioavailability; 2) less of a maximum effect (see next paragraph), including fewer side effects with overdose (e.g., nausea); 3) no effect on gastric emptying; 4) no direct central nervous system effects (lack of effect on satiety) and thus no weight reduction; 5) nonselectivity (with respect to other enzymes and many other DPP-4 substrates in addition to GLP-1); and 7) nontoxicity of overdose (except liver toxicity and QT prolongation for vildagliptin). For further differences, see Table 1.
The less maximal effect of DPP-4 inhibitors results from the fact that patients cannot be titrated as with GLP-1 agonists. DPP-4 inhibitors moderately increase endogenous GLP-1 levels, which explains some of the differences from GLP-1 agonists shown in Table 1. Thus, the reason for several differences is that the “relative” incretin increase is much smaller using an incretin enhancer than using incretin mimetics or analogs. Future development of DPP-4 inhibitors should concentrate on compounds with higher potency and greater selectivity for DPP-4 over other related enzymes, such as DPP-2, -8, and -9.

Side effects that have not been observed yet in the clinic but have been observed in vitro and in some animal studies should be considered during development of new compounds: skin toxicology for vildagliptin and saxagliptin, decrease in lymphocytes for saxagliptin, increase in transaminases for vildagliptin.

**B. Linagliptin (BI 1356; Tradjenta)**

Linagliptin [I-8-(3-amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione] is a second-generation, xanthine-based DPP-4 inhibitor (Boehringer Ingelheim, Ridgefield, CT), approved by the FDA in May 2011. It is more potent and more selective (Table 3) than the three earlier compounds (Thomas et al., 2008a,b; Graefe-Mody et al., 2009) and has a very high selectivity, except for fibroblast activation protein α. The potential differences from other competitive compounds are described below.

The pharmacokinetics is nonlinear, which may be explained by in vitro experiments on concentration-dependent binding (Retlich et al., 2010); it binds to the target at rather low plasma levels in humans. Linagliptin exposure [area under the plasma concentration–time curve and maximum plasma concentration (Cmax)] increased less than proportionally with dose (Heise et al., 2009).

Because DPP-4 binding capacity is saturated at low doses, accumulation of linagliptin in tissues is unlikely, despite the long persistence of low amounts in the body (Fuchs et al., 2009). The long terminal half-life (113–131 h) leads to a sustained inhibition of DPP-4 activity (Heise et al., 2009; Deacon and Holst, 2010).

Unlike the other inhibitors, linagliptin is extensively protein-bound (>80%) (Scheen, 2010b), is not metabolized in vivo (radioactive compound experiments) (Blech et al., 2010), and the elimination occurs primarily via liver (Deacon and Holst, 2010); biliary and fecal excretion are the dominant excretion pathways, with 84.7% (by mouth) and 58.2% (intravenous) (Blech et al., 2010), and renal excretion accounted for 5.4% (by mouth) and 30.8% (intravenous). This means that dosage adjustments to account for renal insufficiency are not necessary, which may be a major advantage for diabetics with this secondary defect.

Thus a unique profile of linagliptin in the DPP-4 inhibitor class suggests that linagliptin may be superior to competitors. Side effects of Linagliptin are limited; long-term experience is missing. No signs or symptoms of hypoglycemia have been observed, even at high doses of up to 600 mg (Tiwari, 2009).

**C. Alogliptin (SYR-322)**

Alogliptin is a novel quinazolinone-based DPP-4 inhibitor developed by Takeda Healthcare Products Co., Ltd. (Kyoto, Japan) that improves glycemic control (Pratley et al., 2009) and has a kinetic profile that may allow a once-a-day dosing regimen (Christopher et al., 2007; Christopher and Karim, 2009). It exhibits >10,000-fold selectivity for DPP-4 over the closely related serine proteases DPP-2, DPP-8, and DPP-9 (Table 3); fibroblast activation protein (seprase); prolyl endopeptidase; and tryptase. It has an absolute oral bioavailability of between 45 and 88% (Lee et al., 2008), is not metabolized extensively, and >70% is eliminated unchanged. Positive results have been observed in phase III clinical studies (a new drug application was submitted to the FDA in 2008) (for review, see Fredenrich et al., 2009). However, alogliptin approval by the FDA was postponed in 2009 because of insufficient data on cardiovascular risks.

Linagliptin and alogliptin show no relevant drug-drug interactions (Scheen, 2010a,b). A reduction in the dose of sulfonylureas is usually recommended (risk of hypoglycemia) when DPP-4 inhibitors are added (pharmacodynamic rather than a pharmacokinetic interaction) (Scheen, 2010a).

### TABLE 3

Comparison of DPP-4 inhibitors to be developed

<table>
<thead>
<tr>
<th>Enzyme Inhibition</th>
<th>Dissociation Velocity</th>
<th>Selectivity versus</th>
<th>Selectivity versus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In Vivo after 24 h (ED50)</td>
<td>From Enzyme</td>
<td>DPP-8 and DPP-9</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>nM</td>
<td>%</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>&gt;30</td>
<td>19</td>
<td>&gt;80 (max. 97)</td>
</tr>
<tr>
<td>Vildagliptin</td>
<td>14</td>
<td>62</td>
<td>&gt;80 (max. 95)</td>
</tr>
<tr>
<td>Alogliptin</td>
<td>10</td>
<td>24</td>
<td>75 (max. 90)</td>
</tr>
<tr>
<td>Saxagliptin</td>
<td>2.7</td>
<td>50</td>
<td>70 (max. 80)</td>
</tr>
<tr>
<td>Linagliptin (BI 1356)</td>
<td>0.9</td>
<td>1</td>
<td>70 (max. 80)</td>
</tr>
<tr>
<td>Dutogliptin (PHX 1149)</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Degree of inhibition does not translate into dosing.
D. Dutagliptin (PHX 11497)
Dutagliptin ([(2R)-1-[(3R)-pyrroolidin-3-ylamino]acetyl]pyrroloidin-2-yl)boronic acid (originally developed by Phenomix Corporation, San Diego, CA; phase III) can be used as a once-daily oral therapy (100–400 mg) (Garcia-Soria et al., 2008; Pattzi et al., 2010); efficacy and tolerability are positive (O’Farrell et al., 2007; Garcia-Soria et al., 2008; Pattzi et al., 2010). Dutagliptin is highly water soluble (>2000 mg/ml), has low cellular permeability, is rapidly absorbed (with a $T_{\text{max}}$ of 3 to 4 h), has a half-life of 10 to 13 h, exhibits low plasma protein binding (11%) (Pattzi et al., 2010), is not metabolized (Pattzi et al., 2010), and is excreted renally. Further development is not clear because Phenomix was shut down recently.

E. Other Compounds
Many more compounds (for review, see Gupta et al. (2009)) are under investigation. They may be classified as peptidomimetic or nonpeptidomimetic; the peptidomimetic may be divided into glycine-based or β-alanine-based and then again into irreversible and reversible inhibitors. They include:

- ALS2–0426/AMG 222, a small-molecule DPP-4 inhibitor (phase II, 2007): once per day orally, no further publications.
- Carmegliptin (R-1579; phase II completed): reversible inhibitor (Gupta et al., 2009).
- NVP-DPP728 (1-[[2-[(5-cyanopyridin-2-yl)amino]ethyl]amino[acetyl]-2-cyano-(S)-pyrrolidine] is an irreversible inhibitor and can also reverse new-onset diabetes in NOD mice by reducing insulitis, increasing CD4(+)/CD25(+)FoxP3(+) regulatory T cells (immunomodulatory effects), and stimulating β-cell replication (Tian et al., 2010).
- P32/98 (isoleucine thiazolidide di-[3N-(2S,3S)-2-amino-3-methyl-pentanoyl]-1,3-thiazolidine)fumarate): is effective and increased β-cell mass in animal studies (Pospisilik et al., 2003; Wargent et al., 2005).
- Gosogliptin (PF-734200): phase II.
- PSN 9301: discontinued after phase II (Epstein, 2007).
- SK-0405, an N-terminal bis-(2-chloroethyl)amino and fluoro sulfonyl analog of calcitonin gene-related peptide 8-37, has a low IC$_{50}$ of 3.3 nM, a high selectivity (17,000-fold over DPP-8, DPP-9, and fibroblast activation protein), and longer lasting enzyme inhibition compared with vildagliptin (Yasuda et al., 2007).
- SDZ 029-576: belongs to a group of 1-aminomethylisoquinoline-4-carboxylate with substitutions of the isoquinoline at positions 6 and 8.

Many more DPP-4 inhibitors, including those for which the status is not clear, were reviewed by de Meester et al., 2003 and Yu and Wang, 2008:

- Valine pyrrolidide (also investigated with respect to GLP-2 degradation inhibition) (Hansen et al., 2007; Pedersen et al., 2008). This prototype has been used to improve the knowledge of the binding site helping to design DPP-4 inhibitors.
- Fluoro-olefin (Edmondson et al., 2008) and fluoro olefin isosteres of diacyl hydroxylamines.

Three series of DPP-4 inhibitors have been synthesized using a linker for substituted anilines, benzylamines, and phenylethylamines to (2S)-cyanopyrrolidine; more than 20 compounds were thus evaluated (Coumar et al., 2007).

Other reversible inhibitors are as follows:

- Oligopeptides (diprotin A and B): more or less investigated as CD26 inhibitors under immunological aspects (DPP-4 is nearly identical to a functional protein called T-cell antigen CD26; see below).
- Boronic acid derivatives such as Pro-boroPro: investigated with respect to immunological aspects and aspects of food intake by inhibiting degradation of appetite regulatory peptide TMC-2A (Aspergillus oryzae-derived, but not investigated with respect to diabetes).
- Irreversible inhibitors include: diacyl hydroxylamines, dipeptide phosphonates prodipine (Pro-pro diphenyl-phosphonate) (investigated only with respect to their immunological effects and not as compounds to treat diabetes). The new DPP-4 inhibitors mentioned here have practically no risk for causing hypoglycemia (by comparison, e.g., glipizide has approximately a 32% chance of inducing hypoglycemia) but may have unexpected side effects due to nonselectivity, a possibly underestimated aspect. DPP-4 selectively cleaves peptides with a proline or alanine residue in the penultimate amino-terminal position (see beginning of section III), which includes, in addition to GLP-1, approximately 30 other compounds (Table 4), mainly hormones, neuropeptides, cytokines, and chemokines (Mentlein et al., 1993; Mentlein, 1999; De Meester et al., 2000; de Meester et al., 2003), thus influencing the regulation of immune, nervous, and endocrine functions (Durinx et al., 2000; Gorrell, 2005). This should be an issue that needs to be monitored regarding long-term safety.

Other concerns (relevance has to be more strictly evaluated) are lack of selectivity against other DPP enzymes, such as DPP-2, DPP-8, and DPP-9 (Table 3); fibroblast activation protein; and attractin. Inhibition of DPP-8 and DPP-9 can cause thrombocytopenia, anemia, enlarged spleen, skin lesions, bloody diarrhea, and mortality; it also influences hematological and immune cells (Gorrell, 2005; Lankas et al., 2005; reviewed by Ver-
spohl, 2009), although some aspects have not been confirmed (Burkey et al., 2008).

In addition, the link of DPP-4 to immunomodulation has to be considered with respect to T-lymphocyte memory cell activity, cell adhesion, and cell movement (Masuyama et al., 1992, 1999; reviewed by Verspohl, 2009). These effects are mediated by the nearly identical functional protein T-cell antigen CD26, which has no enzymatic activity (Bednarczyk et al., 1991). Therefore, it is not surprising that low DPP-4 may be clinically linked to some immune diseases: systemic lupus erythematosides (Stanciková et al., 1992), rheumatoid arthritis (synovial fluid) (Kamori et al., 1991), and Crohn’s disease (Willehm et al., 1997). The clinical data are not conclusive because DPP-4 has been shown to have contradictory effects: that is, increasing and inhibiting immune responses (reviewed by Hildebrandt, 2004) and long-term safety based on effects on CD26 remains unknown (Richter et al., 2008a) except for a nonspecific increase of infections after sitagliptin therapy (Richter et al., 2008b). In developing new compounds, the aspect of a possible link of sitagliptin to hepatitis should be kept in mind.

Conclusion. No great differences in pharmacological efficacy are expected from newly developed DPP-4 inhibitors; potential differences may only result from data on metabolism, elimination, and enzyme selectivity. DPP-4 inhibitors (as well as GLP-1 receptor agonists) expand type 2 diabetes treatment options and are possibly prophylactic. New DPP-4 inhibitors must be profiled for enzyme selectivity, T-cell proliferation, and animal toxicity. For the new class of antidiabetic agents (incretin enhancers, gliptins), long-term clinical studies (weight durability, β-cell function, and cardiovascular endpoints) need to determine the risk/benefits of targeting the incretin axis. The TECOS (trial evaluating cardiovascular outcomes with sitagliptin) study (http://clinicaltrials.gov/ct2/show/NCT00790205) is aimed at the cardiovascular risk of sitagliptin, which should be kept in mind for the whole class of compounds and developments in the future. So far, no studies have reported on patient-oriented parameters such as mortality, costs of treatment, and health-related quality of life. The ability of either treatment to preserve functional B cell mass in humans needs clinical confirmation. DPP-4 inhibitors are additive to established antidiabetic drugs and may be compatible in the future with first-line therapy. Their exact place in therapy remains to be explored.

1. Neutral Endopeptidase 24.11 Inhibition as a Target? NEP-24.11 is a membrane-bound zinc metallopeptidase (neutral endopeptidase) that cleaves peptides at the N-terminal side of aromatic or hydrophobic amino acids; six potential cleavage sites have been detected in GLP-1. Up to 50% of GLP-1 that enters the circulation may be degraded by NEP-24.11; therefore, combined inhibition of DPP-4 and NEP-24.11 could be superior to DPP-4 inhibition alone in preserving intact GLP-1 (Plamboeck et al., 2005).

IV. Sodium-Coupled Glucose Cotransporter 2 Inhibitors

Glucose enters eukaryotic cells via two different types of membrane-associated carrier proteins, the facilitative type glucose transporters (GLUTs) (Shepherd and Kahn, 1999) and the sodium-coupled glucose cotransporters (SGLTs) (Kanai et al., 1994; Wood and Trayburn, 2003; Asano et al., 2004b). SGLT1s couple the transport of glucose against a concentration gradient with the simultaneous transport of Na+ (1:1 ratio) (Mackenzie et al., 1996). Two major SGLT isoforms, SGLT1 and SGLT2, have been cloned (Wright, 2001). A third form, SGLT3, has also been reported to exist in several tissues. SGLT2, a low-affinity high-capacity transport system (Wells et al., 1992; Kanai et al., 1994; Katsuno et al., 2007), is specifically expressed in the kidney, exclusively at the apical domain of epithelial cells in the early proximal convoluted tubule (S1 segment), is responsible for the reabsorption of the bulk (90–98%) of renally filtrated glucose (Fig. 3) (Wright et al., 2007; Idris and Donnelly, 2009), and is a critical factor for maintenance of glucose balance. SGLT1 is located primarily in small intestinal cells but is also present in kidney (area of Henle’s loop) and heart (cardiac glucose transport) (Zhou et al., 2003;
Asano et al., 2004b). SGLT1 accounts for the additional 10% of renal glucose reabsorption. Inhibition of SGLT1 causes primarily a malabsorption of sugars, resulting in diarrhea (Turk et al., 1991; Martín et al., 1996).

SGLT2 inhibition as a therapeutic target was encouraged and accelerated by the knowledge of mutations in the SGLT2 gene, called “kidney diabetes,” which results in renal glucosuria with no danger of a hypoglycemia (van den Heuvel et al., 2002; Calado et al., 2004). SGLT2 inhibitors have the advantage of being an insulin-independent treatment option (Washburn, 2009). Every 1 g of glucose excreted into the urine equates to ~4 kcal of energy. Approximately 50 to 90% inhibition of SGLT2 is required to elicit an effective glucose-lowering response (O’Connor-Semmes et al., 2007). Various SGLT2 inhibitors are known. The first to be discovered was phlorizin, a natural product and dietary constituent (Starke et al., 1985; Rossetti et al., 1990; Harmon et al., 2001; Ehrenkranz et al., 2005) that has two major drawbacks: nonselectivity with respect to other glucose transporters (Rossetti et al., 1987a,b; Fisher et al., 1997; Oku et al., 1999; Ehrenkranz et al., 2005; Katsuno et al., 2007; Calado, 2009) and low bioavailability because of its tendency to be hydrolyzed in the gut to its aglycone phloretin. Because phlorizin is found in apple tree bark (as well as bark from other fruit trees), this probably explains the use of apple tree bark in ayurvedic medicine.

Many SGLT2 inhibitors are glycosides structurally derived from the prototype phlorizin. Novel SGLT2 inhibitors having different chemical, pharmacodynamic, and pharmacokinetic profiles have been reviewed (Kees et al., 1996; Link and Sorensen, 2000; Ohsumi et al., 2003; Zhang et al., 2005, 2006; Isaji, 2007; Abdul-Ghani and DeFronzo, 2008; Idris and Donnelly, 2009; Washburn, 2009). These compounds comprise O-, C- and N-glycosides generated by attachment of an appropriate lipophilic aglycone component to a suitable glucose analog (Washburn, 2009). 3-(Benzo[b]furan-5-yl)-2,6-dihydroxy-4-methylpropiofuranone-2-O-(6-O-methoxy carbonyl-β-D-glycopyranoside) (T-1095), a phlorizin derivative, overcomes the low bioavailability of phlorizin but has been discontinued because of its nonselectivity versus SGLT1 (Oku et al., 1999; Asano et al., 2004a,b).

After the discovery of T-1095, medicinal chemistry has been used to investigate the addition of various substituents to the glycoside core to enhance potency, selectivity, and oral bioavailability. For example, structural modification from O- to C-glycosides (creating a carbon-carbon bond between the glucose and the aglycone moiety) modified the pharmacokinetic half-life and duration of action. This modification from O- to C-glycosides was part of a clinical evolution. O-Glycosides are sergliflozin and remogliflozin (no longer in development although already in phase II), and C-glycosides are dapagliflozin and canagliflozin.

A. Dapagliflozin (Phase III)

Dapagliflozin [BMS-512148; Bristol-Myers Squibb (Stamford, CT), licensed to AstraZeneca Pharmaceuticals LP (Wilmington, DE)] is the most advanced compound in this class (Han et al., 2008; Calado, 2009; Kipnes, 2009; Komoroski et al., 2009a,b). It has an IC\textsubscript{50} of 1 nM at SGLT2, which is 3000 times higher affinity than for SGLT1 inhibition (high selectivity) (Calado, 2009). Fasting plasma glucose, postprandial plasma glucose, HbA\textsubscript{1c} and body weight (as a result of caloric loss) are reduced (Brooks and Thacker, 2009; Kipnes 2009; Woo, 2009). The renal glucose loss increased to 40, 73, and 82 g/day (5-, 25-, and 100-mg groups, respectively). The diuretic (osmotic) effect from this loss of glucose may also help to control hypertension (Calado, 2009). Dapagliflozin has linear pharmacokinetics over the dose range of 2.5 to 500 mg/day, and its
effect is not influenced by simultaneous food consumption. It is primarily eliminated via urinary excretion (Kipnes, 2009). No serious adverse events have been reported, except an increased occurrence of mycotic genital infections (Calado, 2009), which is typical for this therapeutic group. Very few patients reported polyuria or nocturia. Major hypoglycemic episodes have not been observed; rare reports came from combination use with metformin. Larger, multicenter, randomized, double-blind, placebo-controlled clinical trials are ongoing (http://clinicaltrials.gov/ct2/show/NCT00162305). Dapagliflozin represents the first in a new class of drug that may represent a promising new option in the treatment of type 2 diabetes (Brooks and Thacker, 2009) and is intensively investigated (Poucher et al., 2011). However, because of an increase in breast and bladder cancer, experts did not recommend FDA approval for 2011 (its decision is pending). Whether it originally linked to the compounds or is even a class effect remains unsolved. However, canagliflozin and empagliflozin did not yet show these effects.

B. Additional Compounds

Nagliflozin and canagliflozin (TA-7284) (Rosenstock et al., 2010; Rothenberg et al., 2010; Sarich et al., 2010) are already in phase III trials.

Empagliflozin (BI-10773, phase III) has positive effects, such as loss of body weight. Thirst, pollakiuria, and nasopharyngitis have been reported.

An aryl C-glycoside [Yamanouchi Pharmaceutical (Tokyo, Japan) and Kotobuki Pharmaceutical (Osaka, Japan)] inhibits (IC\textsubscript{50}, 5.7 nM) the uptake of methyl-\alpha-D-glucopyranoside in Chinese hamster ovary cells stably expressing human SGLT2. The blood glucose concentration-time profiles are lowered over an 8-h period by 45% (Handlon, 2005).

AVE2268 (Sanofi-Aventis, Bridgewater, NJ), a substituted glucopyranoside of which the full structure is described by Bickel et al. (2008), was in phase II (Mezzetti et al., 2008), but this study is now discontinued. It had selectivity for SGLT2 (IC\textsubscript{50}, 13 nM) compared with SGLT1 (IC\textsubscript{50}, >10 \mu M) (Bickel et al., 2008).

Other compounds in clinical trials have been reviewed (Ferranini et al., 2010; Rajesh et al., 2010). Two worth mentioning are ipragliflozin (ASP-1941) and LX-4211 (phase II), a phlorizin derivative with a structure described by Chao and Henry (2010). They are being tested in both monotherapy and combination therapy with approved antidiabetic agents. Favorable clinical data were provided for [(S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol hydrate (TS-071) (Seino et al., 2011) and (1R,2S,3S,4R,5R)-5-(4-chloro-3-(4-ethoxybenzyl)phenyl)-1-(hydroxymethyl)-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol (PF04971729; Amin et al., 2011)].

C. General Safety and Tolerability Aspects

There is no doubt about safety because patients with familial renal glucosuria (SGLT2 inhibition by gene defect; see section IV) maintain normal long-term kidney function (Santer et al., 2003). In addition, patients with other renal abnormalities, inducing glucosuria (SLC5A2 gene mutation), are known to live a normal life (Rajesh et al., 2010).

Osmotic diuresis (because of glucose loss) may be slightly induced, accompanied by a slight increase in hematocrit along with some exsiccosis. It is not clear, however, whether the risk of urinary and/or genital tract infection is increased (Geerlings et al., 2000).

The advantages of SGLT2 inhibitors may be summarized as follows: 1) improvement of blood sugar control without insulin involvement; 2) weight loss (or at least weight maintenance); 3) improvement of insulin sensitivity; 4) indirect preservation of β cells by depletion of toxic blood glucose concentration; 5) No hypoglycemias (because there is no insulinotropic effect or inhibition of hepatic glucose production) (note: the SGLT1 transporter capacity is sufficient to reabsorb a minimum amount of glucose); 6) Reduction of blood pressure; and 7) possible reduction of comorbidities such as obesity, dyslipidemia, and heart failure.

Disadvantages could include the following: 1) risk of a negative effect of glucosuria on the kidneys, polyuria, and increased thirst; 2) risk of bacterial or fungal infection of the urinary or genital tract; 3) salt-wasting (disturbances of electrolytes are not anticipated unless SGLT1 is also inhibited as a result of lack of selectivity, because sodium is reabsorbed to a high degree by SGLT1); and 4) compensatory increase in feeding due to calorie loss.

Conclusion. SGLT2 inhibitors may be useful add-on agents with a low risk of hypoglycemia and good potential for weight loss. SGLT2 represents a promising drug target and an innovative approach to treatment of type 2 diabetes (and type 1 diabetes). The main advantage of these inhibitors is that they act independently of the severity of β-cell dysfunction or insulin resistance, i.e., irrespective of the underlying pathogenesis of hyperglycemia or even “glucose toxicity”; their advantage is to act independently of insulin. It has to be elucidated whether SGLT2 inhibitors may have therapeutic effects on other components of the metabolic syndrome (e.g., lipids, hypertension, obesity) and whether they positively affect diabetic microvascular complications (especially of the kidneys). The risk/benefit ratio of this new class of drug will decide their place for clinical use in the future.

In addition, SGLT1 inhibitors are being investigated. Their efficacy is questionable because they will also influence heart glucose uptake (see first paragraph of section IV). There is no consensus as to whether SGLT1 inhibition is needed in addition to SGLT2 inhibition. It
is uncertain how patients will be titrated correctly for inhibition of glucose absorption. Antisense oligonucleotides against SGLT2 (see section XII.E) are going to be developed.

V. Pramlintide (Amyloid Deposits, Amylin)

A hundred years ago, white amyloid deposits were isolated from the pancreas that were later called amyloid polypeptide (IAPP). This lesion is found in ~90% of patients with type 2 diabetes at autopsy and is associated with toxicity, decreased islet β-cell mass, and progressive loss of function (Marzban et al., 2005). IAPP expression may not be an indicator of cell death induction, but IAPP, including its oligomer, may be an important determinant of the fate of β cells (Park et al., 2010).

Amylin is a neuroendocrine hormone that is produced and cosecreted with insulin from β cells. In patients with type 2 diabetes, plasma amylin levels after a meal are half those in persons without diabetes (16 pM). It must be stressed here that the basis of type 2 diabetes is an amylin fault in addition to the well known insulin fault and glucagon surplus. The therapeutic approach is to compensate for this lack by substituting amylin. Amylin cannot be used directly because it aggregates and promotes a viscous solution. Pramlintide (Fig. 4) is an amylin mimic, marketed in a few countries (e.g., as Symlin in the United States). Pramlintide is injected subcutaneously as an acetate 15 min before a meal. It allows a dose reduction of quickly acting insulins and reduces/smoothens glucose levels after a meal in a dose-dependent manner (up to 300 µg are used). It has additional positive effects: lowering HbA1c, inducing satiety, inhibiting glucagon release, and reducing weight. This is why pramlintide is also effective in patients with type 1 diabetes (Weyer et al., 2003; Ogbru, 2005; Manzella, 2007).

VI. Peroxisome Proliferator-Activated Receptor Agonists (New Glitazones and Glitazars); Glitazars Are Dual Peroxisome Proliferator-Activated Receptor Agonists

PPARγ agonists (thiazolidinediones) were introduced to overcome insulin resistance, which, in the early stages of diabetes is more relevant than inadequate insulin release. Two were withdrawn (rosiglitazone and troglitazone) and some were not pursued. Thiazolidinediones have a genomic effect regulating more than 100 genes; however, there exists only a ~30% overlap of these 100 genes, which explains their differing effects [e.g., on high-density lipoprotein (HD) and low-density lipoprotein cholesterol] and differing safety profiles (for more details, see Rizos et al., 2009; Saha et al., 2010; Shah et al., 2010). There also exist receptor-independent effects that differ from one glitazone to another (Feinstein et al., 2005). Their nonselectivity enforces careful examination of new ones to be developed and may even include indications not yet used: polycystic ovary syndrome, ovarian hyperstimulation syndrome [by vascular endothelial growth factor (VEGF) inhibition in granulosa cells] (Shah et al., 2010), nonalcoholic steatohepatitis (Belfort et al., 2006), psoriasis (Krentz and Friedmann, 2006), and autism (Boris et al., 2007). New compounds should be tested for side effects already observed or discussed for established PPARγ agonists: heart failure, osteoporosis, and bladder cancer. New developments regarding PPAR agonists are summarized in Table 5.

Rivoglitazone is in phase III clinical trials (Schimke and Davis, 2007; Rohatagi et al., 2008; Truitt et al., 2010). There were many dropouts within 6 months during the trials because of side effects (Truitt et al., 2010), and its efficacy has been questioned, although some reported a much stronger HbA1c reduction compared with pioglitazone. Its ED50 (0.20 mg/kg) for the glucose-lowering effect was a hundred times lower than that of pioglitazone and rosiglitazone (Kanda et al., 2009). It is selective in that it has only a small effect on PPARα and PPARδ activity (Kanda et al., 2009). The dose-limiting side effects such as expansion of the plasma volume (low hematocrit) and weight gain should be overcome: mitiglitazone (MSDC-0160) is an insulin sensitizer not directly interacting with PPARγ and may lack these side effects.

Although PPARγ is present mostly in adipocytes, PPARα is expressed at high levels in liver, heart, and muscle. Dual PPARα and PPARγ activators, called glitazars, are being developed. PPAR-α agonists, marketed as fibrates and first used in the 1970s, lower plasma triglycerides and very-low-density lipoprotein and increase HDL cholesterol, which altogether is associated with a cardiovascular benefit. PPAR-α and PPAR-γ agonists also positively affect inflammation and vascular remodeling (Staels and Fruchart, 2005). Neither fibrate

![Fig. 4. Symlin (positions 25, 28, and 29 are phosphorylated).](image-url)
given alone nor any glitazone on its own is able to combine all aspects of the above-mentioned benefits. This was the reason for synthesizing dual PPARα and PPARγ activators (Sauerberg et al., 2002; Balakumar et al., 2007).

The design and synthesis of dual-acting PPARα/γ agonists has been described for aleglitazar, muraglitazar, ragaglitazar, and tesaglitazar (Ramachandran et al., 2008). Ragaglitazar was discontinued as a result of bladder tumors, and tesaglitazar because of a decrease in renal filtration. Muraglitazar has side effects with respect to increased cardiovascular risk and no pharmacological advantage. Thus, only aleglitazar is in phase III. T33 (a nonthiazolidinedione benzopyran derivative) has different affinities for PPARα and PPARγ (EC50, 19 and 148 nM, respectively) (Hu et al., 2007), making therapy unreasonable. MBX213, a nonthiazolidinedione, is as effective as fenofibrate and less potent than rosiglitazone (KATP and Ca2+ channels), although their improved pharmacokinetics correspond more to the postprandial situation.

The profile of mitiglinide (KAD-1229), a third meglinide analog and derivative of benzylsuccinic acid (Bakkali-Nadi et al., 1994a,b; Mogami et al., 1994; Ohnota et al., 1994) is not much different from the already known rapid-acting glinides (reviewed by Malaisse, 2008): plasma concentrations (maximum after approximately 20 min), linear pharmacokinetics, metabolism, uptake by pancreatic islets, insulinotropic actions, effect on ion channels, glucose uptake by hepatocytes (note that there is a possible advantage of this extra-pancreatic effect), preclinical investigations, and cardiovascular effects (possibly weaker than those of glibenclamide or glimepiride). Its receptor binding was unaffected by 100 μM glibenclamide (glyburide) (Malaisse, 2008), indicating no cross-reaction with their binding sites. Its selectivity with respect to ATP-sensitive potassium (KATP) channels in different tissues is 1000-fold for the β-cell type over cardiac and smooth muscle cell types (Reimann et al., 2001; Malaisse, 2003; Malaisse, 2008). Mitiglinide, although marketed in Japan (Glufast; 5, 10, and 20 mg), was not approved by the FDA. Phase II/III clinical trials with a combination of mitiglinide and an α-glucosidase inhibitor are under way. On a molar basis, mitiglinide is more potent than nateglinide and its effect is more rapidly reversible in perfused rat islets than that of repaglinide (Malaisse, 2003). Another from the meglitinide family is S3075, which is not marketed although it is a much more potent insulinotropic agent than its parent compounds (Geisen et al., 1985; Jijakali et al., 1996).

### VII. New Glinides

Meglitinide is the still biologically active nonsulfonylurea miety of glibenclamide. The meglitinide analogs repaglinide and nateglinide possess rather the same mechanism of action compared with sulfonylureas (KATP and Ca2+ channels), although their improved pharmacokinetics correspond more to the postprandial situation.

The profile of mitiglinide (KAD-1229), a third meglitinide analog and derivative of benzylsuccinic acid (Bakkali-Nadi et al., 1994a,b; Mogami et al., 1994; Ohnota et al., 1994) is not much different from the already known rapid-acting glinides (reviewed by Malaisse, 2008): plasma concentrations (maximum after approximately 20 min), linear pharmacokinetics, metabolism, uptake by pancreatic islets, insulinotropic actions, effect on ion channels, glucose uptake by hepatocytes (note that there is a possible advantage of this extra-pancreatic effect), preclinical investigations, and cardiovascular effects (possibly weaker than those of glibenclamide or glimepiride). Its receptor binding was unaffected by 100 μM glibenclamide (glyburide) (Malaisse, 2008), indicating no cross-reaction with their binding sites. Its selectivity with respect to ATP-sensitive potassium (KATP) channels in different tissues is 1000-fold for the β-cell type over cardiac and smooth muscle cell types (Reimann et al., 2001; Malaisse, 2003; Malaisse, 2008). Mitiglinide, although marketed in Japan (Glufast; 5, 10, and 20 mg), was not approved by the FDA. Phase II/III clinical trials with a combination of mitiglinide and an α-glucosidase inhibitor are under way. On a molar basis, mitiglinide is more potent than nateglinide and its effect is more rapidly reversible in perfused rat islets than that of repaglinide (Malaisse, 2003). Another from the meglitinide family is S3075, which is not marketed although it is a much more potent insulinotropic agent than its parent compounds (Geisen et al., 1985; Jijakali et al., 1996).
Conclusion. There are no newer results published in this field within the last four years and a necessity for further compounds within this family is not obvious. There is no positive prophylactic outcome (e.g., for nateglinide) with respect to the occurrence of diabetes or cardiovascular effects.

VIII. Enzymes as Targets (Signaling Systems)

A. α-Glucosidase Inhibitors

α-Glucosidase inhibitors such as acarbose and miglitol delay the digestion and absorption of carbohydrates by blocking oligosaccharide catabolism: postprandial hyperglycemia and hyperinsulinemia are smoothened (weak effect) and even a prophylactic effect is evident (NIDDM-STOP study for acarbose; Chiasson et al., 1998). They have yet more positive effects: decline of triglyceride, cholesterol, and apolipoprotein A-1, elevation of HDL. Several criticisms have been put forward: 1) the effect on improving HbA1c by acarbose is not dose-dependent; 2) there is no significant effect on body weight loss (Van de Laar et al., 2005); and 3) important parameters and endpoints such as mortality, morbidity, and quality of life are not sufficiently addressed (Van de Laar et al., 2005). It may be concluded that the true therapeutic value of these agents is not yet clear.

Nevertheless, another compound, voglibose ([1S]-[1(OH),2,4,5/H]-5-{[2-hydroxy-1-(hydroxymethyl)ethyl]crobial 1996). More compounds are being developed: these mit- adverse GI symptoms than acarbose (Shinozaki et al., et al., 1992; Chen et al., 2006). Voglibose has fewer zaki et al., 1996), albeit somewhat more effective (Odaka are similar to those of established compounds (Shino- kinds of diabetes of the young (Edghill and Hattersley, 2008) glucokinase mutations may be related to maturity onset (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis- ease (Wilms et al., 1970; Caro et al., 1995; Agius, 2008). Glucokinase mutations may be related to maturity onset of diabetes of the young (Edghill and Hattersley, 2008) and permanent neonatal diabetes mellitus (Meglasson and Matschinsky, 1986; Gloyn, 2003; Johnson et al., 2007). All together, a broad range of “glucokinase dis-
region of the enzyme (Efanov et al., 2005) and phosphorylation of the substrate glucose (Grimsby et al., 2004). Glucokinase activators increase the affinity for glucose, and some additionally increase the $V_{\text{max}}$ of the enzyme (Matschinsky, 2009). Glucokinase displays a positive kinetic cooperativity with glucose (Hill coefficient, $h \approx 1.7$). Some tested compounds reduce the Hill coefficient from 1.7 to 1. Note that a decrease of the Hill coefficient to 1 confers “hexokinase-like” characteristics and results in the loss of its unique glucose-sensing capability. Glucokinase activators, therefore, should be designed in a way that they preserve the basic kinetic features of glucokinase.

Some test compounds stimulate 2-deoxy-D-[$^3$H]glucose uptake by 300%. Some activators lower the concentration of glucose that allows half-maximal activity of the enzyme, from $8 \text{ mM}$ to 2 mM or less, shifting the concentration-dependence curve of glucose-stimulated insulin release to the left without augmenting maximal secretory activity in pancreatic islets (Grimsby et al., 2003; Efanov et al., 2005; Futamura et al., 2006; Johnson et al., 2007), pancreatic cell lines (Brocklehurst et al., 2004; Efanov et al., 2005; Pyke et al., 2007; Johnson et al., 2007), and also human islets (Johnson et al., 2007). Experimental glucokinase inhibitors, such as mannoheptulose and 5-thioglucose, or diazoxide, an opener of the ATP-sensitive K$^+$ channel, reverse this effect (Johnson et al., 2007).

Glucokinase activators not only reduce fasting and basal blood glucose levels but also improve glucose tolerance. The phenomenon has been described as “resetting the glucokinase glucostat,” which means that glucokinase activators will restore balance to abnormal glucose appearance and glucose disposal rates found in diabetes, whereas in conditions with intact glucose homeostasis, glucokinase activators will tip the glucose appearance/glucose disposal balance in favor of disposal, which leads to glucose lowering toward the hypoglycemic range (Coghlan and Leighton, 2008). The in vivo pharmacological outcome is well investigated in animals and humans with and without diabetes (Grimsby et al., 2003, 2004; Efanov et al., 2005; Sarabu and Grimsby, 2005; Guertin and Grimsby, 2006; Pyke et al., 2007; Coghlan and Leighton, 2008; Sarabu et al., 2008; Nakamura et al., 2009; Bonadonna et al., 2010).

In addition to $\beta$ cells and liver, the glucokinase enzyme is expressed in many other cells, which has to be remembered with respect to selectivity: entero-endocrine K and L cells (see section II), glucose-excited/glucose-inhibited neurons of the hypothalamus and brainstem (Levin, 2006), and anterior pituitary cells (fertility or other gonadal functions) (Zelent et al., 2006). Glucose-responsive neurons are believed to play an important role in body weight control; administration of glucose into rat brain reduces feeding consumption.

4. Compounds. The search for glucokinase activators started in 1990 and has a long history (Matschinsky, 1996, 2009; Grimsby et al., 2003, 2004; Matschinsky et al., 2006; Pal, 2009a). A pharmacophore model of the heterogeneous chemical group of most known classes of glucokinase activators has been described previously (Grimsby et al., 2008; Sarabu et al., 2008), summarizing key structural features common to both single atom-centered (carbon or nitrogen) and aromatic ring-centered glucokinase activators, including three attachments, two of which are hydrophobic groups (with at least one consisting of an aromatic ring structure) and the other contributes a hydrogen bond donor-acceptor pair. The establishment of a crystal structure of recombinant human glucokinase was mainly put forward by

![Role of glucokinase in various tissues](image)
the availability of glucokinase activators (Grimsby et al., 2003; Dunten et al., 2004). For the allosteric activator site, as many as nine contact amino acids, depending on the chemistry of the drug, have been identified, encompassing Val162, Arg63, Glu210, Ile211, Tyr214, Tyr215, Met235, Val452, and Val455 (Grimsby et al., 2003, 2004; Dunten et al., 2004; Kamata et al., 2004; Efano et al., 2005). The link between enzyme kinetic and structure-activity relationship (SAR) has not yet been sufficiently investigated.

The patent literature with almost 100 compounds has been reviewed (Sarabu and Grimsby, 2005; Guertin and Grimsby, 2006; Coghlan and Leighton, 2008; Grimsby et al., 2008; Sarabu et al., 2008; Matschinsky, 2009; Pal, 2009). The compounds can be grouped into four classes (Table 6).

It has to be mentioned that some glucokinase activators have been developed as potential treatments against obesity [e.g., AZD6370 (Sarabu and Grimsby, 2005; Nilsson & Andersen, 2010) and 4-hydroxyisoleucine (ID1101) (Broca et al., 2004)], which is not a major focus of this review. It is noteworthy that some glucokinase activators act as activators at low glucose levels and as inhibitors at high glucose levels, which has recently been explained by a model (Grimsby et al., 2008).

Some glucokinase activators bind to an allosteric regulatory site located 20 Å from the glucose binding site, at the interface between the large and small domains (Grimsby et al., 2003; Dunten et al., 2004; Kamata et al., 2004; Efano et al., 2005). More details with respect to the superopen conformation, glucose-bound open and closed forms have been reviewed (Grimsby et al., 2008). Dual-acting compounds are also under investigation; they activate glucokinase and inhibit glycogen phosphorylase (Zhang et al., 2009).

5. Potential Side Effects. The key risk of many of the (early) glucokinase activator candidates is hypoglycemia, which includes a narrow therapeutic window and therefore requires exact dosing. This potential risk is due to a leftward shift of glucose responsiveness. Other unwanted side effects include nausea, hyperlipidemia, and fat accumulation in the liver or even liver toxicity. Another problem is nonselectivity, because the presence of glucokinase in various tissues was shown (see section VIII.B.3) in biochemical and physiological studies; however, selectivity for liver and pancreas is important.

Conclusion. All together, glucokinase activators resemble a promising new paradigm combining a dual effect on β cells and liver. The number of currently available glucokinase activators is chemically extremely heterogeneous, making a preference difficult. Glucokinase activators that activate the enzyme by binding to its allosteric site to increase glucose affinity, catalytic rate and change cooperativity with regard to glucose, are milestones of accomplishment. However, prediction of their long-term usefulness is difficult with respect to the molecular defects of the diabetic β-cell, the liver and other glucokinase-containing cells. Other aspects await investigation: what is the role of this enzyme in pancreatic A cells or enteric L cells, in central nervous system neurons? How useful are their combinations with established antidiabetic drugs? Full development of glucokinase-based antidiabetic therapy has not been achieved despite efforts for 2 decades.

C. AMP Kinase

AMPK, a phylogenetically highly conserved multisubstrate serine/threonine protein kinase, is a nutrient sensor and an integrator of regulatory signals that monitor and regulate the systemic and cellular energy balance (“fuel gauge”) (Viollet et al., 2007): during a low-energy status, ATP-consuming catabolic pathways (such as fatty acid oxidation and glycolysis) are switched on, and ATP-consuming anabolic pathways (such as lipogenesis) are switched off. In these processes, short-term effects are mediated by phosphorylation of regulatory proteins and long-term effects via modulation of gene expression. AMPK, a heterotrimeric enzyme complex consisting of a catalytic α subunit and two regulatory subunits, is activated by rising AMP and falling ATP levels. AMPK complexes are activated by phosphorylation of the α subunit on threonine-172 both by Ca2+/calcium MAPK-kinase β and by AMP/liver kinase B1 [STK11-dependent (LKB-1-dependent) signals after metabolic stresses (Leclerc et al., 2011)].

AMPK has an antidiabetic effect by acting on muscle and liver (Gruzman et al., 2009); glucose transport/uptake and fatty acid oxidation are stimulated, and glucose output and gluconeogenesis are decreased. AMPK activated by low glucose in pancreatic α cells stimulates glucagon secretion, thus counteracting hypoglycemia (Leclerc et al., 2011). In addition, antiangiogenesis effects managed by reducing cholesterol and triglyceride synthesis have been observed (Hirabayashi et al., 2008; Gruzman et al., 2009; Yu et al., 2010a). AMPK has also been established to be a link between long-term low-grade inflammation and metabolic regulation in peripheral metabolic tissue (Nerstedt et al., 2010). All together, AMPK represents an attractive concept and target for type 2 diabetes therapy. Activation of AMPK has been investigated for 25 years and is now demonstrated for many compounds (Viollet et al., 2007; Yu et al., 2010a; Leclerc et al., 2011):

- Cytokines (leptin, adiponectin, IL-8, ciliary neutrophic factor);
- Drugs [metformin, phenformin, 6,7-dihydro-4-hydroxy-3-(2′-hydroxy[1,1′-biphenyl]-4-yl)-6-oxo-thieno[2,3-b]pyridine-5-carbonitrile (A-769662), thiazolidinediones, berberine];
- Natural products (Table 7);
- Direct activators (Table 7).

For AMPK activity testing, human recombinant (Escherichia coli) AMPK enzyme is used with a fluores-
TABLE 6
Status summary of some selected glucokinase activators (more than 100 patents; only some important compounds are listed and many were stopped)

<table>
<thead>
<tr>
<th>Glucokinase Activators</th>
<th>Pharmacological Data</th>
<th>Status</th>
</tr>
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<tbody>
<tr>
<td>Carbon-Centered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RO 0281675 (Roche)</td>
<td>Lead compound&lt;sup&gt;a&lt;/sup&gt;: in vivo profile was positively characterized in rodent diabetes models (Grimsbys et al., 2003, 2004); glucose reductions paralleled by an increase in insulin levels (Matschinsky, 2009)</td>
<td>2006: First to be published and thoroughly clinically tested; with side effects because of its thioamide metabolite (Kester et al., 2009), the first to progress to the clinic; halted in clinical trial</td>
</tr>
<tr>
<td>Seven derivatives of lead compound RO 0281675</td>
<td>Effective in rodent models (Grimbsby et al., 2003, 2004, 2008; Efanov et al., 2005; Coepe et al., 2006; McKercher et al., 2006; Fyne et al., 2007)</td>
<td>2004: Well investigated</td>
</tr>
<tr>
<td>LY2121260 (Eli Lilly)</td>
<td>Stimulation of insulin secretion and increasing glucose usage in rat hepatocytes (Efanov et al., 2005; Matschinsky, 2009); increase of both cell replication (via upregulation of insulin receptor substrate-2 and subsequent activation of protein kinase B phosphorylation) (tested in INS-1 cells) (Wei et al., 2009)</td>
<td>2004: Well investigated</td>
</tr>
<tr>
<td>PSN-GK1 (OSI)</td>
<td>One of the most potent (Matschinsky, 2009)</td>
<td>2004; Well investigated</td>
</tr>
<tr>
<td>PSN 010 (Prosidion/Lilly LY2599506)</td>
<td>Fyne et al. (2007); Bertram et al. (2008); Briner et al. (2007)</td>
<td>Phase I</td>
</tr>
<tr>
<td>Fraglhatin (RO 438620, Roche) (5, 10, or 25 mg)</td>
<td>The second with progress to the clinic; well tolerated (Daniewski et al., 2007; Kester et al., 2009; Matschinsky, 2009; Bonadonna et al., 2010)</td>
<td>2007: Phase II; thereafter trials discontinued probably for priority reasons</td>
</tr>
<tr>
<td>Aromatic (benzene- and pyridine)-centered Nishimura et al. (2003)</td>
<td>Potent</td>
<td>2003: Model compound for glucokinase crystalization experiments. Structural information was interpreted according to the “mnemonic” or “slow transition” models of cooperative glucokinase kinetics (Matschinsky, 2009)</td>
</tr>
<tr>
<td>GKA-50 (Caulkett et al., 2005)</td>
<td>Behaves similarly to LY2121260 (Wei et al., 2009); also prevents INS-1 cell apoptosis when induced by chronic high glucose conditions, probably via normalization of the apoptotic protein BCL2-associated agonist of cell death (BAD) and its phosphorylation (Johnson et al. 2007; reviewed by Matschinsky, 2009; Wei et al., 2009; J.)</td>
<td>2005: Preclinical</td>
</tr>
<tr>
<td>Bai et al. (2007) Amino acid-based</td>
<td>No biological data reported</td>
<td>2007</td>
</tr>
<tr>
<td>WO200710434 (patent number, Takeda) Pyrrolene-based</td>
<td>No biological data known/disclosed</td>
<td>2007</td>
</tr>
<tr>
<td>Feng et al. (2007) Other structures</td>
<td>No biological data known/disclosed</td>
<td>2007</td>
</tr>
<tr>
<td>AZD1636 (AstraZeneca&lt;sup&gt;b&lt;/sup&gt;) Other structures</td>
<td>Pyrazine derivative</td>
<td>Phase I (Sarabu and Grimsby, 2005); more than 20 clinical trials</td>
</tr>
<tr>
<td>MK-0941</td>
<td>Three phase II studies: the outcome was not absolutely positive</td>
<td>Three phase II studies: the outcome was not absolutely positive</td>
</tr>
<tr>
<td>PSN105</td>
<td>Halted in preclinical phase (Sarabu and Grimsby, 2005)</td>
<td>Halted in preclinical phase (Sarabu and Grimsby, 2005)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Also effective in reducing basal blood glucose levels and/or had antihyperglycemic effects in three animal models of type 2 diabetes (ohob mice, KKMpgj-AkD mice, Goto-Kakizaki rats). Derivatives were developed that led to new insights: chirality of the molecule is important. However, because of its potential cardiovascular risk (hERG IC<sub>50</sub> 2.8 μM; Purkinje fiber ΔAP<sub>D50</sub> 20%), further development of this compound was abandoned. http://diabetesdigest.com/development/deepphase.htm.

<sup>b</sup> A novel, additional role of glucokinase activators was detected: promoting β-cell growth and preventing chronic hyperglycemia-induced β-cell apoptosis.

<sup>c</sup> Promising therapeutic compound because serum lipids and insulin were reduced and there was no weight gain.

Conclusion. Activating AMPK represents a promising approach for the treatment of type 2 diabetes and the metabolic syndrome. Because antidiabetic compounds such as metformin and thiazolidinediones ex-
ert many, if not all, of their therapeutic effects by activating AMPK, the question is whether the newly described compounds exhibit fewer side effects (GI disturbance as for metformin and weight gain as for thiazolidinediones). The challenge for the development of a compound is huge because AMPK has non-selective effects in many cells, isoforms of AMPK exist, its AMP binding site is not well defined, and the activator $N^\text{1}$-(β-D-ribofuranosyl)-5-aminoimidazole-4-carboxamide is a daping compound listed by the World Antidoping Agency, because power is increased in mice by 40%.

D. Carnitine Palmitoyltransferase-1 Inhibitors

The control of fatty acid translocation across the mitochondrial membrane is mediated by the carnitine palmitoyltransferase (CPT) system. Modulation of this enzyme’s activity, either by using a CPT-1 inhibitor or by genetically engineered modification via plasmid technique (Obici et al., 2003), has positive effects on fatty acid oxidation and glucose metabolism (reduction of glucose production). Inhibitors of this enzyme lead to a decrease in glycogen phosphorylase activity is critical for skeletal muscle function. Fatigue induced by enzyme inhibition may be a major developmental hurdle for this therapeutic strategy. Some concern exists with respect to hepatomegaly.

E. Glycogen Phosphorylase Inhibitors

Glycogen phosphorylase is one step in glucose-1-phosphate formation from glycogen leading to glucose production. Inhibitors of this enzyme lead to a decrease in blood glucose. Understanding the mechanism of action of these inhibitors has been achieved through X-ray crystallographic studies (Oikonomakos and Somsák, 2008).

Several inhibitors have been described: CP-91149 ([R-(R*S*)]-5-chloro-N-[3-(dimethylamino)-2-hydroxy-3-oxo-1-(phenylmethyl)propyl]-1H-indole-2-carboxamide) has an IC$_{50}$ of 0.13 µM and resembles caffeine, a known allosteric phosphorylase inhibitor (Martin et al., 1998). PSN 357 (phase I) rapidly lowers blood glucose (Bradley et al., 2005; Fredenrich et al., 2009). Other compounds are Gpi688 and Gpi921.

A drawback of these inhibitors is the lack of selectivity between skeletal muscle and liver (Baker et al., 2005); glycogen phosphorylase activity is critical for skeletal muscle function. Fatigue induced by enzyme inhibition may be a major developmental hurdle for this therapeutic strategy. Some concern exists with respect to hepatomegaly.
Conclusion. Because glycogen phosphorylase is an important enzyme in glycogen metabolism, inhibitors may become a potential key target for controlling hyperglycemia.

F. Inhibitors of Glycogen Synthase Kinase-3 and Glycogen Synthesis Activation

GSK-3 is a critical kinase in the insulin signaling pathway (a vital regulatory serine/threonine kinase) and is a key enzyme involved in glycogen metabolism (Eldar-Finkelman, 2002). It has a broad regulatory effect because more than 40 proteins are phosphorylated by GSK-3, including more than a dozen transcriptional factors. GSK-3 constitutively phosphorylates insulin receptor substrate-1 (IRS-1) and serves as a “gatekeeper” to limit activation of insulin receptor signaling. In the absence of insulin, GSK-3 maintains the phosphorylation state of the multiple serine residues on IRS-1 and is involved in processes of glucose uptake, glycogen synthesis (glycogen synthase), insulin resistance, obesity, and type 2 diabetes (Cross et al., 1995; Nikoulina et al., 2000). Inhibitors of GSK-3 enhance response to insulin (e.g., stimulate glucose transport and glycogen synthesis in skeletal muscle and lower blood glucose (Plotkin et al., 2003; Ring et al., 2003) and increase IRS-1 expression (Nikoulina et al., 2002)) and therefore could have therapeutic implications for type 2 diabetes. GSK-3, however, is linked to many other diseases (e.g., chronic inflammatory processes, cancer, stroke, and neurological diseases such as bipolar disorder or Alzheimer’s disease) (Frame and Cohen, 2001). It is also a central regulator of embryonic cardiomyocyte proliferation and differentiation (Kerkela et al., 2008).

To suppress enzyme activity three distinct regions on the GSK-3 molecule may be targeted: 1) the ATP-binding pocket, phosphate interaction site (Nikoulina et al., 2002); 2) the metal ion (Mg$^{2+}$) binding site; Li$^+$ is effective (Ryves and Harwood, 2001; Zhang et al., 2003); and 3) the substrate interaction domain. Several phosphopeptides (e.g., Thr–Thr–pSer–Phe–Ala–Glu–Ser–Cys), derived from the amino-terminal end of GSK-3β, compete with substrate binding to the phosphate interaction site of the enzyme. Compounds already known for other biological properties must be mentioned: hymenialdisine (a marine sponge constituent) and its derivatives, paullones and indirubins (reviewed by Vats et al., 2005). Their impact may be only hypothetical, on the basis of quantitative SAR studies, because nonselectivity is obvious and clinical investigations are lacking.

Glycogen synthesis, thus inducing lowering of blood glucose, is stimulated by several GSK-3 inhibitors.

1. Maleimides.

- 3-Anilino-4-arylmaleimide (Smith et al., 2001), 3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione (SB-216763), 3-(3-chloro-4-hydroxyphenyl)-4-(2-nitrophenyl)-1H-pyrrole-2,5-dione (SB-415286), and SB-517955;
- Macrocyclic bisindolylmaleimides (Shen, 2009);
- Polyoxygenated bis-7-azaindole maleimides (Kuo et al., 2003; Shen et al., 2004; Zhang et al., 2004).

2. Pyrimidines.

- 4-Arylpyrimidine-2-amines (Cochran et al., 2002; Moon et al., 2002);
- Substituted 2-aminopyrazines (Savithri and Nuss, 2001);
- 2-Aminopyrimidines (Nuss et al., 2002).

3. Pyrazoles.

- Pyrazolo[3,4-b]pyridazines and pyrazolo[3,4-b]pyridazines (IC$_{50}$ in the nanomolar range) (reviewed by Vats et al., 2005), 4,5 dihydro-1H-pyrazole-5-one (Green et al., 2003).

4. Others.

- Substituted oxa diazepines (Bowler and Hansen, 2003);
- 1-(4-Amino-1,2,5-oxadiazolyl)-1,2,3-triazole derivatives (Olesen et al., 2002, 2003) and 2,4-diaminothiazoles (Bowler et al., 2001; Bowler and Hansen, 2003);
- 2-Aminopyridines (Nuss et al., 1999);
- Conclusion. With respect to glycogen synthesis, many compounds have been described without substantial translation into clinical use. GSK-3 is not expected to be a good target because it is involved in several signaling pathways (Jope and Johnson, 2004) in diseases and cell proliferation.

G. Inhibitors of Protein Tyrosine Phosphatase 1B and Protein Tyrosine Phosphatase Localized to Mitochondrion 1

Protein tyrosine phosphorylation is a fundamental mechanism for the intracellular control of cell growth and differentiation. It is governed by the opposing activities of protein tyrosine kinases, which catalyze phosphorylation, and protein tyrosine phosphatases (PTPs), which are responsible for dephosphorylation. Defective or inappropriate operation of this network leads to many diseases, such as diabetes and cancer.

The insulin receptor is autophosphorylated, which can be reversed by PT Pases, including receptor PTP-α, leukocyte antigen-related tyrosine phosphatase, SH2-domain-containing phosphotyrosine phosphatase-1, and especially PTP1B (Wälchli et al., 2000; Zhang, 2001; Xie et al., 2002). PTP1B also dephosphorylates and thereby inactivates (down-regulation) IRS-1 (Ahmad et al., 1995; Kenner et al., 1996; Chen et al., 1999; Goldstein et al., 2000; Liu, 2003).

Nonspecificity of inhibitory compounds may be expected, because PTP1B is ubiquitously expressed and is involved in various cellular responses (e.g., responses to activation of leptin receptor, epidermal growth factor receptor (Flint et al., 1997), insulin-like growth factor
type I receptor (Buckley et al., 2002), erythropoietin receptors, cadherin (Balsamo et al., 1998), integrin signaling pathways (Arregui et al., 1998), c-Src tyrosine kinase (Bjorge et al., 2000), and cell cycle (Flint et al., 1993; Shifrin et al., 1997)]. PTP-1B inhibition represents a therapeutic approach for insulin resistance and obesity in type 2 diabetes (Burke and Zhang, 1998; Møller et al., 2000; Burke et al., 2001; Blaskovich and Kim, 2002; Johnson et al., 2002). This is underlined by the fact that disruption of the PTP1B gene in mice results in improved insulin sensitivity and resistance to diet-induced obesity (Elchebly et al., 1999; Klaman et al., 2000).

Because the pTyr residue of the substrate provides unique and defining functions through binding and destruction by PTP1B, pTyr mimetics provide useful general starting points for designing competitive, reversible inhibitors. The highly charged nature of the catalytic site of PTP1B has presented tremendous challenge for identifying drug-like inhibitors by targeting the active site. Noncompetitive inhibitors of PTP1B have the advantage of achieving good inhibition via interacting with less charged binding sites: pyridazine analogs are examples. Various compounds are summarized in Table 8, some of which reached IC$_{50}$ values in the low micromolar range.

**Conclusion.** Inhibition of the PTP1B activity is a therapeutic approach for the treatment of type 2 diabetes, insulin resistance, and obesity. Even after decades of investigations, its impact on the market is not clear. Highly charged molecules are necessary, which inevitably poses tremendous challenge in achieving reasonable oral bioavailability and cellular permeability. Protein tyrosine phosphatase localized to mitochondrion 1 (PTPMT1) may be involved in the regulation of insulin secretion, because ATP and subsequently released insulin are increased in PTPMT1 knockdown rat islet experiments. Inhibitors were detected, such as the dibiguandis azide lexidine (IC$_{50}$, 1.08 μM) and chlorhexidine (more potent) (Doughty-Shenton et al., 2010). Phosphorylation of mitochondrial proteins is decreased by these compounds similar to PTPMT1 knockdown experiments (Doughty-Shenton et al., 2010). This enzyme may be a target for future therapies.

**H. Pyruvate Dehydrogenase Kinase Inhibitors**

Pyruvate dehydrogenase inhibitors increase oxidative glucose metabolism and decrease gluconeogenesis, which results in an improvement of blood glucose levels. AZD7545 has been described previously (Mayers et al., 2003; Kato et al., 2007), but no further developments are obvious.

**I. Fructose-1,6-bisphosphatase Inhibitors**

Fructose-1,6-bisphosphatase (FBPase with two isomers) is a rate-limiting enzyme e.g., in liver and muscle converting fructose-1,6-bisphosphate to fructose-6-phosphate, which is part of gluconeogenesis and pentose phosphate shunt. Enzyme activity is pathophysiologically increased in animal models of insulin resistance and obesity (Visinoni et al., 2008). Inhibition of this enzyme may be important in maintaining normoglycemia during fasting.

For inhibiting FBPase, a variety of pyrrole-, pyrazole-, and indole-based (most promising) compounds have been evaluated (Rudnitskaya et al., 2010), with IC$_{50}$ values in the nanomolar range. l-Alanine, N,N’-[5-[2-amino-5-(2-methylpropyl)-4-thiazolyl]-2-furanyl]phosphinylidenebis-, diethyl ester (CS-917; the first candidate) suppresses hyperglycemia and gluconeogenesis (Yoshida et al., 2008). MB07803 (second compound; first-in-class drug, phase II) is highly efficient and has good oral bioavailability and low metabolism (Dang et al., 2008). Managlinat dialanelit, a purine nucleotide analog, is in phase II (Wang and Tomlinson, 2007; no new data thereafter).

**Conclusion.** FBPase inhibition is a promising target and may turn out to be important especially for patients who do not tolerate metformin.

**TABLE 8**

<table>
<thead>
<tr>
<th>Structural Class</th>
<th>Site of Interaction</th>
<th>Development Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis-F$_2$Pmps and Mono-F$_2$Pmps (phosphonodifluoromethyl)phenylalanine</td>
<td>Catalytic site/additional site</td>
<td>Preclinical</td>
</tr>
<tr>
<td>O-carboxymethyl salicylic acid</td>
<td>Catalytic site</td>
<td>Preclinical</td>
</tr>
<tr>
<td>O-carboxymethyl salicylic acid o-malic-acid</td>
<td>Catalytic site</td>
<td>Preclinical</td>
</tr>
<tr>
<td>2-(Oxalylamino)-benzoic acid</td>
<td>Catalytic site</td>
<td>Preclinical</td>
</tr>
<tr>
<td>2-(Oxalylamino)-benzoic acid (derivatives are tested)*</td>
<td>Catalytic site/additional site</td>
<td>Preclinical, reversible</td>
</tr>
<tr>
<td>Pyridazine (analog) (Liljebris et al., 2002)*</td>
<td>Catalytic site/additional site, noncompetitive, reversible</td>
<td>Preclinical, reversible</td>
</tr>
<tr>
<td>Thiazole &amp; trifluoromethyl sulfonamido compounds 1,2-Naphthoquinones (=lead molecule) (Yats et al., 2005)</td>
<td>Noncatalytic site, competitive, reversible</td>
<td>Research</td>
</tr>
<tr>
<td>Formylchromones (best: 6-biphenyl-3-formylchromone (Shim et al., 2003)</td>
<td>Unknown</td>
<td>Research</td>
</tr>
<tr>
<td>Aryl α-ketocarboxylic acids</td>
<td>unknown</td>
<td>Research</td>
</tr>
<tr>
<td>α-Bromoacetophenones (derivatives)</td>
<td>unknown</td>
<td>Research</td>
</tr>
<tr>
<td>Azolidinediones (derivatives) (Malamas et al., 2000a)</td>
<td>Covalently alkylation the site (cysteine)</td>
<td>Phase I</td>
</tr>
<tr>
<td>Others: Trodusquemine</td>
<td>unknown</td>
<td>Research</td>
</tr>
</tbody>
</table>

* As the number of acid groups increases, the chance for the inhibitor to penetrate the cell membrane via passive diffusion is dramatically reduced.
* Unlike many other tyrosine phosphatase inhibitors, a pyridazine analog lacks negative charge and thus easily penetrates the cell membrane (Liljebris et al., 2002).
J. 11β-Hydroxysteroid Dehydrogenase-1 Inhibitors (and Hexose-6-phosphate Dehydrogenase Inhibitors)

11β-HSD1, a bidirectional NADP(H)-dependent enzyme, catalyzes the reduction of the glucocorticoid cortisone to cortisol. Cortisol is known to be involved in increasing gluconeogenesis and weight and to be a functional antagonist of insulin. 11β-HSD1 activity (and cortisol) are elevated in patients with type 2 diabetes, obesity (3–5 times higher enzyme activity in adipose tissue), or metabolic syndrome (Walker, 2006; Wang et al., 2010). Knowing the effects of reversing Cushing’s syndrome (e.g., insulin resistance) suggests that reducing cortisol action by using 11β-HSD1 inhibitors may provide a novel therapeutic approach in the metabolic syndrome and several other diseases. Hexose-6-phosphate dehydrogenase is linked because it provides intracellular NADPH for 11β-HSD1; for this enzyme, no inhibitor is available.

(S)-2-[(1S,2R,4S)-Bicyclo[2.2.1]heptan-2-yl]amino)-5-isopropyl-5-methylthiazol-4(5H)-one (AMG-221; phase I), a thiazolone with an exo-norbornylamine at the 2-position and an isopropyl group on the 5-position, decreases blood glucose and insulin levels after feeding and reduces body weight in mice with dietary obesity (Véniant et al., 2010). N-(Pyridin-2-yl)arylsulfonamide (PF-915275) was a lead compound (Siu et al., 2009), but development was terminated because of formulation problems. INCB13739 (first in class; phase II; reviewed by Hughes et al., 2008) reduces body weight (especially in patients with a BMI of >30 kg/m²), HbA1c (by 0.56%), and fasting glucose levels; normalizes insulin sensitivity; and decreases cholesterol and triglycerides (Hollis and Huber, 2011) and may turn out to malizes insulin sensitivity; and decreases cholesterol and

K. Sirtuin 1 Activators

Sirtuin 1 influences metabolic processes (lipid and glucose metabolism, IRS-2, desacetylase, suppression of the PTP1B gene and others involved in energy maintenance) and influences transcription coactivator PPAR-γ coactivator 1α. Resveratrol (phase II) is active (Vetterli et al., 2010), and compound SRT2104 is in phase IIa.

L. Other Enzymes (Complexes)

Acetyl-CoA-diacylglycerol acyltransferase-1 is an enzyme that catalyzes the final committed step of triglyceride synthesis. trans-4-[4-(4-Amino-7,8-dihydro-5-oxopyrimido[5,4-f][1,4]oxazepin-6(5H)-yl]phenyl)cyclohexanecarboxylic acid (PF-04620110, in phase I) is a selective inhibitor of acyl-CoA-diacylglycerol acyltransferase-1 for the treatment of diabetes.

Phosphoenolpyruvate carboxykinase mRNA and glucose-6-phosphatase mRNA are reduced by 3-chloro-2-methyl-N-[4-[2-(4-methylpiperazin-1-yl)-2-oxoethyl]-1,3-thiazol-2-yl]benzenesulfonamide hydrochloride (BVT.2733); blood glucose is reduced by 25% (Xie et al., 2008).

PPAR-γ coactivator 1α (transcriptional coactivator) has a key role in the modulation of hepatic gluconeogenesis (Yoon et al., 2001). Although this target has been identified, there is still a lack of interacting compounds.

Acetyl-CoA carboxylases, the rate-limiting enzymes in de novo lipid synthesis, play important roles in modulating energy metabolism. Acetyl-CoA carboxylase inhibition has a promising therapeutic potential for treating obesity and type 2 diabetes mellitus in transgenic mice and preclinical animal models. Several compounds have been described previously (Corbett, 2009), and some inhibitors are derived from herbicides (pinoxaden) (Yu et al., 2010b). A novel series of disubstituted (4-piperidinyl)-piperazine derivatives and indole derivatives has been described previously (Chonan et al., 2009, 2010).

Mitochondrial rotenone-sensitive NADH:ubiquitone oxidoreductase (complex I) activity is diminished in patients with type 2 diabetes. (R,S)-(3-piperidino-2-hydroxy-1-propyl)-nicotinic-acid-amidoxime (BGP-15; phase II) increases the activity of this complex when it is inhibited by saturated fatty acids. Complex effects leading to insulin sensitizing are observed (Kolonic et al., 2010).

IX. Physiological Compounds (Hormones)

A. Leptin (Receptor Modulators)

Leptin released from fat cells indicates their substrate overloading and induces insulin resistance. Leptin insufficiency in the hypothalamus induced by either lep-tinopenia or restriction of leptin transport across the blood-brain barrier may initiate antecedent pathophys-
iological sequelae of diabetes type 1 and 2 (Kalra, 2009). Leptin replenishment in vivo, especially by supplying it to the hypothalamus using gene therapy, prevents the antecedent pathophysiological sequelae (hyperinsulinemia, insulin resistance, and hyperglycemia) (Kalra, 2009). Leptin suppresses hyperglucagonemia, normalizes HbA1c, lowers (in contrast to insulin monotherapy) both lipogenic and cholesterologenic transcription factors and enzymes, and reduces plasma and tissue lipids (Wang et al., 2010). Pyridinyl and piperazinyl carbamate compounds have been identified as therapeutic leptin receptor modulators (Simpson et al., 2009).

Possible therapeutic maneuvers may be neutralization of circulating ghrelin (vaccination with ghrelin immunoonjugate), neutralizing of ghrelin receptors, or administration of ghrelin receptor antagonists. (R)-N-(4-(4-Methoxybenzyl)-5-phenethyl-4H-1,2,4-triazol-3-yl)-2-(1H-indol-3-yl)ethyl)-2-aminoacetamide (JMV2959) (ghrelin receptor antagonist) suppressed/blocked the majority of the above-mentioned ghrelin effects, with the notable exception of ghrelin-induced food intake and food efficiency (Ogbodo et al., 2009).

B. Ghrelin Antagonists

Ghrelin is a potent gastric orexigenic factor (Kojima et al., 1999) and is involved in obesity and glucose homeostasis (elevation of blood glucose) (Dezaki et al., 2004). It is mentioned only very briefly because its effects are related more to obesity than to diabetes.

Possible therapeutic maneuvers may be neutralization of circulating ghrelin (vaccination with ghrelin immunoonjugate), neutralizing of ghrelin receptors, or administration of ghrelin receptor antagonists. (R)-N-[3,5-bis(trifluoromethyl)phenyl]-2-[(8aR)-hexahydropryrolo[1,2-a]pyrazin-2(1H)-yl]-2-(3-pyridinyl)ethanohydrazide) and YIL-781 (6-[(4-fluorophenyl)oxy]-2-methyl-3-[(3S)-1-(1-methyl-ethyl)-3-piperidinyl]methyl]-4(3H)quinazolinone), which are effective at low micromolar concentrations (Perdonà et al., 2011). An indolone derivative is effective at nanomolar concentrations (Baroni and Puleo, 2010). In addition, inverse agonists were described previously (Pasternak et al., 2009).

Conclusion. Not very much effort has been put into this field in the past.

C. Resistin

The hormone resistin, released from fat cells, is increased in animal models of diabetes and obesity. Glitazones (e.g., pioglitazone) are the only group yet to decrease resistin levels. Resistin antagonists have been developed but more or less with the focus on reduction of inflammatory effects.

D. Bariatric Surgery

It is noteworthy that bariatric surgery (independent of the type: gastric banding, gastric bypass, resecting the stomach) leads to improvement of diabetic parameters independent and before weight loss: decrease in blood glucose, plasma insulin, and HbA1c (Schauer et al., 2003; Dixon et al., 2008; Frezza et al., 2009). Because adipocytes release more than 100 adipokines, hormones, inflammatory compounds, etc., bariatric surgery normalizes many of released compounds, such as leptin and adiponectin.

X. New Remedies with Respect to Late Complications of Diabetes

A. Late Complications (Nephropathy, Retinopathy, Neuropathy, Vascular Complications)

Hyperglycemia induces various diabetic complications via different mechanisms, which are the basis for therapies. Since many defects overlap between various complications they are first solely listed and afterward the remedies are described in extra chapters:

1. Diabetic Nephropathy. See Gnudi et al. (2003), Conway and Maxwell (2009), and Obrosova (2009):

- Inhibition of increased glucose flux through the polyol pathway (aldose reductase inhibition; see section IX.H);
- Inhibition of increased formation of advanced glycation end-products (AGE) (Fig. 6);
- Inhibition of protein kinase C (PKC) isoforms (Fig. 6);
- Inhibition of increased hexosamine biosynthesis pathway (Fig. 6);
- Inhibition of reactive oxygen species (ROS) and superoxide formation (Fig. 6);
- Inhibition of secretion of transforming growth factor-β (TGF-β) (Fig. 6);
- Activation of transketolase; and
- Inhibition of poly(ADP-ribose) polymerase (PARP) (Fig. 6).

There is evidence for a genetic susceptibility to diabetic nephropathy, but clear targets are not obvious because of the finding of many single nucleotide polymorphisms and hundreds of novel susceptibility variants.

2. Diabetic Retinopathy. See Madsen-Bouterse and Kowluru (2008), Mohamed and Wong (2008), and Wilkinson-Berka and Miller (2008). It is the most feared diabetes complication. The biochemistry and pathogenesis remain speculative (more than a single metabolic disorder), complicating the identification of targets and therapeutic approaches:

- Inhibition of increased retina PKC activity (Xia et al., 1994; Kowluru et al., 1998) (see also vascular complications);
- Inhibition of VEGF;
- Inhibition of AGE accumulation (see section IX.E);
• Inhibition of polyol pathway (aldose reductase inhibition, see section X.E);
• Inhibition of hexosamine biosynthesis pathway (see section X.E); and
• Reduction of oxidative stress/superoxide induced damage.

3. Diabetic Neuropathy. This includes painful paraesthesia and loss of sensation (Mahmood et al., 2009; Obrosova, 2009). There exists a controversial discussion of its cause; e.g., axonal degeneration and probably secondary demyelination (Dyck, 1989; Oates, 2002; Wada and Yagihashi, 2005; Leinninger et al., 2006; Edwards et al., 2008; Zochodne, 2008) complicate the identification of a therapeutic approach:
• C-peptide to overcome its deficiency and its lack of effect;
• Inhibition of glycosylation of structural proteins;
• Inhibition of AGE (see section X.D) and increased AGE receptors;
• Inhibition of ROS and of oxidative-nitrosative stress;
• Inhibition of increased aldose reductase (see section VIII.F);
• Inhibition of increased PKC activity (see section VIII.F);
• inhibition of PARP (see section VIII.F); and
• Correction of growth factor imbalances.

4. Vascular Complications. These exist with the following approaches:
• Increase in nitric oxide (NO; vasodilator) bioavailability (note that the decrease is due mainly to accelerated NO degradation by ROS);
• Reduction of oxidative stress (see section X.A) (Pozenta et al., 2009);
• Inhibition of AGE (see section X.A);
• Inhibition of PKC activity (see section X.C); and
• Decrease of inflammatory signaling.

Many of these pathophysiological parameters are linked to more than one secondary complication. A PKC activation has been associated with abnormalities such as increased vascular permeability, alterations in blood flow, and stimulation of neovascularization (Takagi et al., 1996; Park et al., 2000; Yokota et al., 2003). PKC both induces and responds to VEGF, a primary suspect in induction of retinal neovascularization in diabetes (Aiello et al., 1997; Yokota et al., 2003; Amadio et al., 2008). From all mechanisms involved in endothelial dysfunction, increased oxidative stress seems to be the first alteration. Oxidative stress reduces the bioavailability of NO. Under physiological conditions, $O_2^-$ produced by NADPH oxidases is scavenged by antioxidant enzymes including superoxide dismutase. Imbalance in cell redox status resulting from excessive production of ROS and/or insufficient antioxidant capacity promotes both endothelial dysfunction and insulin resistance; therefore, restoring physiological redox balance is an attractive treatment approach. Insulin resistance with impaired PI3K effects decreases insulin-mediated production of NO and reduces vasodilation, capillary re-
Compensatory hyperinsulinemia enhances activation of intact MAP-kinase pathways and contributes to proatherogenic events by increasing secretion of endothelin-1, stimulating expression of adhesion molecules such as vascular cell adhesion molecule-1 and E-selectin, and inducing production of ROS (Potenza et al., 2009). Overexpression of PKC isoforms can also directly induce insulin resistance (Cortright et al., 2000).

Increased superoxide anion production induced by hyperglycemia leads to decreased activity of glyceraldehyde-3-phosphate dehydrogenase and to consequential increased activity of alternative pathways, including the polyol, hexosamine, diacylglycerol, PKC, and AGE pathways (Fig. 7).

Not discussed here are nonspecific therapies. For example: for diabetic neuropathy (anticonvulsants: phenytoin, carbamazepine, lamotrigine and valproate), antidepressants (tricylic and tetracylic, selective serotonin reuptake inhibitors, serotonin-norepinephrine-reuptake inhibitors), and opioid-based therapies (tramadol), an antiarrhythmic (mexiletine), N-methyl-D-aspartate receptor antagonists (dextromethorphan and memantine), capsaicin (an extract of capsicum peppers) as topical agent binding to TRPV1 receptors (transient receptor potential cation channel, subfamily V, member 1) and substance P, patches containing 5% lidocaine, isosorbide dinitrate (via NO generation), and darifenacin (M3 receptor antagonist). Although facing immense costs of secondary effects of diabetes, only a few clear options are available to eliminate the major causes (Edwards et al., 2008).

**B. C-Peptide (Neuropathy, Vascular Function)**

C-peptide, being a product of the process of insulin biosynthesis, was thought to be biologically inert because its structure is not very conserved among species (Hills and Brunskill, 2009). It has been used mainly as a surrogate marker of endogenously released insulin during insulin therapy of type 2 diabetes because its release into the bloodstream is equimolar with that of insulin. Unlike insulin, C-peptide is subjected to negligible first-pass metabolism by the liver.

It had to be learned that C-peptide actually is a bioactive peptide: the cascade of induced events is summarized in Fig. 8. Shown is its interaction with a cell membrane receptor coupled to a pertussis-sensitive G-protein. The identity of this receptor remains elusive, because gene cloning and proteomic strategies have not been successful (Luzzi et al., 2007). Both phospholipase C and PI3K are induced. Phospholipase C activation evokes an increase in \([Ca^{2+}]_i\), resulting in the concomitant NO generation (Forst et al., 1998b; Jensen and Messina, 1999; Wallerath et al., 2003; Joshua et al., 2005) and PKC. In the stimulation of NO generation, the calcium-Janus tyrosine kinase 2/signal transducer and activator of transcription 1 pathway is involved (Hills and Brunskill, 2009; Lee et al., 2010; Richard and Stephens, 2011). and PKC together with increased de novo synthesis of diacylglycerol (DAG) stimulates activation of Na\(^+\)/K\(^-\)-ATPase via PKCo translocation to the cell membrane. There is a PKC-dependent activation and translocation of RhoA to the plasma membrane and phosphorylation and activation of MAPKs. Stimulation of the MAPK pathway also results in both increased Na\(^+\)/K\(^-\)-ATPase activity and activation of various transcription factors. Together with the elevated PI3K lev-

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**Fig. 7. Pathophophysiological factors (Potenza et al., 2009).**
els, MAPK-induced transcription factor expression is increased, and effects, including reduced apoptosis, and increased endothelial nitric-oxide synthase and CD36 levels, are observed. In addition, gene transcription is activated (e.g., cAMP-response-element-binding protein and activating transcription factor 1). It is noteworthy that specific displaceable binding of C-peptide to pancreatic islet β cells has been described previously (Flatt et al., 1986). All together, C-peptide induces positive effects on diabetic neuropathy, vasodilation, and erythrocyte cell adhesion (Ekberg and Johansson, 2008).

C-peptide supplementation may induce beneficial effects in many tissues that are commonly the target of diabetic complications: circulatory impairments such as decreased blood flow (Nordquist and Stridh, 2009), decrease (7%) of glomerular hyperfiltration in patients with diabetes (Johansson et al., 2000), and improvement of renal function (Forst et al., 1998a). Prevention/improvement of diabetic neuropathy is achieved via increasing endoneural blood flow and by preventing axonal swelling, improvement of sensory nerve conduction, and modulation of neurotrophic factors (Ekberg and Johansson, 2008; Nordquist and Stridh, 2009). Opposing data with respect to vascular inflammation and atherosclerosis and anti-inflammatory and antiatherogenic effects have been described for C-peptide (Luppi et al., 2008). Maximum effects are achieved at plasma C-peptide levels of ~3.5 nM (Forst et al., 2000). Details of important amino sequence groups of C-peptide for biological effects have been reviewed (Hills and Brunskill, 2009); on this basis, a shortened C-peptide sequence could be marketed.

**Conclusion.** C-peptide treatment has the potential to reduce the prevalence of diabetic complications. Long-term effects, however, have not been sufficiently investigated (Johansson et al., 1993, 2000). The necessity for patients with type 1 diabetes to add C-peptide to insulin (Johansson et al., 2003; Ekberg and Johansson, 2008) is an option, albeit not discussed here.

**C. Protein Kinase C Inhibitors**

(Retinopathy, Neuropathy)

Elevated glucose levels stimulate DAG, which in turn activates PKC. PKC both induces and responds to VEGF, a primary suspect in induction of retinal neovascularization in diabetes being an angiogenic protein (Aiello et al., 1997; Yokota et al., 2003; Amadio et al., 2008). Others such as plasminogen activator inhibitor-1, nuclear factor-κB, and TGF-β (Fig. 6) combine development of inflammation and diabetic complications; in particular, the PKC-β isoform has been implicated (Beckman et al., 2002; Arikawa et al., 2007; Das Èvcimen and King, 2007; Edwards et al., 2008).

PKC-β isoform inhibition, therefore, may be a therapeutic option. PKC412, the first PKC inhibitor used in treatment of diabetic macular edema (Campochiaro, 2004), has been abandoned because of hepatotoxic effects. Ruboxistaurin (LY333531; Arxxant), a selective and competitive PKC-β inhibitor, reduced the progression of diabetic retinopathy, improved retinal blood flow,
decreased diabetic macula edema, and improved the symptoms of diabetic peripheral neuropathy (Vinik et al., 2005) and (to a lesser extent) nephropathy (Beckman et al., 2002; Aiello et al., 2006) without significant adverse effects (Strøm et al., 2005; Aiello et al., 2006). Because of the short time allowed for providing requested additional data for getting FDA approval, the development process was postponed in 2006.

Conclusion. There is not sufficient proof of efficacy for some investigated compounds; this does not mean the concept is wrong.

D. Advanced Glycation End-Products Inhibitors
(Retinopathy, Renopathy)

Glucose interacts nonenzymatically with amino groups in proteins, with lipids, and with nucleic acids to form Schiff’s base and Amadori products. After a complex cascade of reactions, AGEs are formed (Ahmed, 2005; Toth et al., 2008). Three main pathways are known for the formation of AGE precursors (reactive dicarbonyls): 1) oxidation of glucose to form glyoxal; 2) degradation of Amadori products (fructose-lysine adducts); and 3) aberrant metabolism of glycolytic intermediates to methylglyoxal (highly reactive; damage of endothelial cells).

AGEs increase inflammation, NF-kB activity, and production of cytokines (IL-1, IL-6, tumor necrosis factor-α). Their accumulation targets the retinal basement membrane and inhibits its function as well as cellular transport and functions of other tissues (Tanzi et al., 2000; Gardiner et al., 2003; Ramasamy et al., 2005).

AGEs enhancement is accompanied by an increase in its receptor RAGE (Schmidt et al., 1996). There exist AGE receptors 1, 2, and 3 (encoded by dolichyl diphospho-oligosaccharide protein glycosyltransferase, protein kinase C substrate 80K-H, and lectin galactoside-binding soluble 3, respectively) (Hoverfelt et al., 2010). RAGE protein is massively up-regulated during diabetes in peripheral nerve and ganglia. S100 proteins as ligands for RAGE exist also in other cells, and S100 proteins as ligands e.g., suppress mineralization of preosteoblastic MC3T3-E1 cells (Yoshida et al., 2009). The therapeutic option in diabetes would be prevention of AGE formation and/or blocking RAGE, or, even better, breaking the AGE-protein cross-links.

Benfotiamine, a highly bioavailable thiamine derivative, reduces AGE levels and markers of endothelial dysfunction in patients with diabetes (Stirban et al., 2006). It is a transketolase activator (indirect effect by elevating a cofactor of this enzyme) that directs glucose to the pentose phosphate pathway (Winkler and Kempler, 2010), which leads to reduction of oxidative stress, phosphorylation/activation of VEGF receptor-2 and Akt, and increased Pim-1, pBad, and Bcl-2 levels (Katare et al., 2010). This identified mechanism helps to identify new targets.

Aminoguanidine (pimagedine) prevents cross-link formation by interacting with post-Amadori reactive inter-

- Alagebrium chloride (ALT-711) was positively tested for vascular benefit (Kass et al., 2001; Little et al., 2005; Coughlan et al., 2007; Zieman et al., 2007).
- ALT-946 (N-(2-acetamidoethyl) hydrazinecarboximidamide hydrochloride; pyridoxamine and pyridoxamine analog) may improve AGE-related complications by preventing AGE-dependent oxidative damage (Voziyan and Hudson, 2005).
- 7-O-galloyl-d-sedoheptulose reduces diabetic oxidative stress and AGE formation (Yamabe et al., 2009). Prenylated flavonoids isolated from Sophora flavescens inhibit AGE formation, although some have a high IC50 (261 μg/ml) (Jung et al., 2008).

RAGE can be blocked either by a soluble RAGE (the extracellular ligand-binding domain of RAGE; scavenging AGEs) or by anti-RAGE antibodies (Hudson and Schmidt, 2004). Preventing the final stages of diabeticogenesis and prevention of sensory deficits has been reviewed (Edwards et al., 2008). Several compounds were not pursued in the last 6 years: (±)-2-isopropylideneydrarzono-4-oxo-thiazolidin-5-yl acetanilide (OPB-9195), N-phenacyltiazolium bromide, and LR-90.

Conclusion. The clinical utility of AGE inhibition remains to be firmly established. No reviews on the clinical outcome of new AGE inhibitors have appeared since 2005. Progress may be hampered because a number of established therapeutics are able to reduce the accumulation of AGEs: angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, metformin, and PPARγ. At present, the focus of AGE inhibitor investigation is for treatment of cancer: a benefit for diabetics should have been observed during treatment if this approach were viable.

E. Hexosamine Pathway Inhibitors
(Retinopathy and Others)

Glutamine:fructose-6-phosphate amidotransferase is the rate-limiting step of the hexosamine biosynthesis pathway, which is activated as an alternative to glycolysis for the utilization of hyperglycemia-induced overproduction of fructose-6-phosphate, ultimately resulting in excess N-acetylgalactosamine and abnormal modification of gene expression of TGF-β1 and plasminogen activator inhibitor-1 (Fig. 6) (Kolm-Litty et al., 1998; Brownlee, 2001; Thornalley, 2003; Du et al., 2010). Its overactivation accounts for some adverse cardiovascular
effects, metabolic diabetic derangements, and endothelial cell and retinal neuron apoptosis (Nakamura et al., 2001; Du et al., 2003).

Compounds have been described that are effective in several ways. Azaserine reduces cardiovascular effects caused by hyperglycemia as an antioxidant rather than by inhibiting only the hexosamine pathway (Grønning et al., 2006; Zheng et al., 2007; Rajapakse et al., 2009). WAS-406 (2-acetamido-1,3,6-tri-O-acetyl-2,4-dideoxy-α-D-xylono-hexopyranose) acts similarly; however, it also acts by inhibition of islet amyloid formation (see section V) (Hull et al., 2007). 6-Diazo-5-oxo-L-norleucine is another inhibitor (Takata et al., 2009). Benfotiamine, by activating transketolase (see section X.D), converts fructose-6-phosphate into pentose-5-phosphates, thus reducing flux through the hexosamine pathway (Hammes et al., 2003). Rhein, an anthraquinone compound isolated from rhubarb, decreases hexosamine pathway and is effective in treatment of experimental diabetic nephropathy (Zheng et al., 2008).

Conclusion. In vitro data have not been sufficiently translated into clinical testing.

F. Poly(ADP-ribose) Polymerase Inhibitors (Neuropathy, Endothelial Dysfunction)

PARP is a nuclear enzyme closely associated with glucotoxicity and oxidative-nitrosative stress; it is activated by free radicals and oxidants (Fig. 6). It acts by cleaving NAD⁺ to nicotinamide and ADP-ribose residues attached to nuclear proteins (Southan and Szabó, 2003), leading to NAD⁺ depletion, changes in gene transcription, and diversion of glycolytic intermediates to formation of pathogenic pathways such as PKC and AGE (Ha et al., 2002; Du et al., 2003; Obrosova et al., 2005a).

PARP inhibitors may protect nerve or ganglia microvessels and neurons by turning down DNA repair enzyme over-reaction induced by oxidative-nitrosative stress. 1,5-Isouquinolinediol and 3-aminobenzamide improve experimental neuropathy dysfunction (Obrosova et al., 2005a; Ilnytska et al., 2006). Nicotinamide (Vitamin B₃) acts both as a PARP inhibitor and as an antioxidant (Gale et al., 2004; Stevens et al., 2007), and its combination with the xanthine oxidase inhibitor allopurinol and the antioxidant DL-α-lipoic acid was planned.

Conclusion. Inhibition of PARP may be a promising target, but convincing clinical trials in addition to animal experiments are missing.

G. Vascular Endothelium Growth Factor Inhibitors (Retinopathy)

VEGF up-regulation, seen in the eye fluid of patients with diabetes, is associated with neovascularization in proliferative diabetic retinopathy as well as diabetic macular edema. VEGF interacts with the tyrosine kinase VEGF receptors 1 and 2 (Shen et al., 1993; Ferrara, 2004). Local, intracocular administration of VEGF inhibitors have to be preferred over systemic approaches. Inhibitors are thought to be useful in the treatment of not only diabetic retinopathy but also of macular edema, age-related macular degeneration, and inflammation (Malm et al., 2010; Zhang and Wu, 2010).

VEGF inhibitors (pegaptanib, ranibizumab, and bevazumab) are already marketed and known to penetrate through the retina with high ability. In addition, low molecular weight compounds such as quinolone derivatives (Malm et al., 2010) have been described: tivozanib (AV-951) and axitinib (also an inhibitor of receptor phosphorylation). Low molecular weight molecules for selectively inhibiting VEGF production have been described previously (Cao et al., 2010).

Unselective inhibitors of either tyrosine kinases [e.g., lenvatinib (E7080); Keizer et al. (2010)] or of other growth factors in addition to VEGF have been described but have a predominant clinical focus on cancer: linifanib (ABT-869), ponatinib (AP24534), regorafenib (BAY 73-4506), and 20 others.

Conclusion. Although effective, antibodies are very expensive and possible systemic effects after intraocular administration (Jorge et al., 2006) limit their use; in particular, hypertension and stroke are a problem. The possible link of long-term VEGF inhibition to aggravation of retinal deterioration has to be investigated. Non-peptide antagonists may be the future. Antisense oligonucleotides (VEGF) and small interfering RNAs are described in section XII.E.

H. Aldose Reductase Inhibitors

The metabolism of glucose and the effect of hyperglycemia are summarized in Fig. 6. In the polyol pathway (Oates and Mylari, 1999; Naruse et al., 2000; Miwa et al., 2003), fructose is enzymatically produced from glucose in two energy-dependent steps (Fig. 6): first, catalyzed by aldose reductase, glucose is converted to sorbitol using NADPH as cofactor; second, catalyzed by sorbitol dehydrogenase, sorbitol is converted into fructose using NAD as a cofactor. This pathway is enforced primarily by hyperglycemia as a mass action of glucose. The increase in intracellular sorbitol levels is pathophysiological and is followed by these elements:

- A relative intracellular hypertonic state;
- A compensatory efflux of other osmolytes, such as myo-inositol and taurine (an antioxidant) (Nakamura et al., 1999; Vincent et al., 2004);
- A decrease, therefore, of myo-inositol levels (important in signal transduction, necessary for Na⁺/K⁺-ATPase function; its impact is addressed in Fig. 8 and section X.B);
- A decrease in cellular NADPH levels, resulting in an increase in redox imbalance, decreased concentrations of glutathione (a free radical scavenger) and
nitric oxide (a vasodilator, impact is addressed in Fig. 8 and section X.A);
- Enhanced formation of AGEs (pathophysiological impact is described in Figs. 6 and 7 and section X.D) due to fructose increase (note that fructose is 10-fold more active than glucose in glycosylation reactions); and
- Increased formation of DAG, which activates the deleterious PKC pathway (discussed in section X.C) (Yamagishi et al., 2003; Uehara et al., 2004).

Both enzymes, aldose reductase and sorbitol dehydrogenase, are abundantly expressed in tissues prone to diabetic complications (Giannoukakis, 2008). Aldose reductase inhibitors are expected to have the following effects:

- Slow or reverse progression of neuropathy (Chalk et al., 2007);
- Reduce eye diseases (cataracts, osmotic stress associated with polyol accumulation in the diabetic lens) (Kinoshita et al., 1968; Chylack et al., 1979); and
- Prevent or even reverse nerve deterioration (Cameron et al., 1986; Yagihashi et al., 1990; Kato et al., 2000).

However, despite the knowledge of pathophysiological details and despite clinical investigations for more than 20 years, the therapeutic efficacy of these compounds is still inclusive (Kador et al., 1990; Sorbinil Retinopathy Trial Research Group, 1990; Engerman and Kern, 1992; Nicolucci et al., 1996).

Several compounds may have major drawbacks, such as the following:

- Symptoms include muscle strength and sensation, improvement of limb numbness and cramping, sensory examination, nerve conduction, neuropathic symptoms, electromyogram, delay of disease progression, quality of life, and occurrence of foot ulcers (Chalk et al., 2007, 2009);
- Many compounds have inconsistent effects; some have been effective only in rodents and not in humans (sorbinil, ponalrestat) or have major side effects (sorbinil), so that they were withdrawn from all or at least many markets [fidarestat (SNK-860), lidorestat, sorbinil, tolrestat, zenarestat, zopolrestat (analog of ponalrestat)];
- High placebo effects may obscure clinical results, although side effects were rather high;
- Some side effects, such as increase in liver enzymes, nausea, and diarrhea, are unrelated to inhibition of aldose reductase; and
- Studies should last many months or even several years to arrive at a convincing Conclusion.

Some important compounds are listed in Table 9.

**Conclusion.** Expectations for new effective aldose reductase inhibitors should not be too high; therefore, it has been suggested that any future clinical trials of aldose reductase inhibitors are restricted (Chalk et al., 2009). Nerve sorbitol level per se is not a convincing indicator of nerve health and drug efficacy (Gabbay, 2004).

**I. Antioxidants (Vitamin C and E) and Radical Scavengers, Including Plant Extracts (Neuropathy, Retinopathy)**

High plasma glucose levels are often associated with high circulating free fatty acid (FFA) concentrations, resulting in enhanced mitochondrial superoxide production and increased exposure of cells to ROS (Fig. 7). Despite high expectations, administration of traditional antioxidants such as ascorbic acid (vitamin C) or tocopheryl (vitamin E) have provided disappointing clinical outcomes (Müller et al., 2003, 2004). Nevertheless other compounds have been tested: trolox (tocopheryl analog), N-acetylcysteine, β-carotene, selenium, benfotiamine (lipid-soluble thiamine derivative; see section X.C), and polyphenols (in green tea), zeaxanthin (primary carotenoid in the retina), curcumin (a polyphenol) (Stahl and Sies, 2004; Madsen-Bouterse and Kowluru, 2008). Improvement of endothelial function by polyphenols in clinical studies is not yet verified (Madsen-Bouterse and Kowluru, 2008). Clinical data on lipoic acid (on the market) as a potent regenerator of other antioxidants are inconsistent and ambiguous. The radical scavenger bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)decandioate di-hydrochloride, a nonconventional cyclic hydroxylamine derivative, improves metabolic alterations by counteracting β-cell dysfunction associated with oxidative stress (Novelli et al., 2010).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Status</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epalrestat (carboxylic acid) 50 mg 3 times/day</td>
<td>1992 (Ramírez and Borja, 2008); no conclusive evidence for efficacy: alleviates neuropathy symptoms (limb numbness and cramping) and delays progression (Hottu et al., 2006); note: it is now the standard drug therapy for diabetic neuropathy in Japan; critics: long-term comparative studies in diverse patient populations are needed.</td>
<td>Only few (↑ liver enzymes, nausea, and diarrhea)</td>
</tr>
<tr>
<td>Ranirestat (AS-3201; spirosuccin-imide)</td>
<td>1998; most promising of all, is effective (nerve conductance velocity) and is perhaps the only agent advanced enough in clinical trials (reviewed by Giannoukakis, 2008) to warrant further consideration (Bril and Buchanan, 2006); promising phase II trials and phase III is underway (Bril and Buchanan, 2006; Oates, 2008)</td>
<td>Safe and well tolerated</td>
</tr>
</tbody>
</table>
Conclusion. Intake of antioxidants is probably not effective, possibly because local concentrations are not sufficient to provide a definitive Conclusion.

J. Agents on the Horizon

Upcoming agents for diabetic neuropathy include neurotrophic factors, growth factors, gene therapy, immunotherapy, and nonimmunosuppressive immunophilin ligands (Mahmood et al., 2009). myo-Inositol as a dietary supplement against decreased Na^+/K^-ATPase function and nerve conductance velocity is not discussed here.

XI. G-Protein-Coupled Receptors

A. General Comments

More than 800 GPCRs are encoded by the human genome. Wide varieties of ligands bind to the GPCRs, but there are also more than 100 orphan GPCRs for which ligands and effects are not yet known. Over the last few years, a number of GPCRs expressed in pancreatic β cells and activated by lipids have been discovered. One, GLP-1, has already been discussed in section II. Other examples are:

- GPR40 and GPR119 (fatty acids as ligands);
- GIP (the second incretin);
- Neurotransmitters acetylcholine (M₃ muscarinic receptors);
- Noradrenaline (β₂-receptors and α₂-adrenoceptors);
- Neuropeptide pituitary adenylate cyclase-activating polypeptide;
- Vasoactive intestinal polypeptide and its receptors VPAC1 and VPAC2;
- Cholecystokinin receptors (Vrsopohl, 2009);
- Neuropeptide Y Y1 receptors (Winzell and Ahren, 2007);
- Cannabinoid receptors;
- Vasopressin receptors; and
- Purinergic receptors (Winzell and Ahren, 2007).

B. Lipid Receptors (Agonists of G-Protein-Coupled Fatty Acid Receptors 40 and 119 and Others)

1. G-Protein-Coupled Fatty Acid Receptor 40. GPR40 (G-protein-coupled fatty acid receptor 40; FFAR1) is activated by medium- to long-chain fatty acids (C12-C22) (Kebede et al., 2009) and acutely amplifies glucose-induced insulin secretion, mediated by signaling via Gq and phospholipase C (Brownlie et al., 2008; Kebede et al., 2009). The GPR40 agonist 4-((3-phenoyxyphenyl)methyl)amino)benzenepranoproic acid (GW9508) opens ATP-dependent K⁺ channels, which suggests that GPR40 activation may also have the potential to inhibit insulin secretion (Zhao et al., 2008). In the short term, insulin release is increased; long term, however, inhibition of insulin release and apoptosis are observed (lipotoxic action). The importance of GPR40 was evaluated using GPR40-deficient mice, which have an impaired acute insulin secretory response to FFAs (Steneberg et al., 2005; Latour et al., 2007; Brownlie et al., 2008). In view of the possibility that GPR40 might be protected from the deleterious effects of a high-fat diet on glucose tolerance, it has also been suggested that GPR40 antagonism may be beneficial for the treatment of type 2 diabetes. It is noteworthy that a loss-of-function mutation of the GPR40 gene exists in 0.75% of healthy subjects and is associated with obesity and increased insulin secretion (Vettor et al., 2008). It should be noted that some controversy exists as to whether GPR40 agonists or antagonists should be designed (Kebede et al., 2009), but the focus is on GRP40 agonists.

GPR40 agonists include various structures: amino-phenyl propionic acid derivatives, alkoxyphenyl propionic acid derivatives, bicyclic compounds, 4,5-diphenylpyrimidylamino-substituted carboxylic acids, phenylaminobenzoxazole-substituted carboxylic acids, oxadiazolidinedione compounds, bicyclic carboxylic acid derivatives, 3-(4-hydroxyphenyl)-substituted propanoic acids, cyclopropane carboxylic acids, phenylpropanoic acid derivatives, and diazeporphoglucinols. In addition, TAK-875 (dihydrobenzofuran derivative) is going to be developed (Takeuchi et al., 2010; Viswanathan et al., 2011).

In particular, low molecular agonists of GPR40 may improve insulin secretion without increasing apoptosis (Pfleiderer et al., 2010): AMG 837 is an example (Yazaki et al., 2011); it is also called glucose-dependent insulin secretion potentiator. For several compounds, lipotoxicity may be anticipated as derived from FFA effects but has not been confirmed (Tan et al., 2008).

Conclusion. GPR40 may be a promising therapeutic target and a valuable therapeutic approach for type 2 diabetes. However, nonselective effects have to be expected because GPR40 is expressed not only in β cells (though predominantly) but also in brain, adipocytes, enteroendocrine cells, and glucagon-secreting A-cells (Floodgren et al., 2007; Brownlie et al., 2008).

2. G-Protein-Coupled Fatty Acid Receptor 119. GPR119 is a class-A (rhodopsin-like) Gα protein-coupled receptor (cAMP) that was recently deorphanized. It is activated by lipid amides as endogenous ligands, such as oleoylethanolamide (OEA), lysophosphatidylcholine, oleoyllysoophatidylcholine, and olvanil (Overton et al., 2006). Nonselective effects may be anticipated when marketed because of the broad tissue distribution (not only β cells, brain, and GI tract) (Ramaekhsnan, 2001; Takeda et al., 2002; Soga et al., 2005; Chu et al., 2007; Overton et al., 2008).

GPR119-deficient mice retained a normal insulin secretory response to glucose and GLP1 but, as expected, had no response to the GPR119 agonist N-(2-fluoro-4-methanesulfonfylphenyl)-(6-[4-(3-isopropyl-1,2,4]oxadiazoil-5-yl)-piperidin-1-yl)-5-nitropyrimidin-4-ylamine (AR231453) as proof of concept (Chu et al., 2007). GPR119 agonists mediate a unique dual elevation of both insulin and glucagon-
like peptide 1/glucose-dependent insulino tropic peptide levels (Chu et al., 2008; Jones et al., 2009; Kebede et al., 2009; Jones, 2010) making them interesting for a combination with DPP-4 inhibitors (see section III), especially with respect to weight loss. It is not yet established whether the primary determinant of GPR119 efficacy results from its direct insulino tropic or incretin-releasing properties. Agonists improved glucose tolerance in diabetic rodents (Jones, 2010). It is noteworthy that anorectic effects of OEA (endogenous ligand of GRP119) are not mediated through GPR119 (Lan et al., 2009). It is noteworthy that anorectic effects of OEA (endogenous ligand of GRP119) are not mediated through GPR119 (Lan et al., 2009).

More than 10 GPR119 agonists have been described previously (Shah, 2009), but only one compound has reached clinical development (Semple et al., 2011), indicating weak effects of this class:

- PSN375963 (5-(4-butyicyclohexyl)-3-pyridin-4-y1-1,2,4-oxadiazole) and PSN632408 (tert-butyl 4-[(3-pyridin-4-yl)-1,2,4-oxadiazol-5-yl) methoxy]piperidine-1-carboxylate) (Ning et al., 2008); These compounds may additionally activate GPR119-independent pathways, which may be criticized as being nonselective (Ning et al., 2008). PSN632408 is a mediator of anorectic effects (Overton et al., 2006), which is consistent with the ability of GPR119 to enhance plasma levels of both GLP-1 and peptide YY(3–36), both well characterized modulators of food intake (Turton et al., 1996; Batterham et al., 2002). This compound is nearly equipotent to OEA, the effects of which on feeding are now clearly not dependent on GPR119 (Lan et al., 2009). The pharmacology is dissimilar in PSN632408 and OEA, and indeed even between the synthetic GPR119 agonists PSN375963 and PSN632408 (Ning et al., 2008);
- AR231453 (a nitropyrimidine) (Semple, 2007; Semple et al., 2008, 2011); PSN119-2 (an oxadiazole) with an EC50 of 18 nM for insulin secretion from HIT cells and an EC50 of 8 nM for GLP-1 release from GLUTag cells (L-cells releasing GLP-1) (Fyfe et al., 2008) was discovered by using SAR studies;
- Cyclic amine derivatives such as pyridinylpiperidine and pyridazinylpiperidine derivatives (Endo et al., 2010) (some showed EC50 of 26.8 nM for increasing cellular cAMP levels);
- Pyridazinyl derivatives (Carpenter et al., 2010);
- Pyridopyrimidnone-based orally effective compounds (Neelamkavil et al., 2010) derived from SAR studies;
- GSK252A (dihydropyrrolopyrimidine,imidazoline, and piperazine derivative); and
- APD668 (phase I) effective in patients with diabetes, but no details have been released (Arena Pharmaceuticals, 2008).

Direct comparison of a distinct GPR119 agonist with the DPP-4 inhibitor sitagliptin indicates that the latter may have a modestly better ability to raise plasma GLP-1 levels, and DPP-4 inhibition seems insufficient to elicit strong effects on feeding or body weight (Lovshin and Drucker, 2009). In addition, combinations with DPP-4 inhibitors (Mark et al., 2010) for treating and/or preventing metabolic diseases, in particular type 2 diabetes, have been investigated. Taken together, some results imply that a GPR119/DPP-4 combination could be an extremely effective add-on in patients whose metformin responsiveness is waning (Jones et al., 2009).

Conclusion. As a stand-alone therapy or in tandem with approved DPP-4 inhibitors (see section II), GPR119 agonists could be an interesting option for type 2 diabetes mellitus (Shah, 2009). In particular, their dual action in improving secretion of both insulin and incretin hormones is unique and may even have the potential of a leading target for the next generation of antidiabetic therapies. Used as monotherapy, their long-term efficacy and safety has to be established. These agents may also be able to protect β-cell mass effectively, as possibly mediated by released incretin (GLP-1) (Baggio and Drucker, 2007), and they would, therefore, accelerate/propagate the relevance of this type of treatment. It will be interesting to see whether a combination with a DPP-4 inhibitor, which would inhibit the degradation of GLP-1 as secreted by GPR119, will be an option (Lauffer et al., 2008).

3. Other G-Protein-Coupled Fatty Acid Receptors. GPR41 and GPR43 (ligands are short-chain fatty acids) may indirectly regulate β-cell function via adipokine secretion, but the relevance is not clear (Kebede et al., 2009). Because they are expressed in many cells (especially immune cells), selective effects may not be expected. Phenylacetamides have been described as agonists (Lee et al., 2008). GPR120 (Gq) mediates fatty acid-stimulated GLP-1 release from L-cells in addition to direct insulin release (Kebede et al., 2009). Nevertheless, GPR41, -43, and -120 are mainly expressed in extraislet tissues. Information on these receptors as potential targets is limited, and further studies are required. GPR109A (HM74A in humans; Homo sapiens G protein-coupled receptor agonist) is expressed in adipocytes and reduces circulating FFAs, thereby ameliorating insulin resistance.

C. Bromocriptine Mesylate (Dopamine-2 Receptor Agonist)

Bromocriptine mesylate (Cycloset; already used in Parkinson’s disease), approved in 2009 by the FDA, is for use alone or with other antidiabetic agents in the management of type 2 diabetes. Bromocriptine is thought to act on circadian neuronal activities within the hypothalamus, thereby resetting in insulin-resistant patients an abnormally elevated hypothalamic drive for increased plasma glucose, triglyceride, and FFA levels in fasting and postprandial states (Waknine, 2009; Kerr et al., 2010). This is the first chronotherapy-based treatment of type 2 diabetes. The link of dopamine to diabetes originated from studying the metabolism of migrating birds, because they develop seasonal insulin resistance.
For adverse effects, its use as an antiparkinson drug must be kept in mind; the FDA warns that bromocriptine can cause orthostatic hypotension and syncope, particularly on initiation of therapy and dose escalation (Waknine et al., 2009). In addition, cardiac and noncardiac fibrotic reactions typical for ergoloids have to be kept in mind.

Conclusion. This concept is questionable, because Levodopa (antiparkinson drug) was reported 30 years ago to induce a diabetic situation via inhibition of insulin release and promotion of glucagon release. No other D2 receptor agonists seem to be developed at present.

D. M3 Subtype Muscarinic Receptor Agonists

Mutation experiments with M3 muscarinic acetylcholine receptor subtype (Gq) in mice have revealed their relevance in regulating important metabolic functions (obesity and associated disorders) as well as glucose homeostasis, insulin sensitivity, food intake, and basal and total energy expenditure (Gautam et al., 2008). The M3 ACh receptor subtype is involved in stimulatory effects on insulin-secreting β cells (Verspohl et al., 1990; Gautam et al., 2010).

Conclusion. Development will be hampered by the fact of nonselectivity; M3 receptors are present in many tissues. In addition, no M3 receptor-selective compound has been detected, not even for other indications.

E. 5-Hydroxytryptamine 2c Subtype Serotonin Receptor Agonists

5-HT2c receptors expressed by pro-opiomelanocorticotropin neurons are physiologically relevant regulators of insulin sensitivity and glucose homeostasis (Xu et al., 2010). There exists an association of −759C/T polymorphism of 5-HT2c receptor with type 2 diabetes and obesity (Gao et al., 2009). Mice lacking 5-HT2c receptors display hepatic insulin resistance, a phenotype normalised by re-expression of these receptors. A deficiency can be overcome by the 5-HT2c receptor agonist such as aminergic receptors imidazoline binding proteins and the precise function of imidazoline receptors? The heterogeneity of effects is obvious, because these two compounds with imidazoline structure (phentolamine and efaxran) do not behave identically (Bleck et al., 2004). The imidazoline ring is probably not the pharmacophore, adding to the unanswered questions. All together the molecular target of “second-generation imidazolines” remains elusive.

S-22068 has a potent effect on glucose tolerance and significantly increased insulin secretion in diabetic rats (Pelé-Tounian et al., 1998; Le Bihan et al., 1999). Unlike earlier imidazoline-based secretagogues, this compound does not have a high affinity for α2-adrenoceptors or the imidazoline binding sites I1 or I2 but may act through a as-yet-unrecognized imidazoline-binding site.

Some compounds exert an antiapoptotic effect on β cells (preservation of β-cell mass). The imidazoline 1-phenyl-2-(imidazoline-2-yl)benzimidazole (RX871024) causes death of highly proliferating insulin-secreting cells, putatively via augmentation of Janus kinase activity (Zaitseva et al., 2008).

Conclusion. The data from the last 20 years are still confusing in several aspects: although effects mediated via α2-adrenoceptors reduce insulin secretion, imidazoline structures (high concentrations) increase insulin secretion. The involvement of KATP channels is uncertain, which opens speculations about an effect on direct triggering of the exocytosis process. No imidazoline compound has been developed clinically for diabetes. This should not be taken as evidence of a flaw in the basic hypothesis, but derives, in part, from continued ignorance about the molecular characteristics of imidazoline binding proteins and the precise structure-activity relationships of their ligands.

G. Glucagon Receptor Antagonists

In addition to a lack of insulin effect, type 2 diabetes is often associated with a glucagon excess. An inappropriately high rate of hepatic glucose production is a predominant cause of fasting hyperglycemia and a major contributor to the postprandial hyperglycemia characteristic of type 2 diabetes. The glucagon receptor is predominantly located in the liver and, upon activation, stimulates hepatic glycogenolysis and gluconeogenesis. Glucagon receptor antagonists, therefore, have the po-
potential to reduce hepatic glucose production and be effective antidiabetic agents.

Testing assays are glucagon receptor binding inhibition assays using, for example, 3-[125I]iodotyrosyl glucagon, and efficacy in suppressing glucagon-induced plasma glucose excursion. Antagonism of the glucagon receptor is associated with increased circulating levels of GLP-1, which is a significant contributor to the glucose-lowering effects during glucagon receptor antagonist treatment. To investigate this, it is necessary to compare effects in wild-type mice and GLP-1 receptor knockout mice (Gu et al., 2010). Compounds have to be tested for selectivity toward type mice and GLP-1 receptor knockout mice (Gu et al., investigate this, it is necessary to compare effects in wild-type mice and GLP-1 receptor knockout mice (Gu et al., 2010). Compounds have to be tested for selectivity toward cardiac ion channels, other family receptors such as hGIP and hGLP1 receptors, other receptors, and various enzymes.

The structures of glucagon receptor antagonist (mostly patents) are summarized (the IC50 values given are derived from either receptor binding studies or assays for inhibition of glucagon-stimulated cAMP levels):

- Naphthyl-β-alanine derivatives (some with an IC50 of 3.3 nM) (Lin et al., 2010a);
- Spiro-imidazoline compounds (IC50, ~450 nM) (Stamford et al., 2010);
- 1,2-Diphenylethane compounds (IC50, 1.0 nM) (Lin et al., 2010b);
- Cyclic compounds with a nitrogen containing heteroaryl ring (IC50, <10 μM) (Greenlee et al., 2009);
- Thiophenylcarbonylaminopropionic acid derivatives (IC50, <50 μM) (Chappell et al., 2006);
- Benzoylaminopropionic acid derivatives (IC50, <100 nM) (Conner et al., 2005);
- Substituted pyrazoles (Parmee et al., 2004);
- N-(4-((4-(1-Cyclohexen-1-yl))((3,5-dichloroanilino)methyl)benzoyl)-2-hydroxy-β-alanine derivatives (the IC50 of II [10] was 0.046 nM (Fujii et al., 2011);
- A novel class of 1,3,5-pyrazoles, some of which have oral bioavailability (Shen et al., 2011).

Conclusion. Glucagon receptor antagonists are effective in reducing glucose levels and improving glucose tolerance (mainly animal experiments). The beneficial effects are achieved mainly on the level of liver but also through improved islet function. Mainly because of toxicological problems, no compound made it into the market. The need for glucagon receptor antagonists has lessened since marketed GLP-1 receptor agonists and DPP-4 inhibitors inhibit glucagon effects (see Table 1). Nevertheless, in addition to treatment of type 2 diabetes, they may be interesting for obesity and dyslipidemia.

XII. Hypothetical New Targets (Emerging Targets) Still Lacking Therapeutic Significance

A. General Comment

Type 2 diabetes is a multifactorial disease, which indicates the need for an unconventional approach to detect new targets. One possibility is the comprehensive gene expression analyses of critical tissues for understanding the molecular signature of type 2 diabetes. Serial analysis of gene expression techniques have made it possible to compare tag levels among independent libraries and to identify previously unrecognized genes with novel functions that may be important in the development of diseases (Takamura et al., 2008). Such serial analysis of gene expression-based approaches may lead to the identification of novel therapeutic targets for the treatment of type 2 diabetes and its complications.

B. Retinoid X Receptors

Retinoid X receptors (RXRs) control lipid and carbohydrate metabolism. They are members of the nuclear
hormone receptor superfamily and are thought to be key regulators in differentiation, cellular growth, and gene expression. Endogenous RXR agonists negatively regulate glucose-stimulated insulin secretion.

Conclusion. The modulation of endogenous RXR in β cells may be a new therapeutic approach for improving impaired insulin secretion (Miyazaki et al., 2010); PPAR-γ-agonists (see section VI) are known to act in tandem with RXR.

C. Colesevelam

In phase III, colesevelam is a bile acid sequestrant, reduces cholesterol by complexing bile acids, and indirectly influences a diabetic situation: a decrease of blood glucose, HbA1c, and low-density lipoprotein-cholesterol levels in patients with prediabetes and type 2 diabetes (Fonseca et al., 2010; Levy and Jellinger, 2010), whereas insulin sensitivity was unchanged during insulin clamp experiments (Schwartz et al., 2010) but increased in whole-body experiments during a postmeal test. Taurocholate, as well as other bile acids, is an endogenous ligand for the RGR5 receptor and stimulates the secretion of anorexigenic hormones GLP-1, peptide YY, and oxyntomodulin (Young et al., 2010).

D. Interleukin-1 Receptor and Chemokine Receptor 2 Antagonists

IL-1β is increased by high glucose concentrations and leads to impaired insulin secretion, decreased cell proliferation, and apoptosis (Larsen et al., 2007). The expression of IL-1 receptor antagonists is decreased in β cells prepared/obtained from patients with type 2 diabetes (Maedler et al., 2004). IL-1 receptor antagonist therapy, therefore, has been investigated as a possible treatment of type 2 diabetes (Larsen et al., 2007, 2009), especially to prevent the progression of deterioration of β-cell function as partly linked to apoptosis (Butler et al., 2003; Donath and Halban, 2004) and low-grade systemic inflammation.

Treatment with the IL-1 receptor antagonist anakinra improves glycemia (HbA1c reduction) and β-cell function (proinsulin/insulin ratio); it also reduces markers of systemic inflammation in patients with type 2 diabetes (Larsen et al., 2007), lasting for 39 weeks after treatment withdrawal (Larsen et al., 2009). Canakinumab (ACZ885) is a monoclonal antibody against IL-1β (phase II). CCX140-B is a chemokine receptor 2 antagonist being in phase II (Sullivan et al., 2010).

Conclusion. Low-grade systemic inflammation is observed in patients with type 2 diabetes; it is probably not only an epiphenomenon (Kolb and Mandrup-Poulsen, 2005) and should be therapeutically addressed.

E. Antisense Strategies

The discovery and development of antisense drugs for the treatment of various metabolic disorders, including type 2 diabetes, have been reviewed (Liu, 2003; Bhanot, 2008; Wancwicz et al., 2008). These drugs are highly selective and safe with respect to therapeutic index. They are mostly 20-base chimeric oligonucleotides, the first and last five bases having a 2′-O-(2-methoxy)-ethyl modification (Crooke, 2004). This chimeric strategy increases both their binding affinity to the complementary sequences and their resistance to the degrading action of nucleases, improving their pharmacodynamic (increase in potency) and pharmacokinetic properties (duration of action).

- Against PTP1B (the role of PTP1B is described is section VII.G): ISIS 113715 (phase II), a 20-base-pair oligonucleotide, lowers glucose without hypoglycemic risk and increases insulin sensitivity in animal models of insulin resistance (Dean et al., 2001; Cowsert et al., 2002; Zinker et al., 2002), including possibly a promotion of weight loss (at least no weight gain). Positive effects have also been shown for monkeys (Swarbrick et al., 2009). It is selective with respect to other phosphatases. Pharmacokinetics have been described: complete absorption from subcutaneous site, half-life of 16 days, and no pharmacokinetic interaction with oral diabetic drugs (Bhanot, 2008).
- Against SGLT2 (the role of SGLT2 is described in section IV): ISIS-388626 selectively inhibits renal SGLT2 gene expression in vivo (positive result from a “proof-of-concept” study) (Patel and Fonseca, 2010). Once-weekly or once-monthly injection reduced gene expression by up to 80% with tissue and gene selectivity; glucose is lowered (Bhanot et al., 2008).
- Against phosphoenolpyruvate carboxykinase [key (rate-limiting) enzyme of gluconeogenesis]: this approach is unfortunately without effect.
- Against glucagon receptor expression/production (the role of glucagon is described in section XI.G): for ISIS 325568, a phase I study was completed in 2008 (no release of details from the company developing this compound, ISIS Pharmaceuticals) (Monia et al., 2011).
- Against glucocorticoid receptor production: ISIS 377131 (Monia et al., 2010) has a unique and preferential distribution to tissues such as liver and fat, thereby potentially minimizing the systemic side effects. It reduces the diabetic effects of glucocorticoids (see section VIII.K).
- Against VEGF (the role of VEGF is described in section X.G): new drugs modulating growth factors (including VEGF) and newer targeted therapeutics using antisense oligonucleotides and small interfering RNAs are currently in clinical trials (Mohamed and Wong, 2008).

Overall conclusion. The unique therapeutic profile of antisense techniques is unmatched by existing therapies. However, long-term effects and safety await to be checked.
F. Angiotensin Receptors

Insulin-regulated aminopeptidase is inhibited by ANG IV, and ANG IV reduced the increase in blood glucose during a glucose tolerance test (Siebelmann et al., 2010). The impact of ANG IV/insulin-regulated aminopeptidase agonists acting via angiotensin receptors may be worth being investigated as antidiabetic agents.

G. Thioredoxin-Interacting Protein

Glucotoxicity plays a major role in pancreatic β-cell apoptosis and diabetes progression, but the mechanisms involved are largely unknown. TXNIP may be a link; it has been identified as a proapoptotic β-cell factor; the experiments were performed with TXNIP-deficient islets of animals harboring a natural nonsense mutation in the TXNIP gene, leading to high glucotoxicity (Shalev, 2008).

Conclusion. Inhibition of TXNIP may protect against glucotoxic β-cell apoptosis and therefore may represent a novel therapeutic approach to halt diabetes progression.

H. Blockers of Channel Systems

Pancreatic β cells depolarize in response to glucose and fire calcium-dependent action potentials that trigger insulin secretion. K⁺ channels play a central role in regulating the resting membrane potential and the shape and duration of the action potential in pancreatic β cells. Voltage-gated outward K⁺ currents from pancreatic islet β cells are known to repolarize the action potential during a glucose stimulus and consequently to modulate Ca²⁺ entry and insulin secretion. At least three types of K⁺ channels are involved (K_{ATP}, K_{Ca}, and Kv2.1 channels). One major responsibility for action potential repolarization in these cells is a delayed rectifier channel (Kv2.1), including subunits expressed in β cells. Hence, blockers of Kv2.1 channels might prolong action potentials and enhance calcium influx and insulin secretion.

Hanatoxin and guangxitoxin-1 have been described as inhibitors (Herrington et al., 2006). Guangxitoxin-1 prolongs glucose-triggered action potentials, enhances glucose-dependent intracellular calcium elevations, and augments glucose-dependent insulin secretion. SNAP-251-180 (S180), a N-terminal SNAP-25 domain (synaptoosomal protein of 25 kDa, 206 amino acids; blocking kv2.1 channels), but not SNAP-251-206 (S206), inhibits Kv current and enhances glucose-dependent insulin secretion mediated by the blockade of the Kv2.1 current (Zhuang et al., 2009).

Conclusion. Blockers of Kv2.1 channels have potential for novel therapeutic agent design. Another type (Kv1.3) is associated with the regulation of insulin sensitivity in peripheral target tissues. There is a rationale for the potential therapeutic use of Kv1.3 blockers in diabetes treatment as well (Choi and Hahn, 2010).

I. Influencing Islet Cell-to-Cell Communication

Intra- and interislet coordination of β cells exist in which the expression of connexin-36 may be involved; there are clusters at gap junction domains of the cell membrane, and adjacent β cells share cytoplasmic ions and small metabolites within individual islets (Bavaman et al., 2007). This may be a basis for developing novel therapeutic approaches to diabetes.

J. Fibroblast Growth Factor 21

It improves deranged nutrient metabolism, including overcoming insulin resistance. It stimulates glucose uptake in adipocytes but not in other cell types (Kharitonenkova et al., 2005). The effect is associated with FRS2 phosphorylation, thereby linking fibroblast growth factor receptor to the Ras/MAPK pathway.

K. ω-3-Polyunsaturated Fatty Acids

ω-3-Polyunsaturated fatty acids (ω-3-PUFAs) play a central role. Genes involved in insulin sensitivity (PPARγ), glucose transport (GLUT-2/GLUT-4), and insulin receptor signaling (IRS-1/IRS-2) are up-regulated by ω-3-PUFAs (González-Pérez et al., 2009). They increase adiponectin, an anti-inflammatory and insulin-sensitizing adipokine, and induce AMPK phosphorylation (González-Pérez et al., 2009), a fuel-sensing enzyme and a gatekeeper of the energy balance (see section VIII.C). ω-3-PUFA-derived lipoxins, resolvins, and protectins, which are all novel biologically active lipid mediators (González-Pérez et al., 2009), have well documented protective effects. They suppress VEGF and tumor necrosis factor-α production and inhibit endothelial cell proliferation, which may account for their beneficial effects in pathological retinal angiogenesis (Das, 2009). Other PUFAs, such as arachidonic, eicosapentaenoic, and docosahexaenoic acids, are important for retinopathy in view of their anti-inflammatory, wound healing, and neuroprotective actions (Das, 2008). Beneficial actions of ω-3-PUFAs and their bioactive lipid autacoids in preventing obesity-induced insulin resistance have also been described.

L. Nutraceuticals

Nutraceuticals may have a prophylactic potential. Glucomannan (soluble fiber) as a supplementary treatment slowed carbohydrate absorption, enhanced prandial ghrelin reduction when given before a glucose load, and impeded the rise of fasting ghrelin after 4-week supplementation (Chearskul et al., 2009). Nutraceuticals already marketed are guar gum and chromium picolinate.

Other nutraceuticals with substantial antidiabetic efficacy are as follows:

- Chlorogenic acid, which may be responsible for reduction in diabetes risk associated with heavy coffee intake;
- Bean-derived α-amylase inhibitors such as phaseo-
lamin (is not equivalent to acarbose) (McCarty, 2005); 

- Extracts of bitter melon and of cinnamon (McCarty, 2005; Verspohl et al., 2005); and 

- The role of zinc and zinc transporters for diabetes is a new field (Rungby, 2010).

**M. Emerging Metabolic Targets**

- Signal transducer and activator of transcription (transcription signaling) is increased by hyperglycemia and linked to diabetic nephropathy;
- Melanin-concentrating hormone receptor antagonists (patents) have been described: spirocylindrical bipyrlyridyridones (Christensen et al., 2010b), bipyrlyridyridones (Christensen et al., 2010a,c), piperdine and piperaizine derivatives (Boyle et al., 2010), arylyridones (Ahmad, 2010; Ahmad et al., 2010), indanylamines, and tetrahydroanthelene amines (Schwink et al., 2010);
- Imeglimin is the first in the new glimin class. By decreasing mitochondrial oxidation, it inhibits glucose production and increases glucose uptake and insulin secretion (Pirags et al., 2010);
- PF-04620110 (phase 1) is an inhibitor of diacylglycerol acyl transferase 1, an enzyme that catalyzes the final committed step of triglyceride synthesis (useful for diabetes and obesity);
- Modulation of ZnT8 activity (a type 2 diabetes-associated zinc transporter) may be a target to control proper glucagon release (Meur et al., 2011).

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