60 Future Drug Treatment for Type 2 Diabetes

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Keypoints

- The heterogeneous and progressive nature of type 2 diabetes presents an ongoing requirement for additional and novel blood glucose-lowering agents.
- Longer-acting (once daily and once weekly injected) analogs of the incretin hormone glucagon-like peptide 1 (GLP-1) and new dipeptidyl peptidase 4 inhibitors are being developed.
- Proof of principle studies have shown potential for non-peptide molecules to act as GLP-1 receptor agonists and insulin mimetics.
- Potentiators of insulin action include specific phosphatase inhibitors and other types of compounds that increase insulin receptor and early post-receptor signaling.

- Selective peroxisome proliferator-activated receptor modulators, glucagon receptor antagonists and cellular glucocorticoid inhibitors have shown potential.
- Agents that directly increase glucose disposal (e.g. glucokinase activators) or reduce glucose output (e.g. fructose 1,6-bisphosphatase inhibitors) are receiving attention.
- Sodium-glucose co-transporter 2 inhibitors can facilitate glycemic control through increased renal glucose elimination.

Introduction

Current strategies for managing type 2 diabetes mellitus (T2DM) are described in Part 6. This chapter reviews new therapeutic approaches to address the metabolic disturbances of T2DM. The main focus is on drugs that have recently become available for clinical use or have entered clinical trials, and established agents that have found new applications in patients with diabetes. Some agents in preclinical development that have particular theoretical promise are also considered.

There is a continuing need for new and improved agents to treat diabetes as available therapies do not reinstate normal glucose homeostasis or eliminate the threat of long-term complications. Indeed, patients with T2DM incur chronic tissue damage and suffer premature death, even with assiduous use of the available therapies [1–3]. Lifestyle measures, notably diet and exercise, remain the foundation therapy [4], while pharmacologic interventions are added to provide further recourse against the multiple, heterogeneous and progressive endocrine and metabolic disturbances of the disease [5]. Recent trial outcomes have re-emphasized the importance of comprehensive risk factor management in which early, effective, individualized and sustained glycemic control can defer the onset and reduce the severity of

complications [1,2]. In particular, "glycemic memory" requires good glycemic control early after diagnosis to minimize the complications of hyperglycemia much later in the disease process [1,2,6].

Development of new antidiabetic agents

As diabetes must usually be treated for the remainder of the patient's life, any new antidiabetic drug must be safe, well-tolerated, conveniently administered and carry minimal risk of serious hypoglycemia. It should offer durable efficacy and preferably other advantages – for example, a novel mode of action or favorable pharmacokinetics that suit a particular group such as the elderly. Ideally, a new agent will be suited to combination therapy with at least some of the existing agents, and confer benefits against conditions that are commonly associated with diabetes, such as abdominal obesity, dyslipidemia, hypertension and other vascular diseases or risk factors. A new drug might correct at least one of the major underlying endocrine or metabolic disturbances such as counter insulin resistance, improve β-cell function, reduce hyperglucagonemia or act directly to decrease glucotoxicity or lipotoxicity.

The development of a drug from a new chemical entity through to marketing approval involves many stages of rigorous preclinical and clinical evaluation (Table 60.1). The process can take 10–15 years and cost US$ 500–2000 million [7]. If there is evidence of increased adverse cardiovascular outcomes during the

Table 60.1 Stages in the development of a new drug.

Preclinical stages

New chemical entity

- Identification, extraction/synthesis, chemical characterization and patenting of compounds
- Genotoxicity testing: screening for biological activity *in vitro* and *in vivo* in animals
- Preclinical pharmacology, mode of action, pharmacodynamics (activity, safety, tolerance), pharmacokinetics (bioavailability, distribution, metabolism, elimination) and toxicity in ≥ two mammalian species

Clinical stages

Investigational new drug application: permission to begin clinical studies

Phase 1

- First administration to small number of healthy human volunteers
- Dose ranging, vital signs, pharmacodynamics, pharmacokinetics, drug interactions, safety

Phase 2

- First trials in small numbers of patients
- Dose ranging, efficacy, further pharmacodynamics, pharmacokinetics and safety

Phase 3

- Trials in larger numbers of patients
- Multicenter trials, comparative trials with other treatments, efficacy, further pharmacodynamics and safety (including meta-analysis of cardiovascular outcomes)

New drug application: permission to market as a drug

Phase 4

- Use in medical practice
- Additional trials (similar to phase 3), post-marketing surveillance, adverse drug reactions, use in special subgroups (e.g. elderly people)

Table 60.2 Established blood glucose-lowering agents and some other agents with blood glucose-lowering activity.

Agents	Main mode of action
Agents used in the treatment of diabetes	
Metformin (biguanide)	Counters insulin resistance
Sulfonylureas	Stimulate insulin secretion
Prandial insulin releasers (meglitinides)	Stimulate insulin secretion (rapid/short acting)
Thiazolidinediones (glitazones)	Increase insulin sensitivity (PPARγ agonists)
GLP-1 analogs	Potentiate insulin secretion
DPP-4 inhibitors (gliptins)	Potentiate insulin secretion
α-Glucosidase inhibitors	Inhibit carbohydrate digestion
Pramlintide	Satiety, slow gastric emptying, decrease glucagon
Insulin	Decrease glucose production, increase glucose disposal, storage and utilization
Other agents with blood glucose-lowering activity	
Acipimox	Decrease lipolysis, reduce NEFAs
Alcohol	Inhibit gluconeogenesis
Niacin	Decrease lipolysis, reduce NEFAs
Quinine	Stimulate insulin secretion
Salicylates	Stimulate insulin secretion
β-Adrenoceptor blockers*	Inhibit adrenergic counter-regulatory response

DPP-4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide 1; NEFA, non-esterified fatty acids; PPARγ, peroxisome proliferator-activated receptor γ.

* β-Adrenoceptor blockers usually raise glucose concentrations, but they can contribute to hypoglycemia by inhibiting the acute counter-regulatory response and impairing hypoglycemia awareness.

pre-authorization studies, depending on the circumstances, either additional pre-authorization or post-authorization trials will be required. To extend the period of marketing exclusivity it is not unusual for companies to seek additional indications and develop new formulations post-authorization. Because many patients with T2DM require combination therapy, there is also considerable interest in the production of single-tablet fixed-dose combinations [8].

Classification of new antidiabetic agents

Blood glucose-lowering agents may be either hypoglycemic or antihyperglycemic [9]. Both reduce hyperglycemia, but hypoglycemic agents can lower blood glucose concentrations below the euglycemic range, and therefore carry the risk of clinical hypoglycemia. Such agents include potent inhibitors of hepatic glucose output, insulin secretagogues that act at low glucose concentrations, potent insulin-mimetic drugs and agents that impair counter-regulatory mechanisms. By contrast, antihyperglycemic agents, when used as monotherapy, do not lower blood glucose

into the range of overt hypoglycemia. These drugs include the inhibitors of carbohydrate digestion and intestinal glucose absorption, anti-obesity agents, weaker suppressors of hepatic glucose output or counter-regulation, mild or glucose-dependent insulin secretagogues, most insulin-sensitizing agents and modulators of lipid metabolism.

The major glucose-lowering drugs currently used to treat diabetes, and some other agents with glucose-lowering activity, are shown in Table 60.2. Examples of other types of compounds under investigation as potential glucose-lowering drugs are listed in Table 60.3 [10,11] and illustrated in Figure 60.1.

Inhibitors of intestinal carbohydrate digestion and absorption

Delaying the digestion and absorption of dietary carbohydrate in the intestine reduces post-prandial hyperglycemia and may have some "carry-over" benefit to reduce basal glycemia. Efficacy is variable but generally modest, although this approach is usually suitable for combination with most other antidiabetic therapies
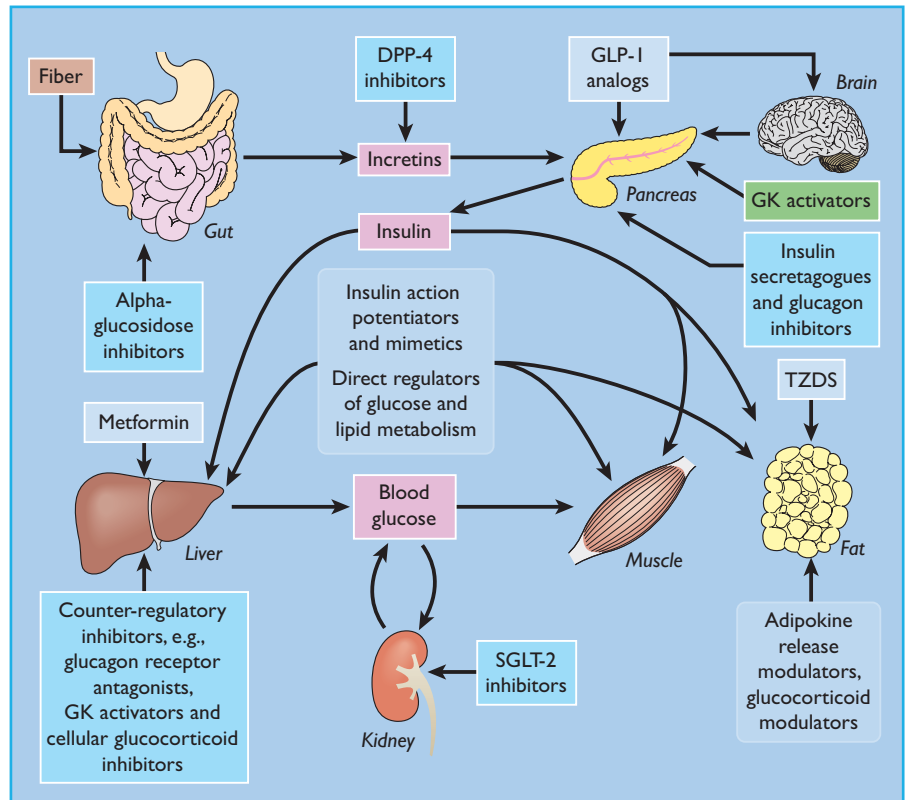
Figure 60.1 Potential sites of action of blood glucose-lowering agents. DPP-4, dipeptidyl peptidase 4; GK, glucokinase; GLP-1, glucagon-like peptide 1; SGLT-2, sodium-glucose co-transporter 2; TZD, thiazolidinedione.

Table 60.3 Examples of some new or investigational blood glucose-lowering agents.

Type of agent	Action	Example
α-glucosidase inhibitors	Inhibit carbohydrate digestion	Voglibose
Insulin secretagogues	Stimulate insulin secretion	Mitiglinide
Insulin secretion potentiators	Enhance nutrient-stimulated insulin secretion	GLP-1 analogs, DPP-4 inhibitors
Insulin mimetics	Insulin-like effects on glucose metabolism	IGF-I
Insulin action potentiators	Enhance actions of insulin	Selective PPAR modulators
Counter-regulatory hormone inhibitors	Suppress secretion/action of counter-regulatory hormones	Glucagon receptor antagonists, cellular glucocorticoid inhibitors
Direct glucose regulators	Increase glucose metabolism	GK activators, F16Pase inhibitors
Lipid regulators	Inhibit oxidation of fatty acids	CPT-1 inhibitors
SGLT2 inhibitors	Increase renal glucose elimination	"Flozins"

CPT-1, carnitine palmitoyl transferase-1; DPP-4, dipeptidyl peptidase 4; F16Pase, fructose 1,6-bisphosphatase; GK, glucokinase; GLP-1, glucagon-like peptide-1; IGF-I, insulin-like growth factor I; PPAR, peroxisome proliferator-activated receptor; SGLT2, secondary active sodium glucose co-transporter 2.

Table 60.4 Soluble and insoluble fiber supplements.

Soluble fiber	Insoluble fiber
Gums	Celluloses
Pectins	Wheat bran
Hemicelluloses	
Mucilages	

including insulin. Extending the period of digestion in this way can also reduce interprandial hypoglycemia in insulin-treated patients. Included here are dietary fiber supplements and inhibitors of digestive enzymes (Table 60.4).
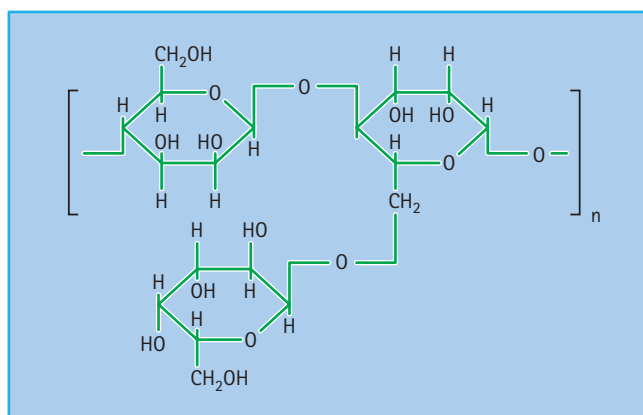
Dietary fiber supplements

Most dietary fiber comprises plant polysaccharides that are not digested or fermented in the large bowel; some are soluble and form bulky viscous gels and gums, while others are coarse and insoluble (Table 60.4). Soluble fiber appears to be more effective than insoluble in reducing post-prandial hyperglycemia and hyperinsulinemia. Fibers can act as a barrier to diffusion within the lumen of the small intestine, where complex starchy carbohydrates are digested. Carbohydrates become entrapped within the matrix, impeding access of digestive enzymes, and restricting the diffusion of liberated saccharides across the unstirred layers of gut contents to the intestinal epithelium [12]. Various fiber

supplements have appeared intermittently on the pharmacy shelves. These include common food thickeners such as soluble guar gum (E412), a galactomannan from the Indian cluster bean (*Cyamopsis tetragonoloba*; Figure 60.2) and fruit pectins. The prandial blood glucose-lowering effect of these supplements is usually small, especially if patients are already consuming a balanced diet containing fruit and vegetables [13].

Inhibitors of carbohydrate digestion

Slowing the digestion of complex carbohydrates by inhibiting amylases and glucosidases can reduce post-prandial hyperglycemia, particularly in individuals consuming a high starch diet such as rice (Figure 60.3). Inhibitors of α-amylases impair the
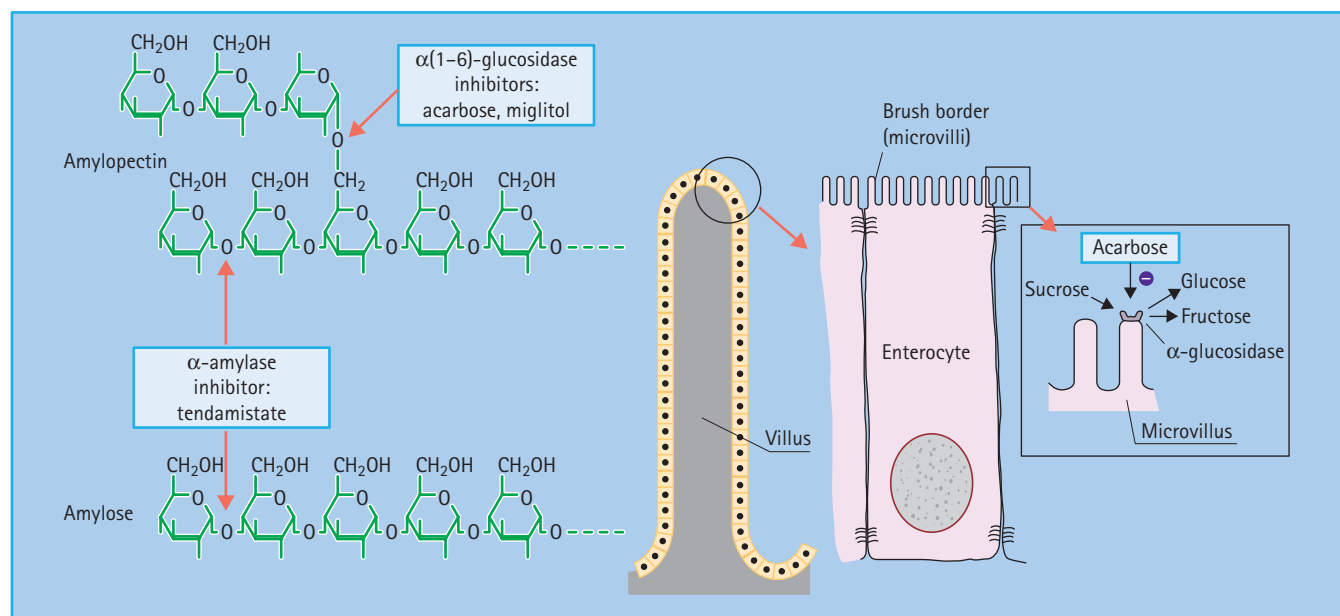
hydrolysis of starch, but such agents have been too unpredictable for routine therapeutic use. Reversible competitive inhibitors of brush-border α-glucosidases such as acarbose, miglitol and voglibose are more predictable (Figure 60.4). These still require appropriate titration against the amount of complex carbohydrate in the diet to prevent undigested sugars from entering the large bowel, where bacterial fermentation can produce carbon dioxide and osmotically active glucose, causing flatulence and diarrhea as side effects [14]. There do not appear to be new α-glucosidase inhibitors in clinical development.

Insulin secretagogues

Several defects in islet β-cell function occur early in the pathogenesis of T2DM: the acute first-phase insulin secretory response to glucose becomes reduced and eventually lost, processing of proinsulin to insulin is impaired, the normal pulsatile rhythm of basal insulin secretion is disturbed, and the second phase of insulin secretion is often extended, albeit diminished in magnitude, as the period of post-prandial hyperglycemia is prolonged (see Chapter 10). In advanced stages of T2DM, β-cell mass and insulin biosynthesis are also compromised. An ideal insulin secretagogue would restore β-cell sensitivity to glucose, and support adequate biosynthesis, processing and secretion of insulin in response to other nutrients, hormones and neural factors. Insulin secretagogues can be categorized into initiators (e.g. sulfonylureas), which stimulate insulin secretion on their own or require only low glucose concentrations, and potentiators (e.g. GLP-1 analogs), which enhance the effect of rising glucose and other secretagogues but do not induce insulin release without glucose.


Figure 60.2 Guar, a galactomannan polysaccharide from the Indian cluster bean, *Cyamopsis tetragonoloba*, used as a dietary fiber supplement.

Figure 60.3 Starch digestion and its inhibitors. α(1–6)-glucosidic linkages are cleaved by α(1–6) glucosidases, and α(1–4) linkages by α-amylase. α-Glucosidase inhibitors such as acarbose act by competitive, reversible inhibition of intestinal brush-border α-glucosidase enzymes. This slows the rate of carbohydrate digestion.

Figure 60.4 α-Glucosidase inhibitors in present use are acarbose and miglitol. Voglibose is available in some countries. These agents have different affinities for specific α-glucosidases: the binding affinity of acarbose is glucoamylase > sucrase > maltase > dextrinase; miglitol and voglibose potently inhibit sucrase, while voglibose shows more potent inhibition of other α-glucosidases than acarbose. Acarbose also weakly inhibits α-amylase.
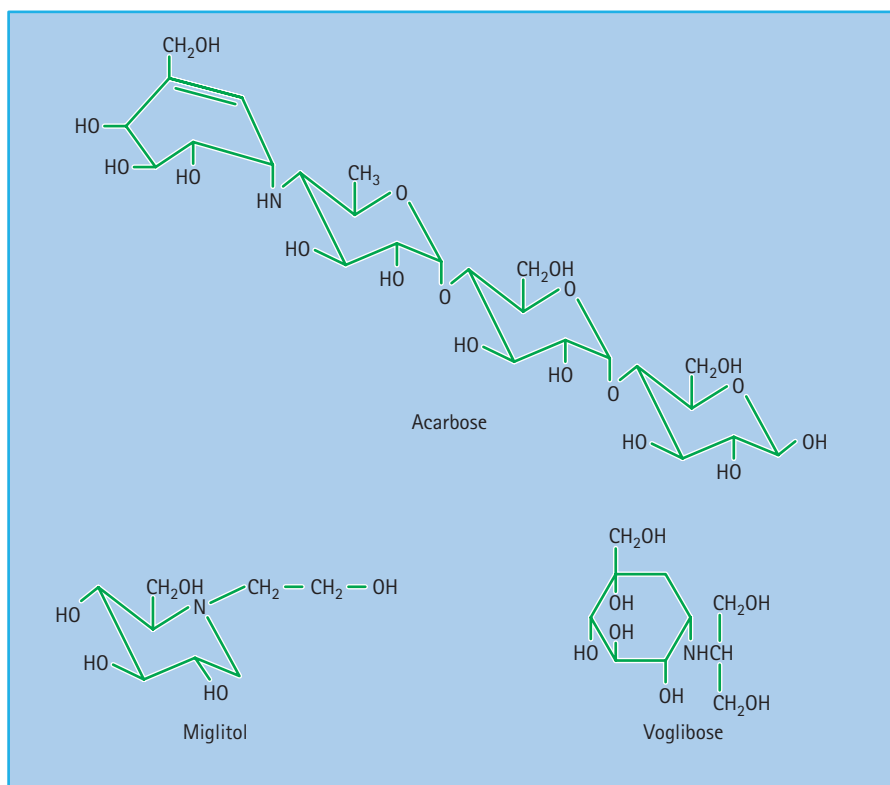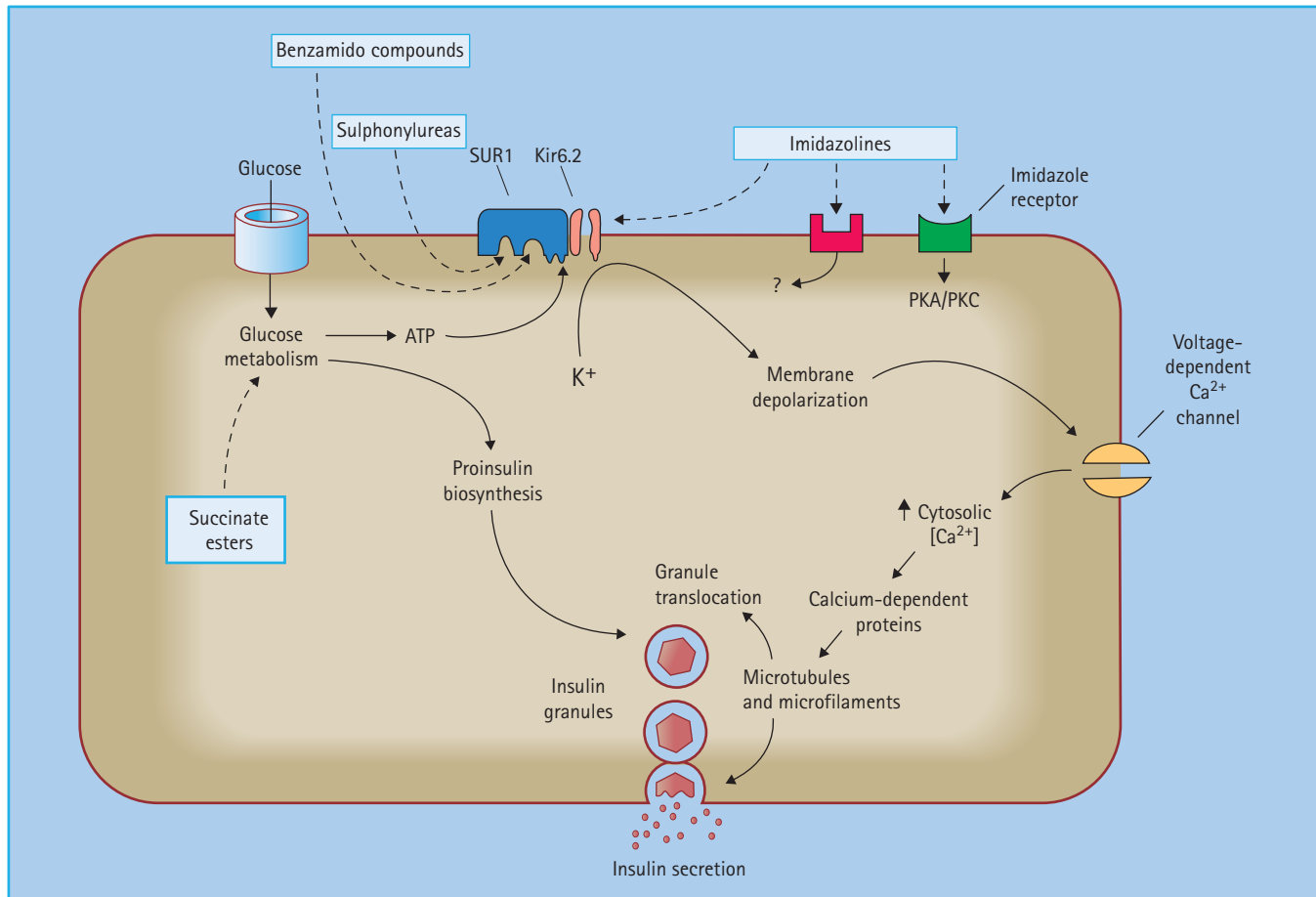

Figure 60.5 The ATP-sensitive (K_{ATP}) channel in the β-cell membrane consists of a large sulfonylurea 1 (SUR1) subunit (17 transmembrane domains) and a smaller pore-forming unit that acts as an inwardly rectifying K channel, Kir6.2. The cytosolic surface of the SUR1 subunit has separate binding sites for sulfonylureas (S), benzamido compounds (B) and the nucleotides, ADP and ATP (N). Four complete channel units self-associate to form an octameric complex in the membrane.
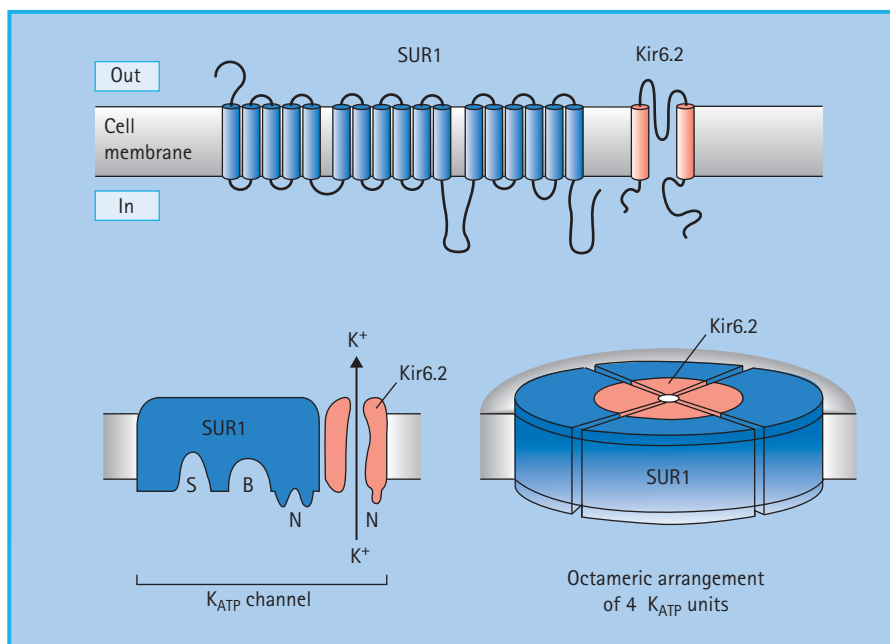
Initiators of insulin secretion

Currently available sulfonylureas and meglitinides are oral insulin secretagogues that initiate insulin secretion (see Chapter 29). They bind to either the sulfonylurea site or the benzamide site (meglitinides) on the sulfonylurea receptor 1 (SUR1) in the plasma membrane of the β-cell. The SUR1 is part of the ATP-sensitive potassium channel (K_{ATP} channel), which consists of an octameric complex of four Kir6.2 pores (inwardly rectifying potassium channels) surrounded by four SUR1 molecules (Figure 60.5). Binding of ligands to the sulfonylurea or benzamide sites

Figure 60.6 Sulfonylureas and benzamido compounds stimulate insulin release mainly by binding to sites on the sulfonylurea receptor 1 (SUR1) of the adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP} channels). This closes the Kir6.2 pores of the K_{ATP} channels, causing localized depolarization, opening of voltage-dependent calcium channels, increasing Ca^{2+} influx, and activation of calcium-dependent proteins that promote exocytosis of insulin granules. Imidazoline derivatives can act on I_1 and I_2 receptors, as well as other (unclassified) receptors and directly on the Kir6.2 pores to enhance insulin secretion. Succinate esters fuel glucose metabolism.

on SUR1 elicits the same response as binding of ATP to the nucleotide-binding domains of the channel, namely closure of the Kir6.2 pore [15]. This prevents K^+ efflux, which leads to localized depolarization of the plasma membrane. In turn, this opens voltage-gated (L-type) calcium channels, allowing influx of Ca^{2+} ions, which increases the cytosolic calcium ion concentration. Calcium-sensitive proteins are thereby activated, triggering the exocytosis of insulin-containing secretory granules (Figure 60.6). SUR1–Kir6.2 channels have also been identified within the membranes of mitochondria and possibly other organelles, suggesting that sulfonylureas and meglitinides could act at these and other sites in the cell as part of their insulin-releasing action.

Several novel insulin secretagogues have been reported to act by closing the K_{ATP} channels. The meglitinide derivative mitiglinide (KAD-1229) appears to bind at the benzamide site on SUR1 (Figure 60.6), and this agent has proceeded in clinical development [16]. Several other types of compounds have been shown to interact with K_{ATP} channels, but these do not appear to have proceeded beyond early clinical studies (Figure 60.7). These include

the morpholinoguanidine BTS67582 [17] and certain imidazolines such as S22068 [18]. Other imidazoline compounds, which bind to I_1, I_2 and probably other receptors, may also close K_{ATP} channels as part of their mechanisms to stimulate insulin secretion [19]; however, there are imidazolines such as BL11282 that stimulate glucose-induced (but not basal) insulin secretion by effects on protein kinases without closing K_{ATP} channels [20]. Various α_2-adrenergic receptor antagonists such as phentolamine, which can reduce the tonic suppression of insulin secretion mediated through α_2-adrenergic activation, may also act to close K_{ATP} channels.

Although closure of K_{ATP} channels initiates insulin secretion, it does not promote insulin biosynthesis. Agents that increase nutrient metabolism, and close K_{ATP} channels through increased ATP production, additionally enhance proinsulin biosynthesis. This capability is illustrated by esters of succinic acid which provide a metabolisable substrate to the mitochondria [21]. These compounds have low enteral bioavailability and a short duration of action, and they also fuel gluconeogenesis in the liver. A related approach could be to stimulate mitochondrial succinyl-
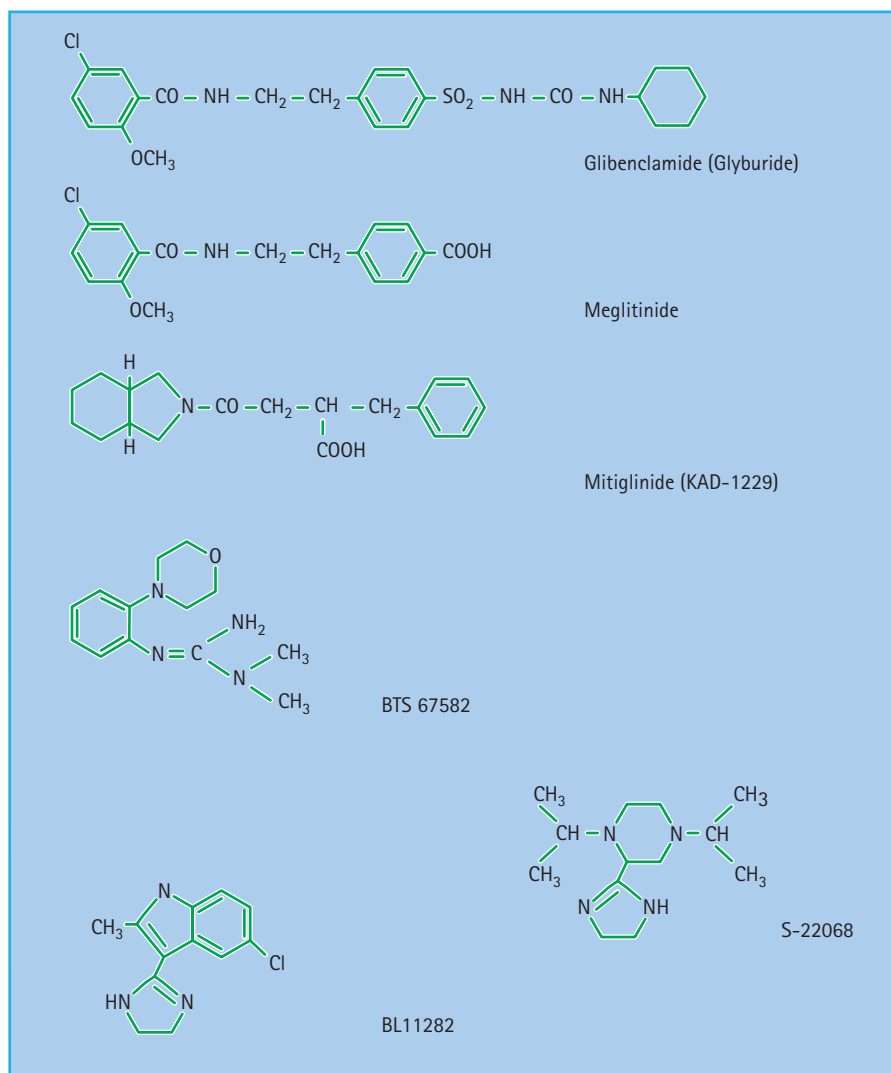
Figure 60.7 Structures of some established and novel insulin secretagogues. Glibenclamide (glyburide) has both a sulfonylurea and a benzamide moiety. Meglitinide derivatives exhibit a benzamide moiety. BTS 67582 is a morpholinoguanidine, while S-22068 and BL11282 are examples of imidazoline compounds.

coenzyme A (CoA) synthetase to generate ATP and guanosine triphosphate (GTP) [22].

Because the islet β-cell takes up glucose approximately in proportion to the circulating glucose concentration and phosphorylates the glucose via glucokinase (GK; EC 2.7.1.1), the β-cell is amenable to stimulation of glycolysis with agents that enhance the activity of GK [23]. Although this effect is actually potentiating the action of glucose (Figure 60.8), it can operate at low glucose concentrations, so it has been categorized here as an initiator. Several specific small molecule activators of GK have been shown to increase insulin secretion and improve glucose homeostasis in models of T2DM. These include allosteric activators and molecules that prevent association of GK with or cause dissociation of GK from inhibitory protein complexes [24–29]. Several of these agents are now in clinical trial, and it will be interesting to see if this approach can benefit other aspects of islet function and be regulated to avoid hypoglycemia. Because liver cells express GK and take up glucose approximately in proportion

to the circulating concentration, GK activators will also stimulate hepatic glucose utilization and reduce hepatic glucose production, adding to their blood glucose-lowering potency.

Potentiators of insulin secretion

Potential opportunities to increase nutrient-induced insulin secretion are shown in Figure 60.8. In principle, these agents should predominantly decrease post-prandial hyperglycemia and carry less risk of interprandial hypoglycemia.

Incretins

Insulin secretion is enhanced by several hormones released from the gut during feeding – so-called incretin hormones. The main incretins are glucagon-like peptide 1 (7–36) amide (GLP-1) and gastric inhibitory polypeptide (GIP; also known as glucose-dependent insulinotropic peptide). These hormones activate specific G-protein coupled receptors in the β-cell membrane and potentiate nutrient-induced insulin secretion and insulin biosyn-
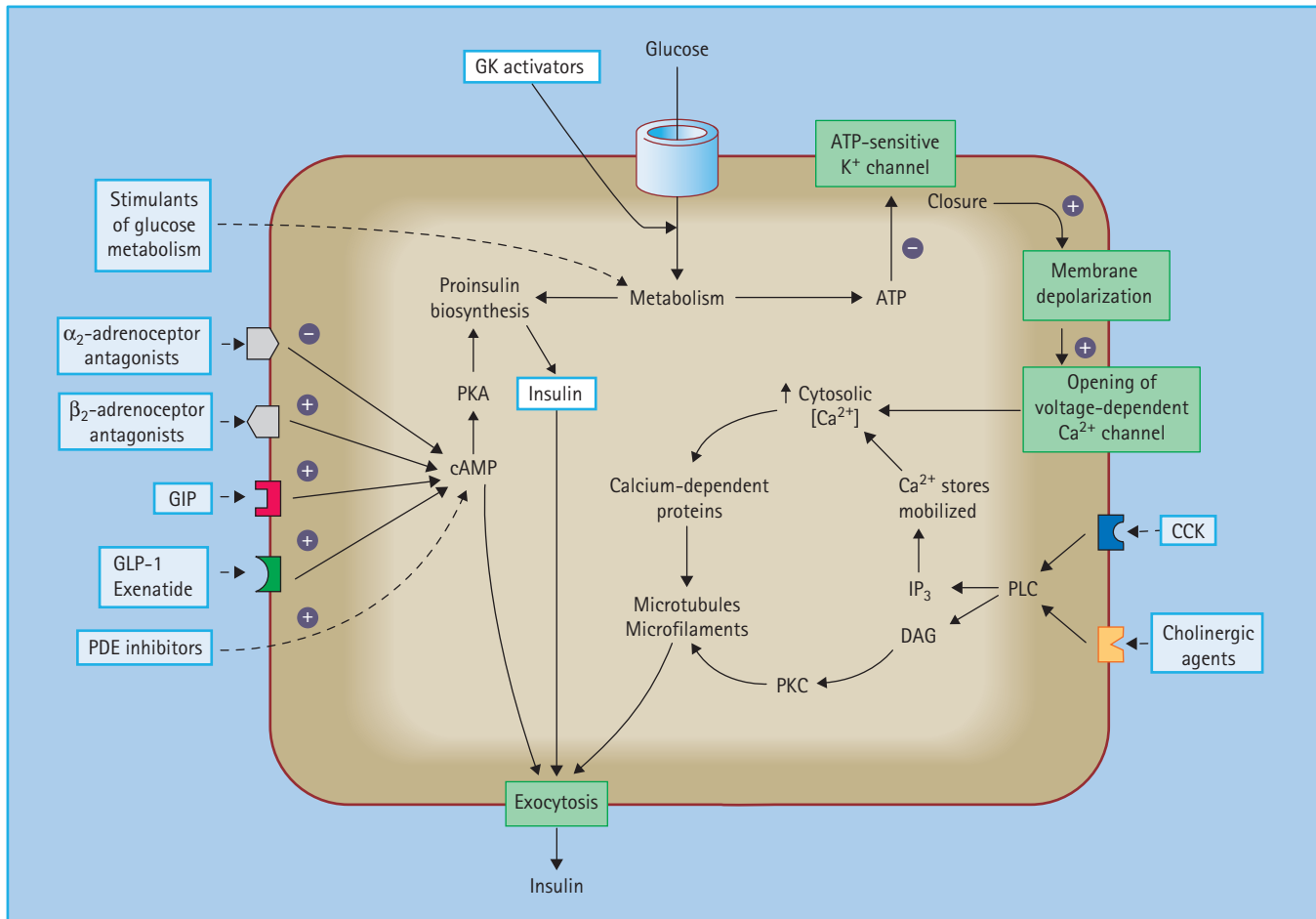
Figure 60.8 Sites on the pancreatic β-cell that offer potential targets to potentiate nutrient-induced insulin secretion. cAMP, cyclic adenosine monophosphate; CCK, cholecystokinin; DAG, diacylglycerol; GIP, gastric inhibitory polypeptide, also known as glucose-dependent insulinotropic peptide; GK, glucokinase; IP$_3$, inositol-1,4,5-trisphosphate; PDE, phosphodiesterase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C. Activated β$_2$-adrenoceptors and GIP and glucagon-like peptide-1 (GLP-1) receptors stimulate insulin secretion, as do antagonists of the inhibitory α$_2$-adrenoceptors.
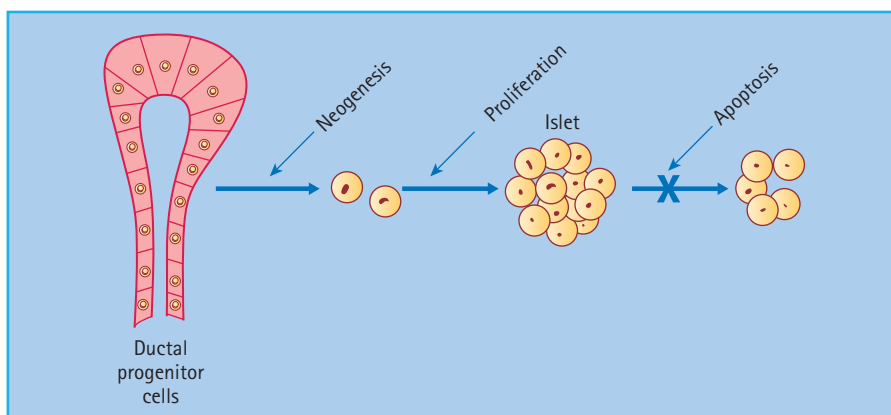
Figure 60.9 Incretin hormones GLP-1 and GIP have been shown to increase β-cell mass in animal models. Possible mechanisms include increased β-cell proliferation and differentiation, slowing of β-cell apoptosis and increased β-cell neogenesis from ductal progenitor cells.

thesis, at least partly through increased cyclic adenosine monophosphate (cAMP) and production of protein kinase A (PKA) [30,31]. Both hormones have also been shown to promote β-cell mass in animal models, possibly slowing β-cell apoptosis and increasing β-cell neogenesis by increased expression of the transcription factor PDX-1 (pancreatic duodenal homeobox 1), which promotes proliferation and differentiation of ductal progenitor cells (Figure 60.9). GLP-1 has been favored as a treatment

for T2DM because it also suppresses glucagon secretion in a glucose-dependent manner, slows gastric empting and exerts a satiety effect that can facilitate weight loss [32]. Moreover, GLP-1 concentrations appear to be reduced in T2DM but the ability of the peptide to enhance insulin release is maintained (Table 60.5). Thus, when injected subcutaneously immediately before a meal, GLP-1 can substantially reduce the post-prandial rise in blood glucose concentrations in T2DM (Figure 60.10). However, the rapid degradation of GLP-1, mostly by the enzyme dipeptidyl peptidase 4 (DPP-4) and the renal clearance of GLP-1 (plasma half-life < 2 minutes) makes the peptide itself an inconvenient form of therapy [33].

DPP-4 cleaves off an N-terminal dipeptide where there is an alanine residue (as in GLP-1) or a proline residue at the N2 position. Exenatide (exendin-4) is a GLP-1 analog with a glycine residue at the N2 position (Figure 60.11), making it resistant to degradation by DPP-4, and extending the half-life to several
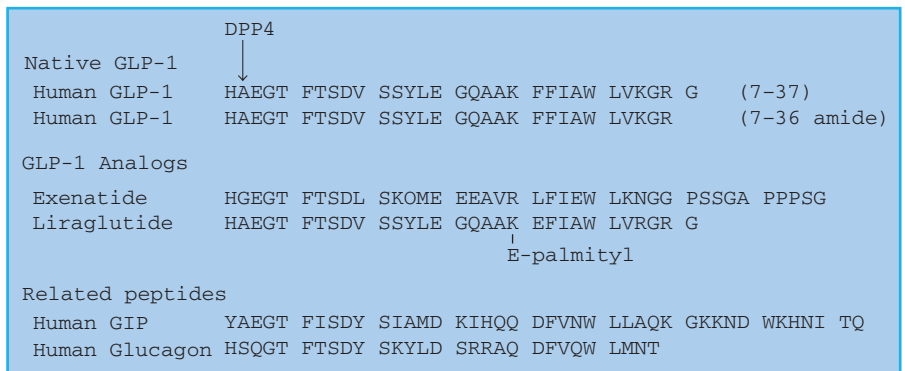
Table 60.5 Effects of the incretin hormones glucagon-like peptide 1 (7–36) amide (GLP-1) and gastric inhibitory polypeptide (GIP; also known as glucose-dependent insulinotropic peptide).

	GIP	GLP-1
Pancreatic		
Glucose-induced insulin secretion	Increase	Increase
Proinsulin biosynthesis	Increase	Increase
β-Cell mass (rodents)	Increase	Increase
Glucagon secretion	Increase or no effect	Decrease
Other actions		
Gastric emptying	No/slight effect	Decrease
Appetite/feeding	No significant effect	Decrease
Weight gain	No effect or increase	Decrease
Myocardial metabolism	No established effect	Possible benefits
Type 2 diabetes		
Plasma concentrations	Normal or slightly reduced	Reduced late phase
Insulin-releasing effect	Reduced	Mostly retained
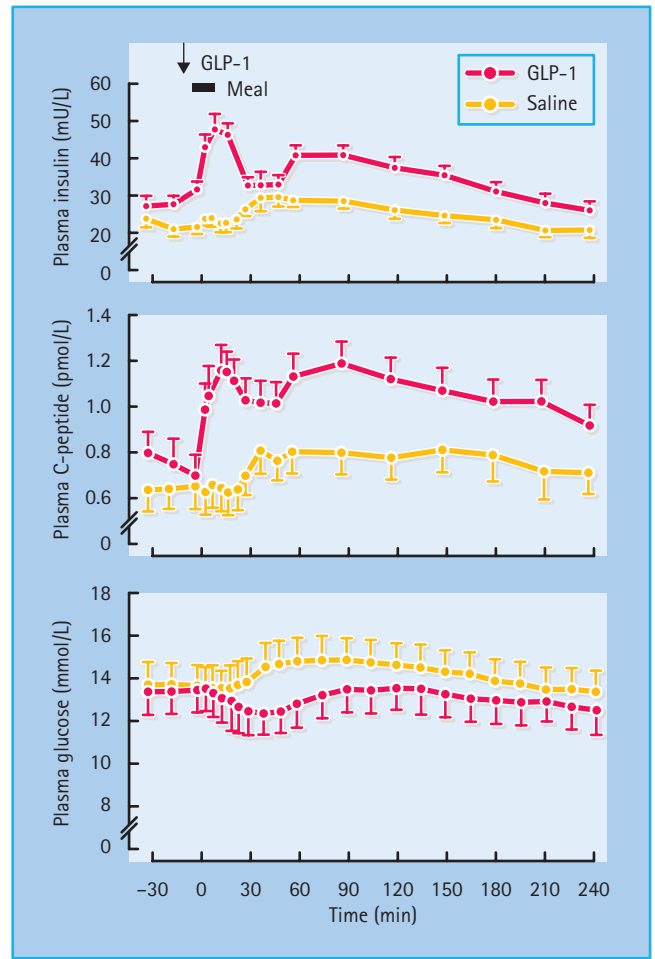
Figure 60.10 Insulin-releasing and glucose-lowering effects of glucagon-like peptide 1 (GLP-1) in eight obese patients with T2DM. A dose of 25 mmol was injected subcutaneously into the gluteal region 5 minutes before a standard meal. The plasma insulin and C-peptide responses were significantly greater, and the plasma glucose response significantly flatter (all $P < 0.001$), compared with a control (saline) injection. Reproduced from Glutniak *et al. Diabetes Care* 1994; 17:1039–1044, with permission from the American Diabetes Association.

Figure 60.11 The amino acid structure of GLP-1, and the GLP-1 analogs exenatide and liraglutide. GIP and glucagon are shown for comparison. DPP-4 cleaves off an N-terminal dipeptide where there is an alanine residue (as in GLP-1 and GIP) or a proline residue at the N2 position. Liraglutide is protected from degradation by DPP-4 when the palmitoyl fatty acid moiety binds to albumin.


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                        DPP4
Native GLP-1             ↓
 Human GLP-1      HAEGT FTSDV SSYLE GQAAK FFIAW LVKGR G     (7-37)
 Human GLP-1      HAEGT FTSDV SSYLE GQAAK FFIAW LVKGR       (7-36 amide)

GLP-1 Analogs
 Exenatide        HGEGT FTSDL SKOME EEAVR LFIEW LKNGG PSSGA PPPSG
 Liraglutide      HAEGT FTSDV SSYLE GQAAK EFIAW LVRGR G
                                          |
                                     E-palmityl

Related peptides
 Human GIP        YAEGT FISDY SIAMD KIHQQ DFVNW LLAQK GKKND WKHNI TQ
 Human Glucagon HSQGT FTSDY SKYLD SRRAQ DFVQW LMNT
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Table 60.6 Peptide glucagon-like peptide 1 (GLP-1) agonists in clinical development.

Compound	Name	Company	Structure
NN2211	Liraglutide* (Victoza)	NovoNordisk	GLP-1 analog linked to palmitoyl fatty acid, t½ 12–13 hours (once daily, s.c.)
Exendin-4	Exenatide-LAR	Amylin/Lilly	Long-acting release exendin (once weekly, s.c.)
?	Albiglutide (Syncria)	GSK	Complex of dimeric GLP-1 bound to albumin (once weekly, s.c.)
LY548806	?	Lilly	DPP-4 resistant GLP-1 analog
LY315902	?	Lilly	GLP-1 analog linked to a fatty acid
BIM-51077, R1583	Taspoglutide	Ipsen/Roche	Long-acting GLP-1 analog (once weekly, s.c.)
CJC-1131	?	ConjuChem	GLP-1 analog linked to a chemical complex
ZP10A, AVE0010	Lixisenatide	Zealand/ Sanofi-Aventis	GLP-1 analog

Other GLP-1 preparations in development include GLP1-INT (GLP-1 analog; Transition Therap/NovoNordisk) and MKC253 (GLP-1-technospheres for inhalation, MannKind Corp).
* Liraglutide was launched in Europe in July 2009.

hours. Because exenatide retains the glucose-lowering efficacy of GLP-1 it has become established as an incretin therapy, injected subcutaneously twice daily before the main meals [34]. The glucose-dependent nature of the insulin-releasing and glucagon-suppressing effects is reflected in the limited risk of hypoglycemia, while the satiety effect has assisted weight loss and favored use in obese patients.

Among other GLP-1 analogs (Table 60.6), liraglutide (NN2211) recognized regulatory approval in Europe in July 2009. Liraglutide is GLP-1 (7-37) with Lys34 replaced by Arg34, and Lys26 attached via a glutamate residue to a C16 hexadecanoyl (palmitoyl) fatty acid chain. The fatty acid chain facilitates association into heptamers and attachment of the molecule to albumin, protecting it from degradation by DPP-4 and enabling once daily injection [35].

A once-weekly depot formulation of exenatide, new long-acting GLP-1 analogs, slow-release formulations and different administration routes (e.g. transdermal, buccal and inhaled) are receiving clinical assessment [32]. Hybrid peptides are also being explored to increase glucose-lowering efficacy, for example DAPD, a peptide that is a GLP-1 receptor agonist and a glucagon receptor antagonist [36]. To circumvent the need for injections, non-peptide GLP-1 receptor agonists (e.g. Boc5) have been identified. These bind to the GLP-1 receptor on islet β-cells and enhance glucose-dependent insulin secretion [37,38].

Although GIP potentiates nutrient-induced insulin secretion it also increases glucagon release and promotes lipid deposition [39]. Moreover, GIP receptor knockout (KO) mice and the administration of GIP receptor antagonists (both peptide and non-peptide) have been shown to prevent the development of obesity, improve glucose homeostasis and reduce insulin resistance in animal models [40–44]. This is consistent with evidence from bariatric surgery: a reduced supply of nutrients through the proximal small intestine (location of GIP-secreting K-cells) rapidly improves glycemic control in obese patients with diabetes [45]. Thus, GIP antagonism by pharmacologic means or "metabolic surgery" may provide a new antidiabetic mechanism [46].

DPP-4 inhibitors

Inhibiting the enzyme DPP-4 (EC 3.4.14.5) prevents the rapid inactivation of the endogenous incretins GLP-1 and GIP, thereby raising their circulating concentrations and increasing nutrient-stimulated insulin release and other effects of these incretins (Table 60.5). DPP-4 is found "loose" in the circulation and tethered to cell membranes, especially endothelia in capillaries of the gastrointestinal tract, a location that facilitates the degradation of incretins. The protease activity of DPP-4 degrades a range of biologically active peptides in addition to incretins, including substance P, bradykinin, peptide YY, neuropeptide Y, pituitary adenylate cyclase-activating peptide, insulin-like growth factor I (IGF-I) and various interleukins and monocyte chemo-attractant proteins [33,47]. Despite affecting this wide range of peptides, to date, specific DPP-4 inhibitors have not shown significant adverse effects during substantial clinical use [32]. Also, DPP-4 is the lymphocyte cell surface protein CD26 required for the co-stimulation response to recall antigens, but its immunologic role does not appear to be interrupted by small molecule inhibitors of its peptidase activity.

Currently available DPP-4 inhibitors (gliptins) include sitagliptin, vildagliptin and saxagliptin (see Chapter 30). These agents improve glycemic control similarly to GLP-1 analogs but there are subtle differences that may partly reflect the concomitantly increased GIP, and the likelihood that raised endogenous incretin concentrations will not achieve the high concentrations of exogenously administered GLP-1 analogs. In consequence, DPP-4 inhibitors are unlikely to cause initial nausea through delayed gastric emptying, and they may exert a lesser satiety effect resulting in little change of body weight. Among the DPP-4 inhibitors in clinical development (Table 60.7), trials with alogliptin are well advanced (Figure 60.12). These agents have shown high specificity for DPP-4 inhibition, and preliminary information indicates that therapeutic concentrations cause almost complete inhibition of DPP-4 activity for about 12 hours, producing similar glucose-lowering efficacy to existing gliptins [32].

Phosphodiesterase inhibitors and other approaches

The β-cell expresses several phosphodiesterases (PDEs) that degrade cAMP and so reduce insulin release. Selective and

Table 60.7 DPP-4 inhibitors recently available and in clinical development.

Compound	Name	Company	Clinical phase	Specificity data (nmol)
MK-0431	Sitagliptin* (Januvia)	MSD	Marketed 2007	$IC_{50} = 18$ $K_i = 9$
LAF237	Vildagliptin (Galvus)	Novartis	Marketed 2008	$IC_{50} = 3.5$ $K_i = 17$
BMS-477118	Saxagliptin (Onglyza)	BMS/AZ	Marketed 2009	$IC_{50} = 26$ $K_i = <1$
SYR-322	Alogliptin	Takeda	3	?
R1438	An aminomethylpyridine	Roche	2	$K_i = 0.1$
P32/98	Isoleucine thiazolidide	Probiodrug	2	$K_i = 80$

AZ, AstraZeneca; BMS, Bristol Myers Squibb; MSD, Merck Sharp Dohme; ?, not reported.

Other compounds under consideration include PSN9301 (Prosidion), GR-8200 (Glenmark), PHX-1149 (Phenomix), SSR-162369 (Sanofi-Aventis), ALS 2-0426 (Alantos/Amgen), and NN-7201 (NovoNordisk).

* Launched in Mexico in 2006, and in USA, UK and several other European countries in 2007.
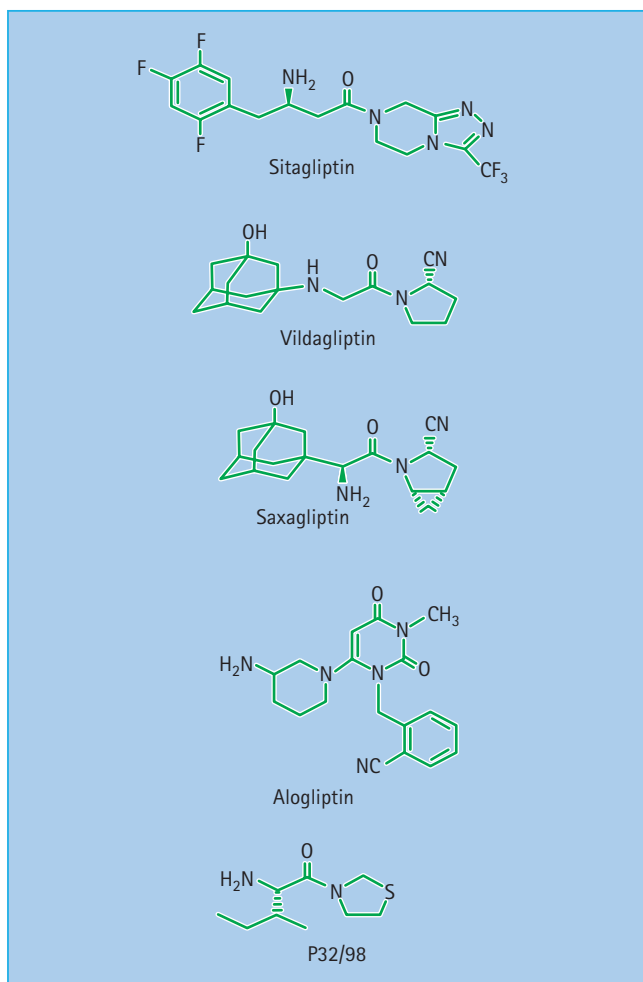
Figure 60.12 Structures of the DPP-4 inhibitors sitagliptin, vildagliptin, saxagliptin, alogliptin and P32/98.
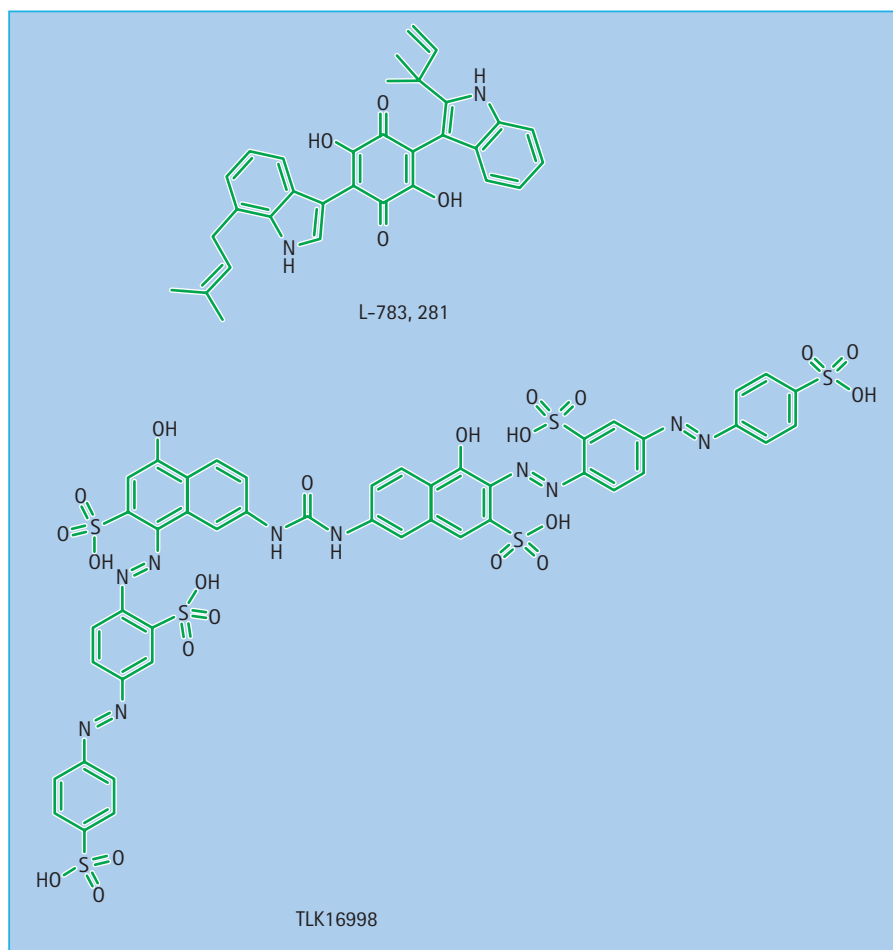
transient inhibition of these enzymes in β-cells, especially isoform PDE-3B, which exerts most influence on glucose-induced insulin secretion, could be a possible intervention [48], but the problem of specifically targeting the β-cells has yet to be overcome. Other theoretical approaches to potentiate insulin secretion (Figure 60.8), such as antagonism of α_2-adrenoceptors and activators of phospholipase C (PLC), have not been possible to target specifically at the β-cell.

Insulin-mimetic drugs

Insulin resistance (impaired insulin action) is a typical feature of T2DM, but it is highly heterogeneous, initially progressive (excepting some monogenic forms), and susceptible to many different genetic and environmental factors [49,50]. Most patients with T2DM probably incur multiple defects that impinge on insulin receptor function and/or various post-receptor signaling pathways, as well as independent disturbances in the activities of substrate transporters and metabolic enzymes consequent to glucotoxicity and lipotoxicity [51]. Indeed, insulin has important genomic effects that determine the expression levels of many cellular components that are directly and indirectly involved in metabolic homeostasis. Defects of insulin receptor structure are uncommon, and reductions in insulin receptor number are not usually rate limiting. Thus, the therapeutic challenge of insulin resistance appears to require interventions at diverse intracellular targets [52].

In theory, agents that address defects of insulin receptor signaling or early post-receptor lesions might be expected to produce a broader spectrum of benefits, but if the rate-limiting defects occur at more distal locations their therapeutic efficacy will be compromised. Potential target sites to obviate cellular defects of insulin action are shown in Figure 60.13.

Figure 60.13 Pathways of intracellular insulin signaling showing some of the potential sites for therapeutic intervention. Adapted from Bailey [52]. Akt, protein kinase B (PKB); AMPK, adenosine monophosphate-activated protein kinase; DAG, diacylglycerol; eNOS, endothelial nitric oxide synthase; FA, fatty acid; FOXO1, forkhead box protein O1A; GLUT-4, glucose transporter 4; Grb, growth factor receptor binding protein; GSK3, glycogen synthase kinase 3; IKKB, inhibitor κ-B kinase-β; IL-6, interleukin 6; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; PC-1/NNP1, glycoprotein-1; PDK, phosphoinositide-dependent protein kinase; PGC-1α, PPAR coactivator 1α; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol-3,4-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; PP2C, pyruvate dehydrogenase phosphatase (protein phosphatase 2C); PTEN, protein phosphatase PTEN; PTP-1B, protein tyrosine phosphatase-1B; Raf, a serine-threonine protein kinase; Ras, a guanosine triphosphatase; ROS, reactive oxygen species; RXR, retinoid X receptor; SHIP-2, src homology-2-inositol phosphatase; SOCS-3, suppressor of cytokine signaling-3; SOS, sons of sevenless; STAT, signal transducer and activator of transcription; TNF-α, tumour necrosis factor α; ↑, increase; →, stimulatory effect; ⊣, inhibitory effect.

Insulin receptor activation

Evidence that an orally active, non-peptide molecule can mimic the gluco-regulatory actions of insulin was obtained with L-783,281 (demethylasterriquinone; Figure 60.14), a metabolite from cultures of a *Pseudomassaria* fungus [53]. L-783,281 initiated phosphorylation and tyrosine kinase activity of the β-subunit of the human insulin receptor expressed in Chinese hamster ovary (CHO) cells [54]. This induced tyrosine phosphorylation and activation of insulin receptor substrate 1 (IRS1), increased activity of phosphatidylinositol 3-kinase (PI3K) and increased phosphorylation of Akt (protein kinase B). Studies with mutated subunits of the insulin receptor showed that L-783,281 interacted selectively with the β-subunit (without requiring insulin to bind

to the α-subunit), and its activity could not be attributed to inhibition of protein tyrosine phosphatases. Low (3–6) μmol/L concentrations of L-783,281 elicited about 50% of the maximum tyrosine kinase activity (TKA) generated by insulin in CHO cells, and initiated a range of insulin-like effects in normal tissues including increased glucose uptake by isolated rodent adipocytes and skeletal muscle [53]. Oral administration of L-783,281 (5–25 mg/kg/day) lowered blood glucose in insulin-resistant obese-diabetic *db/db* mice providing proof of therapeutic concept, although other features of this particular compound are not suited to clinical development.

Insulin-like growth factor I
IGF-I can weakly mimic the effects of insulin through low affinity binding to the insulin receptor [55]. Cross-talk between the IGF-I

Figure 60.14 Structures of compounds that improve insulin action by direct interaction with insulin receptors: L-783,281 and TLK16998.

receptor and early post-receptor components of the insulin-signaling cascades can ameliorate rare cases of severe insulin resistance caused by genetic defects of the insulin receptor. IGF-I can assist glycemic control in type 1 diabetes mellitus (T1DM) and T2DM, but such therapy is generally discounted by potential proliferative effects and other unwanted side effects of IGF-I. Circulating IGF-I is mostly bound to IGF binding proteins, especially IGFBP-3, and the side effects probably reflect an increase in unbound IGF-I after injection. To address this, mixtures of recombinant human IGF-I and recombinant human IGFBP-3 have been employed [56].

Insulin receptor potentiation

Insulin receptor signaling after initial activation by insulin binding at the α-subunit can be enhanced and/or prolonged by several different mechanisms (Figure 60.13). For example, a non-peptide molecule TLK16998 (Figure 60.14) that does not interact with the insulin receptor α-subunit increased insulin-induced phosphorylation of the insulin receptor β-subunit [54,57]. TLK16998 also potentiated β-subunit phosphorylation initiated

by L-783,281. In cultured mouse 3T3-L1 adipocytes, low μmol/L concentrations of TLK16998 increased insulin-induced phosphorylation of IRS-1 and PI3K, increased translocation of GLUT-4 glucose transporters into the plasma membrane and increased glucose uptake during submaximal stimulation by insulin. TLK16998 (30 mg/kg by intraperitoneal injection) also lowered glucose concentrations in insulin resistant obese-diabetic *db/db* mice [57].

C peptide

Insulin C-peptide, which is secreted from pancreatic β-cells along with insulin, appears to bind to G-protein coupled receptors in several insulin-sensitive tissues. In cultured L6 muscle cells, physiologic concentrations of C-peptide (0.3–3.0 nmol/L) increase glycogen synthesis during submaximal (but not maximal) stimulation with insulin, accompanied by increased insulin receptor tyrosine kinase activity and phosphorylation of IRS1 [58]. Activation of PI3K, mitogen activated protein kinase (MAPK) and glycogen synthase kinase 3 (GSK3) was also noted, suggesting that the ability of C-peptide to potentiate insulin receptor signaling might modestly be exploitable as a possible approach to improve insulin action in C-peptide deficient states.

Protein tyrosine phosphatase 1B

Several protein tyrosine phosphatases (PTPs), most notably PTP1B, dephosphorylate the insulin receptor β-subunit, terminating insulin-induced receptor TKA [59]. These phosphatases also dephosphorylate and deactivate IRS1 and IRS2. The therapeutic potential of inhibiting PTP1B has been demonstrated by PTP-1B KO mice which are highly sensitive to insulin [60]. These mice also show an increased metabolic rate and resistance to diet-induced obesity. Antisense oligonucleotides against PTP1B increased insulin sensitivity in *ob/ob* mice, associated with increased phosphorylation of the insulin receptor, IRS1/2 and GSK3, and increased activity of PI3K and Akt [61]. Selective inhibitors of PTP-1B, including certain benzonaphthofurans, thiophenes and acylsulfonamido compounds, improved glycemic control in insulin-resistant diabetic animals and have been considered as templates for potential new therapies [62–64]. PTP-1B inhibitors could also assist weight control by increasing the satiety effect of leptin, because hypothalamic PTP1B normally reduces leptin receptor signaling [65]. Inhibition of PTP1B might also improve endothelial function by improving insulin-induced endothelial nitric oxide synthase (eNOS) production [66]. Vanadium salts inhibit protein tyrosine phosphatases including PTP1B, and have been shown to enhance the actions of insulin and possibly leptin.

Other insulin receptor potentiators

Various substances that increase the number of insulin receptors and/or appear to improve insulin receptor function have been mooted as possible therapeutic leads but have not given rise to new therapeutic entities. These include N-terminal fragments of human growth hormone (GH) and mosapride, a benzamide derivative that activates serotonin 5HT-4 receptors and promotes gastrointestinal motility [52,67].

Insulin receptor and early post-receptor potentiation

The divergent pathways of post-receptor insulin signaling contain many potential rate-limiting steps for insulin action. Most of these pathways are not specific to insulin and impact activities as disparate as cell differentiation and apoptosis. They also include a controlling influence exerted through the feedback of more distal signaling components on more proximal steps [50,51].

Protein kinase C

The IRS–PI3K route of insulin signaling (Figure 60.13) increases the formation of phosphatidylinositol-3,4,5 trisphosphate (PIP3) and phosphoinositide-dependent kinases PDK1/2. These signaling intermediates activate some isoforms of protein kinase C (PKC), which exert a negative feedback on insulin receptor and probably early post-receptor phosphorylation steps [68,69]. Inhibition of specific isoforms of PKC provides a potential means to improve insulin action, although appropriately selective inhib-
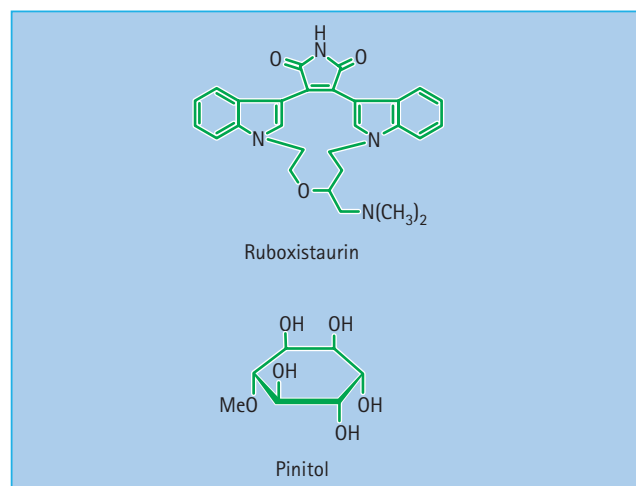
Figure 60.15 Structure of the PKC-β inhibitor LY333531 (ruboxistaurin), the inositol derivative 3-methoxy analog of D-*chiro*-inositol (pinitol).

itors have proved difficult. The PKC-β inhibitor LY333531 (ruboxistaurin; Figure 60.15) is in clinical trial as a potential treatment for diabetic retinal and glomerular microvascular disease [70]. Excess fatty acids, diacylglycerol and chronic hyperglycemia also appear to reduce early insulin signaling via activation of PKC isoforms.

Other signaling feedbacks

Serine phosphorylation of the insulin receptor β-subunit and IRS proteins, which inhibits their signaling activity, is brought about by several serine kinases such as inhibitor kappa-B kinase-β (IKKβ) and c-Jun N-terminal kinase (JNK; Figure 60.13). These kinases contribute to the insulin resistance produced by the cytokine tumor necrosis factor α (TNF-α) [71,72]. The potential opportunity to prevent these routes of insulin resistance is indicated by the ability of salicylates, which inhibit IKKβ, and a cell-permeable inhibitor of JNK to relieve insulin resistance in part [73,74].

The insulin signaling intermediate Akt appears to participate in a negative feedback effect on the signaling pathway. Akt activates the mammalian target of rapamycin (mTOR) which promotes phosphorylation of serine residues on IRS proteins [75]. The membrane ectoenzyme glycoprotein-1 (PC-1/NNP1) binds to the insulin receptor and prevents the conformational changes required for receptor autophosphorylation [76]. These interactions present potential sites for therapeutic intervention.

Potentiation of phosphatidylinositol-3 kinase

PI3K promotes phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) to the 3,4,5-trisphosphate (PIP3). This step is often reduced in insulin-resistant states [77], but direct targeting of PI3K is difficult because the regulatory (p85) subunit suppresses the catalytic (p110) subunit [78]; however, increasing the availability of precursor inositol substrates and prevention of product breakdown might offer therapeutic mechanisms.
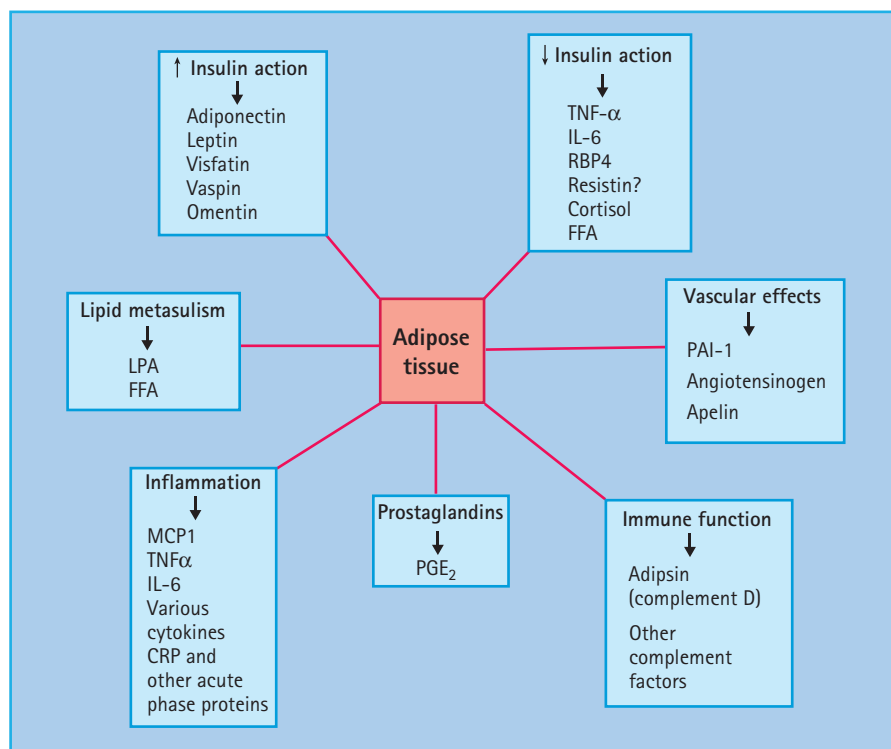
Figure 60.16 Examples of the autocrine, paracrine and endocrine products of adipose tissue that influence insulin action, metabolic control and vascular, inflammatory and immune processes that could impinge on diabetic control. CRP, C-reactive protein; FFA, free fatty acid; IL-6, interleukin 6; LPL, lipoprotein lipase; MCP1, monocyte chemoattractant protein 1; PAI-1, plasminogen activator inhibitor 1; PGE₂, prostaglandin E2; RBP4, retinol-binding protein 4: TNF-α, tumor necrosis factor α.

Inositol derivatives

Inositol derivatives such as D-*chiro*-inositol (INS-1) and the 3-methoxy analog of D-*chiro*-inositol (pinitol; Figure 60.15) improve muscle glucose uptake and reduce hyperglycemia in diabetic animal models and patients with T2DM [79,80]. D-*chiro*-inositol-galactosamine (INS-2) also increased insulin action in diabetic rats, possibly involving direct activation of pyruvate dehydrogenase phosphatase (protein phosphatase 2C [PP2C]) [81].

PTEN and other inositol phosphatases

Preventing the dephosphorylation of PIP3 has been identified as a possible approach to improve insulin action (Figure 60.13). The phosphatase PTEN, which dephosphorylates PIP3, can be inhibited by an antisense oligonucleotide: when administered (ip, once weekly) to *db/db* and *ob/ob* mice, the oligonucleotide increased signaling downstream of PIP3, improved insulin sensitivity and improved glycemic control [82]. Also, an adipose tissue specific PTEN knockout mouse was lean and insulin sensitive with high energy expenditure and increased biogenesis of adipocyte mitochondria [83]; however, substantial disruption of PTEN carries a risk of tumor formation [84]. PIP3 is 5′-dephosphorylated to form phosphatidylinositol 3,4-bisphosphate by the SH2-inositol phosphatases (SHIP-1 and SHIP-2). Partial disruption of the SHIP-2 gene in mice improves insulin sensitivity [85], suggesting another potential target to enhance the effectiveness of PI3K.

Adipokines

Adipose tissue is a source of many autocrine, paracrine and endocrine factors that affect insulin action (Figure 60.16), and obesity is a well-recognized risk factor for insulin resistance and T2DM [86,87]. Thus, some of these factors have been assessed as potential therapeutic targets.

Proinflammatory cytokines

Proinflammatory cytokines produced by adipose tissue, notably TNF-α and interleukin 6 (IL-6), contribute to insulin resistance. They activate or induce JNK, IKKβ and the suppressor of cytokine signaling-3 (SOCS-3), each of which can disrupt TKA of the β-subunit of the insulin receptor (Figure 60.13); however, preliminary evaluation of a TNF-α antibody in patients with T2DM showed little effect on insulin sensitivity, and the risk of susceptibility to infection has called this approach into question [52].

Adipocyte hormones
Leptin

Leptin is an adipocyte hormone that exerts centrally mediated satiety and thermogenic effects, as well as direct effects on cellular nutrient metabolism. Administration of large doses of leptin can produce weight loss and improve insulin action, but the development of leptin resistance and leptin antibodies has compromised long-term efficacy [88]. Leptin analogs and non-peptide receptor agonists remain under consideration.
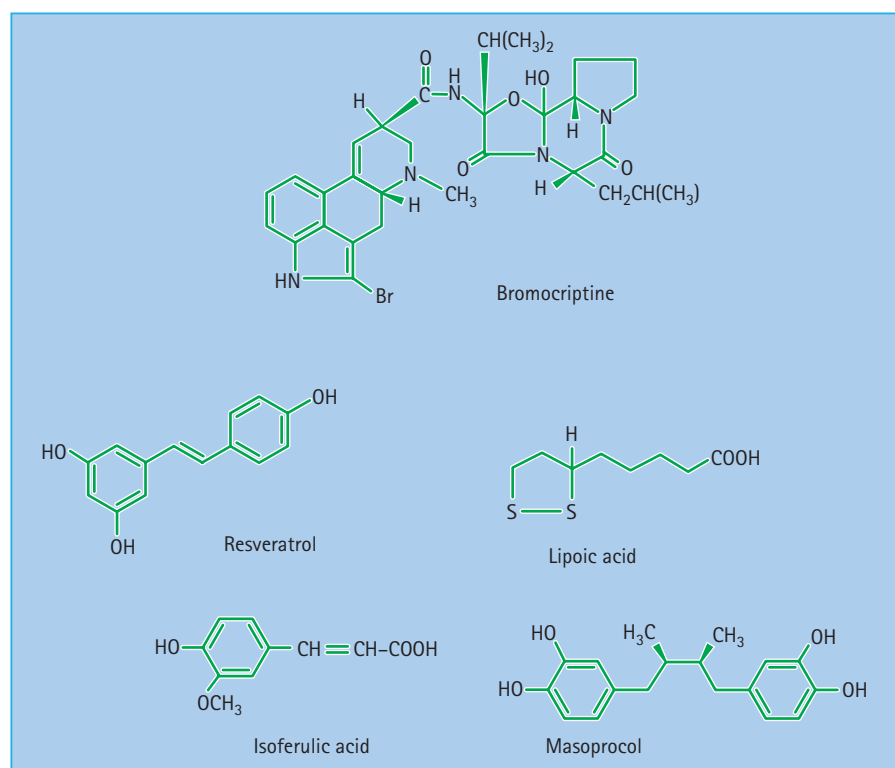
Figure 60.17 Structures of some compounds that potentiate insulin action: bromocriptine, lipoic acid, isoferulic acid, masoprocol and resveratrol.

Resistin

Resistin (FIZZ3), an adipocyte hormone, reduces insulin sensitivity in obese rodents, and immuno-neutralization of resistin improved insulin sensitivity; however, resistin is expressed at low levels by human adipocytes, and may be involved in inflammatory processes, limiting its potential as a therapeutic target [89].

Adiponectin

Adiponectin (Acrp30), produced exclusively and in large amounts by adipocytes, improves insulin sensitivity with increased insulin receptor tyrosine phosphorylation and activation of adenosine monophosphate-activated protein kinase (AMPK) [90]. It also has anti-inflammatory activity and improves vascular reactivity. Adiponectin concentrations become reduced as adipose mass increases, and therapeutic approaches to raise adiponectin levels, develop analogs and non-peptide receptor agonists are under investigation. Part of the insulin-sensitizing effect of thiazolidinediones may reflect increased adiponectin production.

Visfatin

Visfatin (PBEF), mainly from visceral adipocytes, was initially reported to activate insulin receptors. Subsequently, however, no correlation was observed between plasma visfatin concentrations and insulin sensitivity in human subjects [91].

Retinol-binding protein

Retinol-binding protein 4 (RBP4) is one of several retinoid-binding proteins produced by adipocytes and liver that transports retinoids in plasma. Increased RBP4 has been noted in insulin resistant states, while RBP4 gene knockout increases insulin sensitivity [92], suggesting that a reduction of RBP4 might be considered to reduce insulin resistance.

Vaspin

Vaspin (visceral adipose tissue-derived serpin) is an adipocyte serine protease inhibitor which improved insulin sensitivity and glucose homeostasis in obese insulin-resistant rodents, and might therefore offer a therapeutic lead [93].

Omentin

Omentin, a peptide from visceral adipose tissue, increased insulin-stimulated glucose uptake by adipocytes [94], and might indicate a potential therapeutic approach.

Other potentiators of insulin action

Bromocriptine

The dopamine D2 receptor agonist bromocriptine (Figure 60.17), used in the treatment of Parkinson disease, galactorrhea and prolactinomas, has long been known to improve insulin sensitivity and glycemic control in T2DM [95,96]. Bromocriptine as monotherapy or an adjunct to other antidiabetic agents for up to 1 year has reduced HbA$_{1c}$ by 0.5–1.2% (5–13 mmol/mol), lowered triglyceride and non-esterified fatty acid concentrations, reduced some cardiovascular events, not caused serious hypoglycemia,

and facilitated weight loss. However, side effects including nausea, hypotension and psychiatric symptoms should be appreciated. Bromocriptine has recently received marketing authorization to treat diabetes in the USA.

Lipoic acid, isoferulic acid and angiotensin-converting enzyme inhibitors

The antioxidant α-lipoic acid (Figure 60.17), used in some countries to treat diabetic neuropathy, increases insulin sensitivity and improves glycemic control, probably brought about in part by its action as a co-factor for dehydrogenases involved in glycolysis and the Krebs cycle. Additionally, α-lipoic acid increases insulin receptor TKA and IRS1 tyrosine phosphorylation, with increased signaling via PI3K and increased GLUT-4 translocation into the plasma membrane [11]. Isoferulic acid increases expression of GLUT-4 and decreases gluconeogenesis by reducing phospho-enolpyruvate carboxykinase (PEPCK) [11].

Modest improvements of insulin sensitivity have been recorded during treatment with angiotensin-converting enzyme (ACE) inhibitors, possibly because of improved hemodynamics resulting from increased bradykinin. ACE is one of the circulating enzymes that normally degrades bradykinin. ACE inhibitors may also help to counter the effects of insulin resistance by reducing inflammation and increasing vascular reactivity [97].

Plant-derived compounds

Plant extracts and herbal preparations remain commonplace as treatments for diabetes, especially in low and middle income countries, and plant-derived compounds have provided templates for the synthesis of potential antidiabetic agents [98]. For example, the herbal use of *Bougainvillaea spectabilis* led to studies on the antidiabetic action of pinitol [79], and the traditional use creosote bush (*Larrea tridentate*) prompted studies on the glucose-lowering effect of masoprocol [99].

Many current pharmaceuticals have their origins in traditional herbal medicines, and phytochemical approaches continue to attract interest. Recent attention has been given to resveratrol (Figure 60.17), a phytophenol (3,4,5 trihydroxystilbene) found in many plants, especially red grape skins [100]. It appears to offer cardioprotective properties, improving the lipid profile, increasing eNOS-dependent vasorelaxation and exerting antioxidant and anti-inflammatory effects. Resveratrol and related phytophenols may improve insulin action and prevent diet-induced obesity by increasing mitochondrial biogenesis through activation of the sirtuin SIRT1 and by increased activity of the PPAR co-activator PGC-1α [101].

Anti-obesity agents

Appetite control, reduced and delayed nutrient absorption and weight loss are well appreciated to reduce insulin resistance and hyperglycemia in the overweight and obese T2DM. Several centrally acting appetite-suppressing and satiety-inducing anti-obesity agents also exert peripheral effects that improve some actions of insulin and assist glycemic control in overweight patients.

Sibutramine

Sibutramine, which is a serotonin-noradrenaline reuptake inhibitor that induces satiety, acts in part through the primary amine metabolite M2. This metabolite increases glucose uptake by muscle tissue independently of weight loss after treatment *in vivo* [102]. Sibutramine was withdrawn in Europe in January 2010.

Rimonabant

Rimonabant (SR141716), an endocannabinoid receptor-1 (CB1) antagonist that suppresses appetite, was recently withdrawn because of neural side effects soon after introduction in some countries as an anti-obesity agent. In overweight and obese diabetic individuals rimonabant induced a greater reduction in HbA_{1c} than expected for the extent of weight loss, possibly explained in part by increased adiponectin production [103].

β₃-Adrenoceptor agonists

β_3-Adrenoceptor agonists act on β_3-adrenoceptors, expressed mainly by brown and white adipose tissue, to assist weight loss by stimulating lipolysis and thermogenesis. Several selective agonists were developed for rodents, but minor differences in the structure of the human form of the receptor rendered these agents relatively ineffective in humans. Various β_3-adrenoceptor agonists have been shown to stimulate insulin release, improve insulin-mediated glucose disposal and improve glycemic control in obese diabetic rodents, but adequate efficacy and a very high level of selectivity have yet to be demonstrated in humans.

Peroxisome proliferator-activated receptor γ agonists

Current thiazolidinediones (pioglitazone and rosiglitazone) exert their "insulin-sensitizing" effects largely by stimulating the peroxisome proliferator-activated receptor γ (PPARγ; Figure 60.18). Stimulation of this nuclear receptor increases transcription of a selection of insulin-sensitive genes and other genes (Table 60.8; see Chapter 29): these collectively increase adipogenesis, enhance insulin sensitivity, improve glycemic control and exert anti-inflammatory and vascular effects [104,105]. Additional thiazolidinediones that stimulate PPARγ continue in development (e.g. rivoglitazone), and non-thazolidinedione PPARγ agonists have been reported (Figure 60.19) [105]. The latter might alter conformation of the PPARγ binding epitope or other regions of the receptor such that different coactivators are recruited, affecting the selection of genes transcribed and the profile of biologic effects. Thus, non-thazolidinedione PPARγ agonists that partially modulate PPARγ, such as halofenate/metaglidasen and FK614, do not activate exactly the same set of genes as a full PPARγ agonist [106]. Through selective modulation of PPARγ binding it should be possible to retain desired therapeutic effects and reduce undesirable side effects [105]. A similar outcome might be achieved by stimulating genes for selected co-activators of PPARγ such as PGC-1α [107].
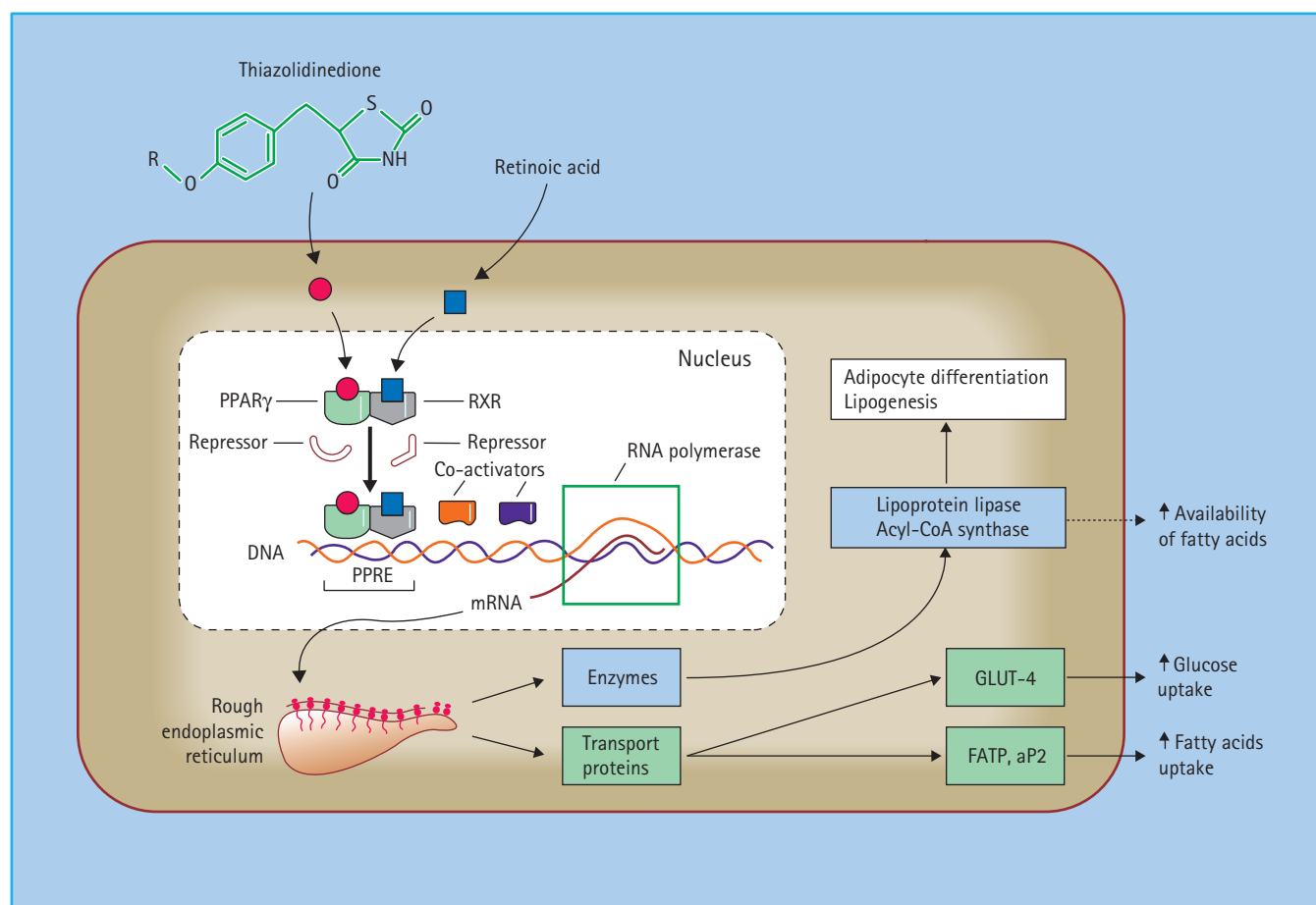
Figure 60.18 Mechanism of action of a thiazolidinedione in an adipocyte. Thiazolidinediones are peroxisome proliferator-activated receptor γ (PPARγ) agonists. They bind to the nuclear PPARγ receptor, which forms a heterodimer with the retinoid X receptor (RXR). Ligand binding releases repressors, attracts co-activators and exposes the active site of the receptors which binds with a specific DNA nucleotide sequence (AGGTCAXAGGTCA) termed the peroxisome proliferator response element (PPRE). RNA polymerase is recruited and transcription of mRNA takes place for genes that carry the PPRE sequence in a promoter region. The mRNA is translated into the enzymes and transporters that bring about the biologic effects of thiazolidinediones in the cell. These include the fatty acid transporter protein (FATP), adipocyte fatty acid binding protein (aP2), acyl-CoA synthase, lipoprotein lipase and glucose transporter 4 (GLUT 4). Stimulation of PPARγ also activates genes encoding proteins with anti-inflammatory and vascular effects.

Other peroxisome proliferator-activated receptor agonists

To take advantage of the blood lipid-lowering and anti-inflammatory effects of low affinity binding to PPARα various thiazolidinediones and non-thiazolidinedione molecules have been described with binding affinities for both PPARγ and PPARα – so-called dual PPARα/γ agonists (glitazars) [105]. The two glitazars most advanced in development (muraglitazar, tesaglitazar) were discontinued because of side effects [108].

Genetic overexpression or selective stimulation of PPARδ (e.g. with GW501516 and L-165041) improves insulin sensitivity, increases fatty acid oxidation, raises thermogenesis and prevents weight gain [109]. Thus, a selective PPARδ agonist or an agent with desired levels of selectivity for PPARα, γ and δ (termed a panPPAR agonist or SPPARM – selective PPAR modulator) could offer therapeutic advantages [105]. Various selective modulators of RXR have been reported to reduce hyperglycemia in animal models, but these tend to have diverse effects including hypertriglyceridemia and interference with thyroid function. Because certain RXR agonists can reduce appetite, increase production of mitochondrial uncoupling proteins and decrease weight gain, combinations of these agents with PPARγ (PPARγ-RXR agonists) have received preclinical consideration [110].

Vitamins and minerals

Vitamins

The pathogenic effect of excess reactive oxygen species has become an accepted part of glucotoxicity, lipotoxicity and insulin resistance, and it is appreciated that anti-oxidant defenses are often depleted in diabetic states [111]. Whether supplementation of the anti-oxidant vitamins C (ascorbic acid), E (α-tocopherol) and β-carotene can measurably benefit insulin sensitivity and reduce cardiovascular risk remains in contention [112]. Adequate concentrations of vitamin D and vitamin D receptor function

Table 60.8 Stimulation of peroxisome proliferator-activated receptor γ increases transcription of a selection of insulin-sensitive and other genes affecting adipogenesis, insulin sensitivity, nutrient metabolism, anti-inflammatory and vascular reactions (so-called "pleitropic" effect).

Lipoprotein lipase (LPL)
Fatty acid transporter protein (FATP/CD36)
Adipocyte fatty acid binding protein (aP2)
Acyl-CoA synthetase (ACS)
Malic enzyme
Glycerol kinase (adipocytes ?)
PEPCK (adipocytes), ↑ perilipin
↑ GLUT-4 (derepression), ↑ GLUT-2
↓ Resistin, ↓ RBP4
↑ Adiponectin (↑ leptin, ↑ IRS2 ?)
↓ TNF-α
↓ CRP and some proinflammatory cytokines, ↓ NFκB, ↓ IFG-γ (T cells)
↓ PAI-1, ↓ MMP-9
↑ UCP1 (?)

↑ increase; ↓ decrease; ? unconfirmed.
CRP, C reactive protein; GLUT-4, glucose transporter 4; IFG-γ, interferon gamma; IRS, insulin receptor substrate; MMP-9, matrix metalloproteinase-9; NKκB, nuclear factor κB; PAI-1, plasminogen activator inhibitor-1; PEPCK, phosphoenolpyruvate carboxy kinase; RBP, retinal binding protein; TNF-α, tumor necrosis factor α; UCP1, uncoupling protein 1.

Figure 60.19 Structures of the thiazolidinediones pioglitazone, rosiglitazone, rivoglitazone and the non-thiazolidinedione partial PPARγ agonist metaglidasen.

appear to be necessary for normal insulin production and secretion, and may be required for normal insulin action. Diabetic patients are often deficient in circulating vitamin D_3 (cholecalciferol), and preliminary data suggest that vitamin D supplementation in deficient individuals might benefit glycemic control [113]. Thiamine (vitamin B_1) is a co-factor for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, and facilitates glucose metabolism, while large amounts of biotin (vitamin H) have been claimed to improve glycemic control and insulin sensitivity in T2DM [52,114]. When interpreting vitamin supplement studies it is pertinent to note that correcting a deficiency is more likely to offer benefit than adding excess in individuals with normal values.

Minerals

Reduced and deficiency levels of several minerals, particularly magnesium, chromium and zinc, are not uncommon amongst older patients with diabetes, and replacement supplements may improve glycemic control in such cases [115]. Supplementation of magnesium (necessary for phosphate transfer and kinase reactions) can enhance insulin action in hypomagnesemic patients [116], and trivalent chromium supplements often improve glycemic control without increasing insulin concentrations in chromium-deficient patients [117]. Insulin-like antidiabetic effects have been reported for zinc, lithium, selenium, molybdenum, tungsten, mercury and cadmium, but the therapeutic index is generally narrow and toxicity risks have limited clinical investigations [115]. Zinc (essential for insulin hexamer formation)

appears to assist both the secretion and action of insulin, while molybdenum and tungsten salts act predominantly to decrease hepatic glucose output [13,59,115]. Improved insulin sensitivity with lithium may be offset by decreased insulin secretion resulting in variable effects on glycemic control in T2DM [115].

Vanadium salts have attracted considerable interest because modest vanadium supplements have improved glycemic control and reduced insulin requirements in diabetic animals and in patients with T1DM and T2DM [115,118]. Improved insulin sensitivity has been attributed at least in part to inhibition of protein tyrosine phosphatases, especially PTP-1B, and suppression of glucose 6-phosphatase. Additional effects to reduce appetite and reduce the rate of intestinal glucose absorption may also contribute to the antidiabetic effect of vanadium salts. To address issues of poor absorption and toxicity, low-dose peroxovanadiums, pervanadates and organic vanadium complexes have received attention [119], and an intermittent treatment schedule might be considered because the antidiabetic effects of vanadium salts can persist for weeks after treatment is stopped.

Counter-regulatory hormones

The major counter-regulatory hormones (glucagon, epinephrine, glucocorticoids and GH) raise blood glucose concentrations by increasing hepatic glycogenolysis and gluconeogenesis (see Chapter 13). As hepatic glucose production is inappropriately raised in diabetes, agents that interfere with the secretion or

action of counter-regulatory hormones could potentially be therapeutically useful; however, those with highly potent or prolonged actions are undesirable, as they might impair the life-sustaining protection of the liver to produce glucose in response to severe hypoglycemia. Indeed, patients in advanced stages of T2DM often show delayed or deficient counter-regulatory responses to hypoglycemia.
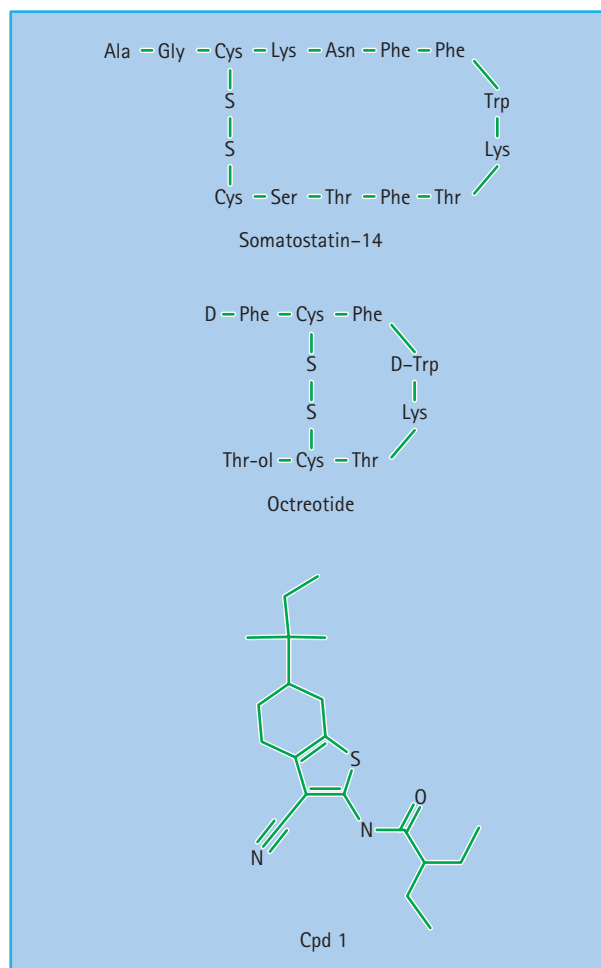
Glucagon antagonists

The concept of reducing hyperglycemia by suppressing glucagon action is illustrated by the use of glucagon antibodies [120], and various peptide antagonists of the glucagon receptor have been described, mostly based on deletion of His1 and replacement of Asp9 with Glu [121]. There are also hybrid peptides that show glucagon receptor antagonism and GLP-1 receptor agonism [36]. Many small molecule glucagon receptor antagonists have been reported. An isopropylfluorobiphenyl (Bay 27–9955) that competitively blocked glucagon binding to its receptor has not proceeded in development [122], but other potent inhibitors such as Cpd1, NNC 25-0926 and MB09975N are being investigated [123,124]. An alternative approach has been to uncouple the glucagon receptor from activation of adenylate cyclase (e.g. with skyrin, a fungal bisanthroquinone) [125]. Inhibitors of glucagon secretion (e.g. MB39890A and somatostatin analogs) have been developed, but have not been sufficiently selective [121]. Thus, the somatostatin analog octreotide (Figure 60.20), suppresses glucagon secretion and delays intestinal glucose absorption as well as preventing GH secretion, but it also inhibits insulin secretion, rendering it unhelpful in T2DM, but potentially useful for glycemic control with insulin in patients with T1DM [121].

Glucocorticoid antagonists

Raised glucocorticoid concentrations can precipitate and aggravate truncal obesity, insulin resistance and hyperglycemia, while maneuvers to reduce glucocorticoid action can prevent and reverse these effects. To avoid lowering overall glucocorticoid production, and to minimize disturbances to the hypothalamic-pituitary-adrenal system, tissue specific inhibition of glucocorticoid action has been investigated. Some inhibitors of glucocorticoid receptor binding show modest degrees of hepatic selectivity [126], but more specific targeting of the liver has been achieved

when glucocorticoid receptor inhibitors are conjugated to bile salts. This retains the inhibitor mostly within the entero-hepatic circulation, reducing hyperglycemia and improving hepatic insulin sensitivity in animal models [127].

Another approach to the cellular targeting of glucocorticoid suppression takes advantage of the normal cellular conversion of less active cortisone to more active cortisol (Figure 60.21). This

Figure 60.20 Structure of native somatostatin-14, its synthetic octapeptide analog, octreotide, and a non-peptide glucagon receptor antagonist Cpd 1.
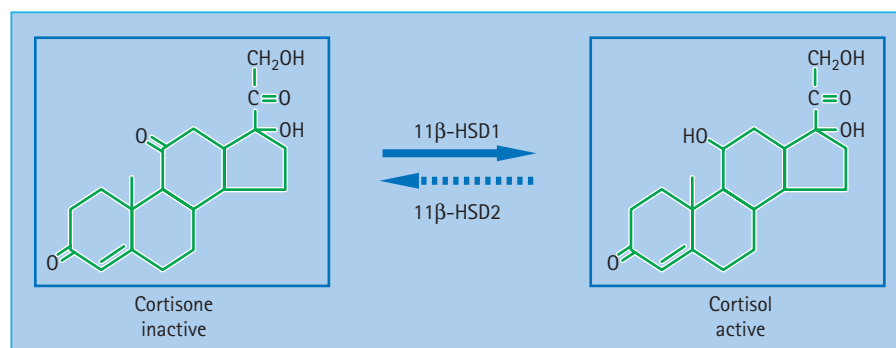
Figure 60.21 Cellular conversion of less active cortisone to more active cortisol through the reductase activity of the enzyme 11β-hydroxysteroid dehydrogenase-1 (11β-HSD1).

reaction is mediated through the predominantly reductase activity of the enzyme 11β-hydroxysteroid dehydrogenase-1 (11β-HSD1) which is strongly expressed in liver and adipose tissue [128]. Selective inhibitors of 11β-HSD1 have been shown to improve insulin sensitivity, glycemic control and plasma lipids in obese-diabetic rodents [129]. Excess glucocorticoids are often associated with some degree of islet hypertrophy, and because 11β-HSD1 is expressed by pancreatic β-cells, possible effects of 11β-HSD1 inhibitors on β-cells require detailed evaluation.

There is a substantial literature documenting that administration of the adrenal androgen dehydroepiandrosterone (DHEA) and an etiocholanolone metabolite (Figure 60.22) can decrease adiposity, improve glycemic control and reduce insulin resistance in obese diabetic animal models [130]. These agents may act in part to elevate tyrosine phosphorylation of IRS proteins and increase translocation of the glucose transporters GLUT-1 and GLUT-4 to the cell membrane [131].

Direct modifiers of glucose metabolism

Many substances directly stimulate glucose uptake and utilization or suppress glucose production. Their metabolic impact is often difficult to control, and their wider effects have precluded therapeutic application for T2DM. Included here are agents that create some insulin-like effects such as deoxyfrenolicin, vitamin K_5, spermine, diamides and peroxides (Figure 60.23) [115]. Okadaic acid and phorbol esters initially imitate certain effects of insulin,

but then prevent tissues from responding further to insulin. Dichloroacetate and its esters increase glucose oxidation by stimulating pyruvate dehydrogenase, and suppress hepatic glucose production by inhibiting pyruvate carboxylase, but may adversely affect neural function through the production of glyoxylate and oxylate [115].
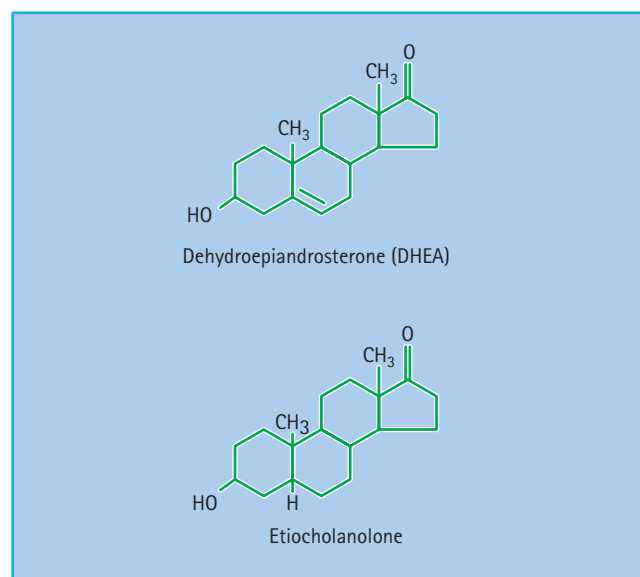
Figure 60.22 Structures of dehydroepiandrosterone (DHEA) and etiocholanolone.
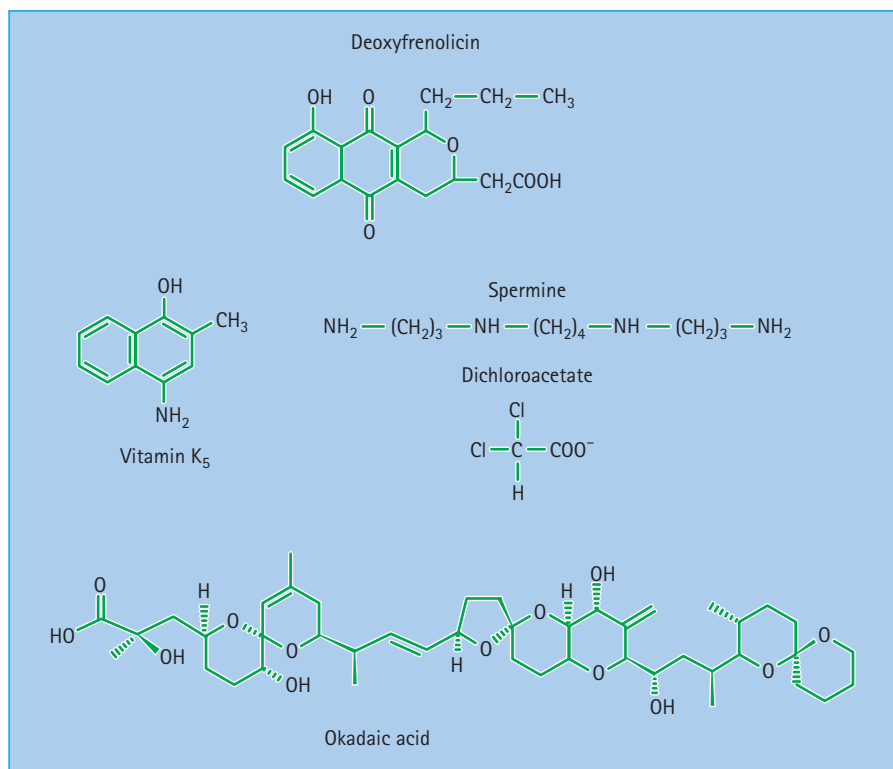
Figure 60.23 Structures of deoxyfrenolicin, vitamin K_5, spermine, okadaic acid and dichloroacetate.
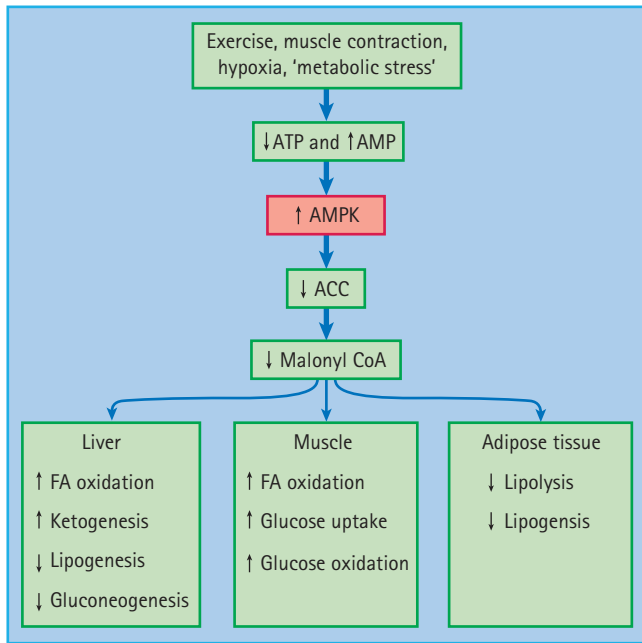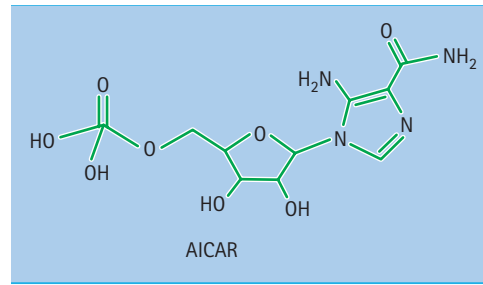
Figure 60.24 Metabolic effects of the energy-regulating enzyme adenosine monophosphate-activated protein kinase (AMPK). ACC, acetyl CoA carboxylase; FA, fatty acid.

Figure 60.25 Structure of AICAR, 5-aminoimidazole-4-carboxamide-1-B-D-ribofuranoside.

Figure 60.26 Glycogen synthase kinase-3 (GSK3) regulation of glycogen synthase (GS). G-6-P glucose 6-phosphate; G-1-P, glucose 1-phosphate; UDPG, uridine diphosphate glucose.

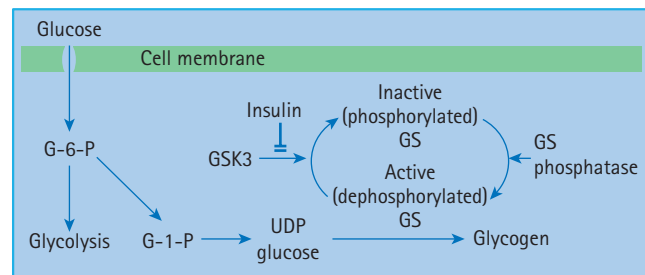## Adenosine monophosphate-activated protein kinase activators

Adenosine monophosphate-activated protein kinase (AMPK) is a key energy-regulating enzyme that is activated when the energy status of cells declines and/or energy demand escalates [132]. When ATP and creatine phosphate become depleted, for example during exercise, AMP concentrations become raised. Rising AMP concentrations activate AMPK, promoting energy production by increasing the uptake and oxidation of glucose and fatty acids, leading to the restoration of ATP (Figure 60.24). AMPK may also reduce expression of PEPCK (key gluconeogenic enzyme), and depending upon associated regulatory factors, suppress cell proliferation [133]. Metformin, adiponectin and thiaziolidinediones have been shown to activate AMPK amongst other actions that contribute to their glucose-lowering effects [134]. Various analogs of AMP such as AICAR (5-aminoimidazole-4-carboxamide-1-B-D-ribofuranoside) have been shown to activate AMPK and improve glycemic control in insulin-resistant diabetic animals (Figure 60.25) [135, 136], and other activators (e.g. DRL-16536) are being developed.

Glycogen synthase kinase inhibitors

Glycogen synthesis is impaired in T2DM, caused in part by reduced activity of glycogen synthase. Glycogen synthase is normally deactivated through phosphorylation by a serine/threonine kinase – GSK3. Glycogen synthesis proceeds when GSK3 is inactivated by insulin via the PI3K-Akt pathway, but expression and activity of GSK3 are increased in insulin-resistant diabetic states.

Small molecule inhibitors of GSK3 have been shown to lower blood glucose and increase insulin-stimulated glucose uptake and glycogenesis by skeletal muscle of insulin-resistant diabetic animals [137,138]. There are two isoforms and several splice variants of GSK3 that have various roles in the signaling of pathways as diverse as cytoskeletal regulation, protein degradation and apoptosis, making it difficult to target glycogen metabolism specifically (Figure 60.26).

Glucokinase activators

Because liver cells, like islet β-cells, can take up glucose approximately in proportion to the circulating glucose concentration, the rate of hepatic glucose disposal is determined mostly through the rate of glucose phosphorylation by GK. Allosteric activators of GK and molecules that prevent GK from binding with its inhibitory regulatory protein have been shown to increase hepatic glucose metabolism and reduce blood glucose concentrations in normal and diabetic rodents [24–29,139]. Clinical studies will establish whether the combined effects of increased hepatic glucose disposal and increased insulin secretion can be titrated to avoid overt hypoglycemia.

Inhibitors of hepatic glucose production

Any therapeutic approach that suppresses gluconeogenesis and/ or glycogenolysis should be partial, readily reversible and should not seriously compromise vital actions of counter-regulatory

hormones and neural signals in times of rapid and severe hypoglycemia.

Glycogen phosphorylase inhibitors

Glycogen phosphorylase inhibitors have received considerable preclinical attention as possible agents to reduce hyperglycemia by preventing the breakdown of glycogen. The agents studied include inhibitors of the active site, the AMP site and other sites on the enzyme [139–141]. An example is the dihydropyridine derivative BAY R3401, a prodrug that is metabolized to an active agent that binds at the AMP site causing allosteric inhibition and dephosphorylation of active glycogen phosphorylase *a* into the inactive *b* form [141]. While most glycogen phosphorylase inhibitors have been shown to improve glycemic control in animal models of T2DM, there have been few reports of clinical studies, and the limited evidence available suggests only modest or unsustained efficacy.

### Glucose 6-phosphatase inhibitors

Glucose 6-phosphatase inhibitors have been considered an attractive approach to address hyperglycemia because they can interrupt the last step in glucose output from both glycogenolysis and gluconeogenesis. Inhibitors of the catalytic subunit and the translocator protein for glucose 6-phosphatase have been shown to reduce hepatic glucose output and lower blood glucose; however, this approach carries a high risk of hypoglycemia. Also, cellular accumulation of glucose 6-phosphate causes excess deposition of glycogen and induction of lipogenic genes which predispose to fatty liver [139].

Fructose 1,6-bisphosphatase (F16BPase) inhibitors

Fructose 1,6-bisphosphatase (F16BPase) inhibitors prevent the dephosphorylation of fructose 1,6-bisphosphate to fructose 6-phosphate as the penultimate step in gluconeogenesis before glucose 6-phosphate. Various F16BPase inhibitors have been described, generally causing only a partial reduction of hepatic glucose output because of increased compensatory glycogenolysis, which helps to guard against hypoglycemia [139]. Although excessive use of a F16BPase inhibitor might cause accumulation of lactate and triglyceride, appropriate titration of such agents in early clinical trials (e.g. MB07803) has given encouraging results.

Modifiers of lipid metabolism

The commonly observed dyslipidemia of T2DM, particularly raised very low density lipoprotein (VLDL), triglyceride and non-esterified fatty acid (NEFA) levels, contribute to insulin resistance and hyperglycemia in several ways [51,142]. These include direct effects of fatty acids and their metabolites to impair cellular insulin signaling pathways, and alterations in fuel selection mediated through the Randle (glucose–fatty acid) cycle. Agents that modify lipid metabolism could therefore benefit glycemic control in T2DM. For example, thiazolidinedione PPARγ agonists act in
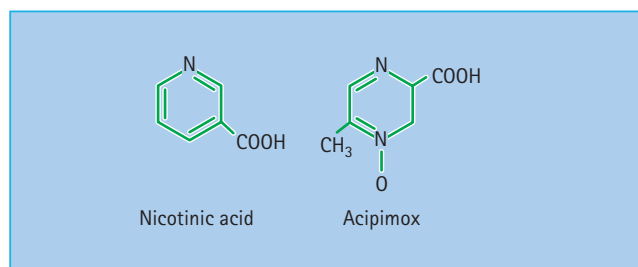

Figure 60.27 Structures of the antilipolytic agents, nicotinic acid and acipimox.

part through an alteration in fatty acid metabolism, and lipid-lowering fibrates, which act via PPARα agonism, can modestly assist glycemic control in some patients. Other agents that lower plasma triglycerides, such as the fenfluramine analog benfluorex and the long-chain dicarboxylic acid, Medica 16, can lower blood glucose concentrations [121]. Reducing circulating NEFA concentrations with conventional antilipolytic agents such as nicotinic acid (niacin) and its analog acipimox (Figure 60.27) can acutely improve glucose tolerance in T2DM, but the effect is not consistent or sustained [121].

Inhibition of fatty acid oxidation interrupts the supply of energy for hepatic gluconeogenesis and enhances the use of glucose as a source of energy in skeletal muscle. Most inhibitors of fatty acid oxidation act by inhibiting carnitine palmitoyltransferase 1 (CPT-1), the rate-limiting enzyme for transfer of long-chain fatty acyl-CoA into the mitochondria. Irreversible inhibitors of CPT-1 such as the oxirane carboxylates (e.g. etomoxir) and the alkylglycidates (e.g. methyl palmoxirate) have effectively lowered glucose concentrations in diabetic animals, mainly through their antigluconeogenic action, but clinical studies have indicated vulnerability to hypoglycemia. Similar concerns have emerged with agents that inhibit intramitochondrial enzymes of fatty acid oxidation [121].

Sodium-glucose co-transporter 2 inhibitors

Glucose is filtered through the renal glomeruli and (almost) all that has been filtered is reabsorbed in the proximal tubules. Reabsorption is mediated mostly via the sodium-glucose co-transporter 2 (SGLT2) system (Figure 60.28). SGLT2 is expressed only in the first segment of the tubules and enables low affinity secondary active glucose transport into the tubular epithelial cells [143]. Thus, specific and appropriately titrated inhibition of these transporters provides an opportunity to reduce hyperglycemia by elimination of excess glucose in the urine. The potential of this approach has long been appreciated because non-specific inhibitors of sodium-glucose co-transporters such as phlorizin (Figure 60.29) from apple tree bark have been shown to reduce hyperglycemia in animals [144]. Non-specific inhibitors can also inhibit SGLT1 which is responsible for intestinal glucose absorption.
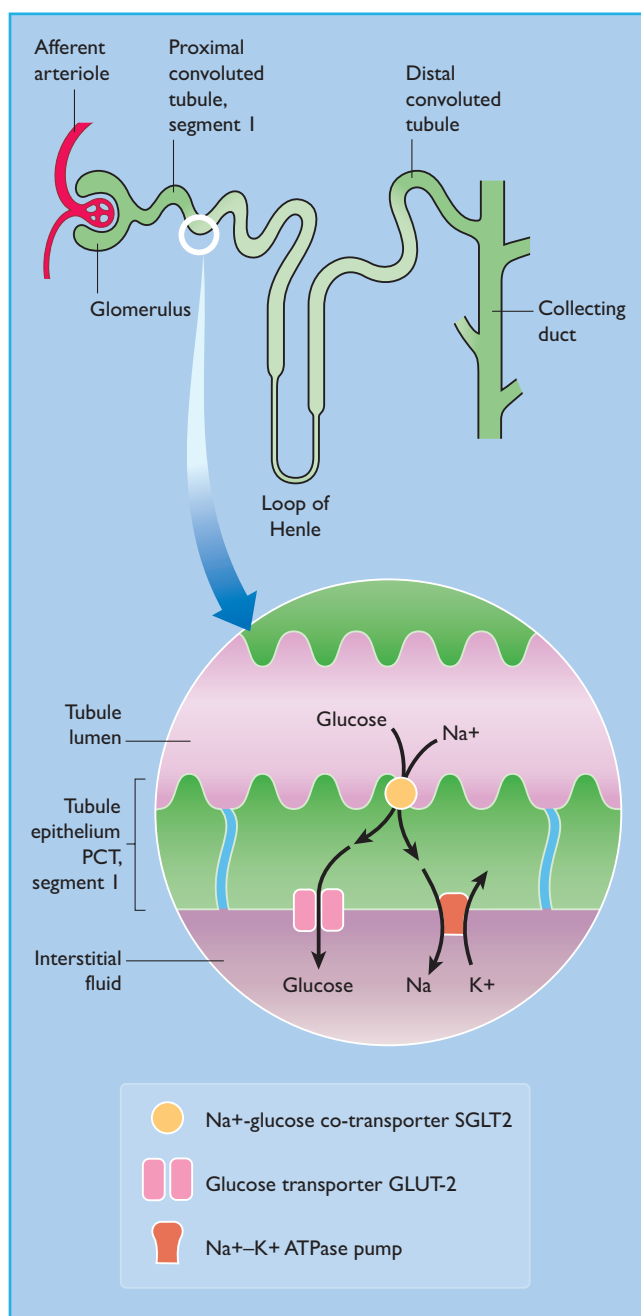
Figure 60.28 After glucose is filtered at the glomerulus into the proximal convoluted tubule (PCT) it is reabsorbed mostly from the first segment of the PCT via the low affinity secondary active sodium glucose co-transporter 2 (SGLT2).

More recently, selective inhibitors of SGLT2 (termed "flozins") have been developed, and these have reduced hyperglycemia in diabetic animals and proceeded into clinical trials [145]. Preliminary evidence suggests that dosages that partially prevent glucose reabsorption to allow elimination of up to about 100 g/day glucose can be used to facilitate glycemic control in patients with T2DM. In principle, reversible SGLT2 inhibitors could be
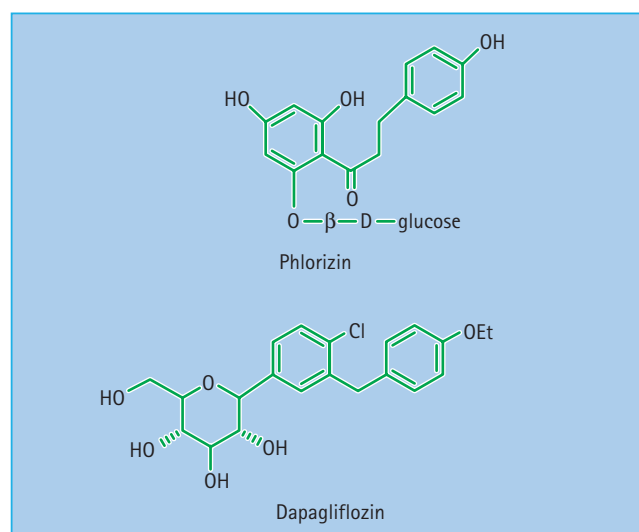
Figure 60.29 Structure of phlorizin (non-specific SGLT1/2 inhibitor) and the selective SGLT2 inhibitor dapagliflozin.

used as adjunctive therapy with any other antidiabetic treatment, and the elimination of glucose should assist weight loss. By lowering glucotoxicity it is anticipated that insulin sensitivity may be improved. Because SGLT2 inhibition does not stimulate insulin secretion or interrupt the counter-regulatory system, serious hypoglycemia should be avoidable if the extent of SGLT2 inhibition is titrated appropriately. Possible adverse effects of osmotic diuresis during SGLT2 inhibition include risk of dehydration and electrolyte imbalance, as well as infection in the urinary tract and urino-genital region.

Sirtuins

Sirtuins comprise a group of seven enzymes that are nicotinamide-adenine-dinucleotide (NAD)-dependent histone deacetylases and/or ADP-ribosyltransferases. They affect gene transcription through chromatin silencing, and mediate metabolic responses that have the potential to extend lifespan similar to chronic caloric restriction [146]. Sirtuin SIRT1 is widely expressed in mammalian tissues including liver, muscle and fat, and appears to promote mitochondrial biogenesis and activity in some tissues, increasing thermogenesis and reducing susceptibility to weight gain, diabetes and cardiovascular disease. AMPK enhances SIRT1 activity, resulting in increased activity of downstream transcription factors such as PGC-1α and the forkhead protein FOXO1 which increase energy production [147]. SIRT1 in pancreatic β-cells may also facilitate insulin secretion. Several small molecule activators of SIRT1 have been described that are structurally unrelated to polyphenols (such as resveratrol). The activators increased mitochondrial capacity, enhanced insulin sensitivity and reduced plasma glucose in animal models [148].

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