

## REVIEW

# Pancreatic regulation of glucose homeostasis

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In order to ensure normal body function, the human body is dependent on a tight control of its blood glucose levels. This is accomplished by a highly sophisticated network of various hormones and neuropeptides released mainly from the brain, pancreas, liver, intestine as well as adipose and muscle tissue. Within this network, the pancreas represents a key player by secreting the blood sugar-lowering hormone insulin and its opponent glucagon. However, disturbances in the interplay of the hormones and peptides involved may lead to metabolic disorders such as type 2 diabetes mellitus (T2DM) whose prevalence, comorbidities and medical costs take on a dramatic scale. Therefore, it is of utmost importance to uncover and understand the mechanisms underlying the various interactions to improve existing anti-diabetic therapies and drugs on the one hand and to develop new therapeutic approaches on the other. This review summarizes the interplay of the pancreas with various other organs and tissues that maintain glucose homeostasis. Furthermore, anti-diabetic drugs and their impact on signaling pathways underlying the network will be discussed.

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## THE PANCREAS IS AN EXOCRINE AND ENDOCRINE ORGAN

The pancreas has key roles in the regulation of macronutrient digestion and hence metabolism/energy homeostasis by releasing various digestive enzymes and pancreatic hormones. It is located behind the stomach within the left upper abdominal cavity and is partitioned into head, body and tail. The majority of this secretory organ consists of acinar—or exocrine—cells that secrete the pancreatic juice containing digestive enzymes, such as amylase, pancreatic lipase and trypsinogen, into the ducts, that is, the main pancreatic and the accessory pancreatic duct. In contrast, pancreatic hormones are released in an endocrine manner, that is, direct secretion into the blood stream. The endocrine cells are clustered together, thereby forming the so-called islets of Langerhans, which are small, island-like structures within the exocrine pancreatic tissue that account for only 1–2% of the entire organ (Figure 1).<sup>1</sup> There are five different cell types releasing various hormones from the endocrine system: glucagon-producing  $\alpha$ -cells,<sup>2</sup> which represent 15–20% of the total islet cells; amylin-, C-peptide- and insulin-producing  $\beta$ -cells,<sup>2</sup> which account for 65–80% of the total cells; pancreatic polypeptide (PP)-producing  $\gamma$ -cells,<sup>3</sup> which comprise 3–5% of the total islet cells; somatostatin-producing  $\delta$ -cells,<sup>2</sup> which constitute 3–10% of the total cells; and ghrelin-producing  $\epsilon$ -cells,<sup>4</sup> which comprise <1% of the

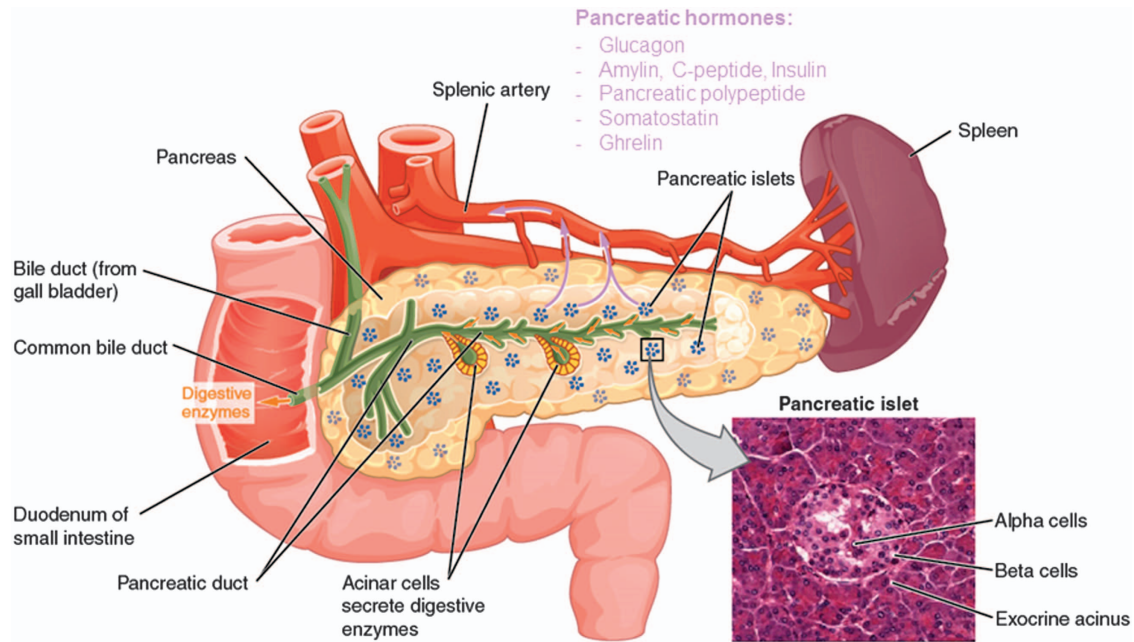
total islet cells. Each of the hormones has distinct functions. Glucagon increases blood glucose levels, whereas insulin decreases them.<sup>5</sup> Somatostatin inhibits both, glucagon and insulin release,<sup>6</sup> whereas PP regulates the exocrine and endocrine secretion activity of the pancreas.<sup>3,7</sup> Altogether, these hormones regulate glucose homeostasis in vertebrates, as described in more detail below. Although the islets have a similar cellular composition among different species, that is, human, rat and mouse, their cytoarchitecture differs greatly. Although islets in rodents are primarily composed of  $\beta$ -cells located in the center with other cell types in the periphery, human islets exhibit interconnected  $\alpha$ - and  $\beta$ -cells.<sup>2,8</sup>

Through its various hormones, particularly glucagon and insulin, the pancreas maintains blood glucose levels within a very narrow range of 4–6 mM. This preservation is accomplished by the opposing and balanced actions of glucagon and insulin, referred to as glucose homeostasis. During sleep or in between meals, when blood glucose levels are low, glucagon is released from  $\alpha$ -cells to promote hepatic glycogenolysis. In addition, glucagon drives hepatic and renal gluconeogenesis to increase endogenous blood glucose levels<sup>9</sup> during prolonged fasting. In contrast, insulin secretion from  $\beta$ -cells is stimulated by elevated exogenous glucose levels, such as those occurring after a meal.<sup>10</sup> After docking to its receptor on muscle and adipose tissue, insulin enables the insulin-dependent uptake of

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**Figure 1** Anatomical organization of the pancreas. The exocrine function of the pancreas is mediated by acinar cells that secrete digestive enzymes into the upper small intestine via the pancreatic duct. Its endocrine function involves the secretion of various hormones from different cell types within the pancreatic islets of Langerhans. The micrograph shows the pancreatic islets. LM×760 (Micrograph provided by the Regents of University of Michigan Medical School © 2012). Adapted from Human Anatomy and Physiology, an OpenStax College resource.<sup>404</sup>

glucose into these tissues and hence lowers blood glucose levels by removing the exogenous glucose from the blood stream (Figure 2).<sup>11–13</sup> Furthermore, insulin promotes glycogenesis,<sup>14–26</sup> lipogenesis<sup>27,28</sup> and the incorporation of amino acids into proteins;<sup>29</sup> thus, it is an anabolic hormone, in contrast to the catabolic activity of glucagon.

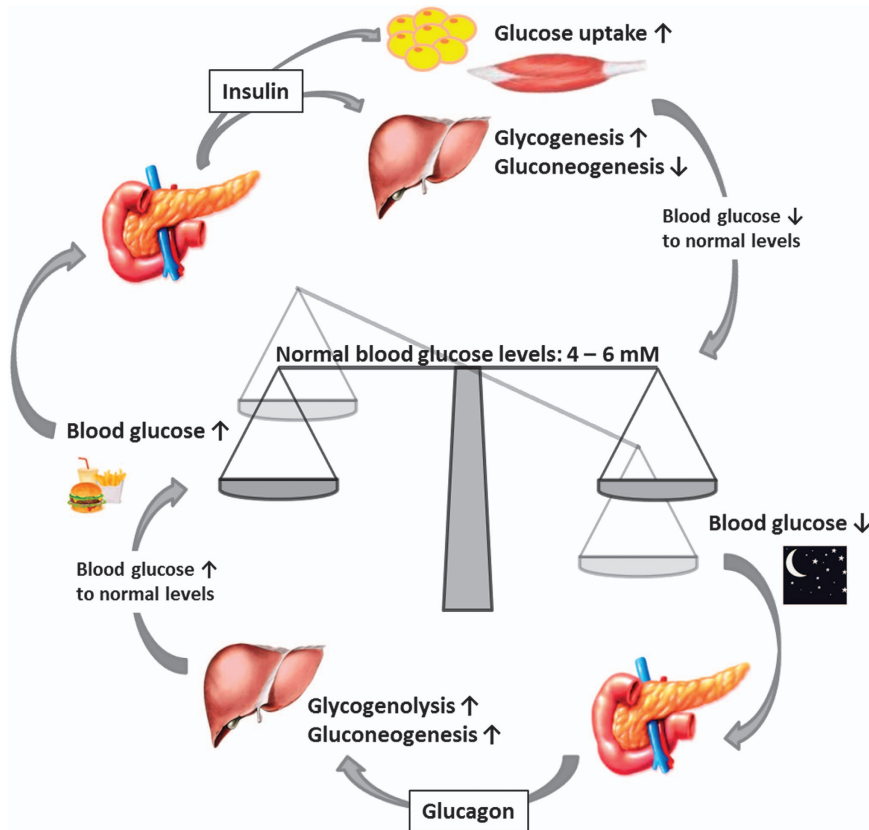
### THE INSULIN SECRETION SIGNALING PATHWAY

Endocrine cells secrete their respective hormones in response to external signals, such as nutrient intake or stress, via humoral, neural or hormonal signaling pathways. The underlying molecular process that translates the stimulus into the actual hormone release is called stimulus-secretion coupling which is known as the stimulus-dependent exocytosis of a particular substance, such as glucose-stimulated  $\beta$ -cell insulin release.<sup>30</sup>

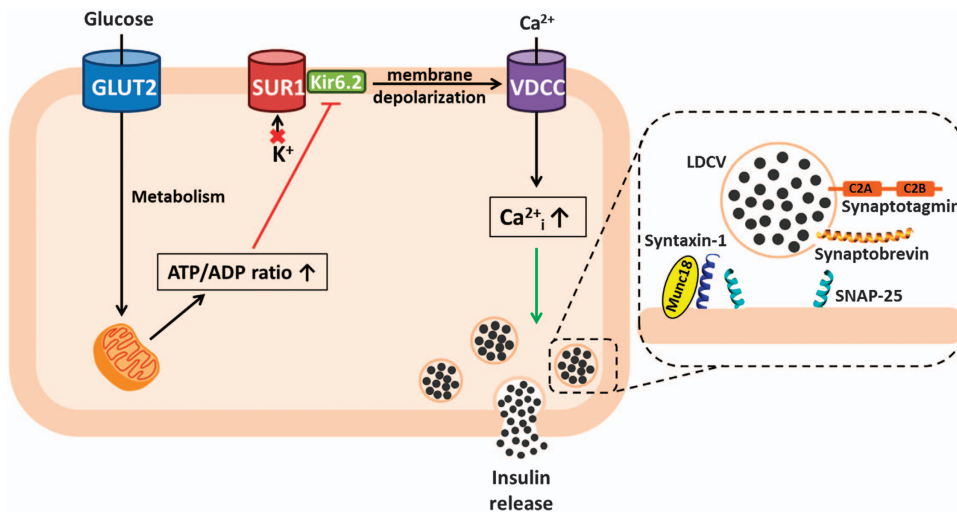
In  $\beta$ -cells, the main stimulus for insulin release are elevated blood glucose levels following a meal.<sup>10</sup> The circulating blood glucose is taken up by the facilitative glucose transporter GLUT2 (SLC2A2), which is located on the surface of the  $\beta$ -cells. Once inside the cell, glucose undergoes glycolysis, thereby generating adenosine triphosphate (ATP), resulting in an increased ATP/ADP ratio. This altered ratio then leads to the closure of ATP-sensitive  $K^+$ -channels ( $K_{ATP}$ -channels). Under non-stimulated conditions, these channels are open to ensure the maintenance of the resting potential by transporting positively charged  $K^+$ -ions down their concentration gradient out of the cell. Upon closure, the subsequent decrease in the magnitude of the outwardly directed  $K^+$ -current elicits the depolarization of the membrane, followed by the opening of

voltage-dependent  $Ca^{2+}$ -channels (VDCCs). The increase in intracellular calcium concentrations eventually triggers the fusion of insulin-containing granules with the membrane and the subsequent release of their content.<sup>31</sup> The whole secretory process is biphasic with the first phase peaking around 5 minutes after the glucose stimulus with the majority of insulin being released during this first phase. In the second, somewhat slower, phase, the remaining insulin is secreted.<sup>32–34</sup> Insulin is stored in large dense-core vesicles that are recruited to the proximity of the plasma membrane following stimulation such that insulin is readily available.<sup>35,36</sup> The key molecules that mediate the fusion of the insulin-containing large dense-core vesicles are the synaptosomal-associated protein of 25 kDa (SNAP-25), syntaxin-1 and synaptobrevin 2 (or vesicle-associated membrane protein VAMP2), all of which belong to the superfamily of the soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor proteins (SNAREs). Together with the Sec1/Munc18-like (SM) proteins they form the so-called SNARE complex.<sup>37</sup> To initiate fusion, synaptobrevin 2, a *vesicle* (*v*-)SNARE that is integrated into the vesicle's membrane, fuses with the *target* (*t*-)SNAREs syntaxin-1 and SNAP-25, which are located in the target cell membrane,<sup>38,39</sup> with mammalian uncoordinated (*munc*)-18 playing a key regulatory role (Figure 3).<sup>40,41</sup>

To date, numerous SNARE isoforms, including syntaxin-1, -3 and -4, SNAP-25 and -23, as well as syntaptobrevins 2 and 3 (VAMP2 and 3), have been shown to be involved in glucose-stimulated insulin secretion,<sup>42–46</sup> whereas VAMP8, a non-essential SNARE protein for glucose-stimulated insulin secretion, has a role in the regulation of the glucagon-like



**Figure 2** Maintenance of blood glucose levels by glucagon and insulin. When blood glucose levels are low, the pancreas secretes glucagon, which increases endogenous blood glucose levels through glycogenolysis. After a meal, when exogenous blood glucose levels are high, insulin is released to trigger glucose uptake into insulin-dependent muscle and adipose tissues as well as to promote glycogenesis.



**Figure 3** Glucose-stimulated insulin release from a pancreatic  $\beta$ -cell. Exogenous glucose is taken up by GLUT2 and undergoes glycolysis inside the cell. Elevated adenosine triphosphate (ATP) levels alter the ATP/ADP ratio, which in turn leads to the closure of ATP-sensitive  $K^+$ -channels. The subsequent membrane depolarization opens voltage-dependent  $Ca^{2+}$ -channels in response to increasing intracellular calcium levels, which eventually trigger insulin secretion following vesicle fusion with the membrane.

peptide-1-potentiated insulin secretion.<sup>47</sup> In addition to SNARE and SM proteins, a calcium sensor is required for the initiation of membrane fusion. Synaptotagmins, which are highly expressed in neurons and endocrine cells, were shown to

participate in  $Ca^{2+}$ -dependent exocytosis processes. To date, 17 synaptotagmins (Syts 1–17) have been identified and only eight of them, namely Syt-1, -2, -3, -5, -6, -7, -9 and -10, are able to bind calcium.<sup>48</sup> Following  $Ca^{2+}$ -binding,

synaptotagmins form a complex with the SNAREs to facilitate and trigger the vesicle-membrane fusion process. Among the synaptotagmin family, Syt-3, -5, -7, -8 and -9 are implicated in insulin exocytosis.<sup>49–52</sup>

## EXTERNAL FACTORS AFFECTING PANCREATIC HORMONE SECRETION

### Metabolism–cAMP coupling

The glucose-triggered stimulus-secretion coupling is an established paradigm of insulin secretion from  $\beta$ -cells and includes a great variety of modulators that trigger, potentiate or inhibit glucose-stimulated insulin secretion, primarily through G-protein-coupled receptors (GPCRs). The most traditional external factor that initiates insulin secretion is glucose. In addition to its trigger function, glucose also induces pathways that amplify insulin secretion through metabolism–cAMP (cyclic adenosine monophosphate) coupling or the incretin hormones glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic peptide (GIP).<sup>31</sup> Metabolism–cAMP coupling refers to the signaling cascade that occurs after the conversion of ATP, which is generated during intracellular glucose metabolism, into cAMP by adenylate cyclase (AC),<sup>53</sup> which in turn activates protein kinase A (PKA)<sup>54</sup> and cAMP-regulated guanine nucleotide exchange factors, also referred to as exchange protein directly activated by cAMP (Epac) 2.<sup>55,56</sup> Although Epac2 activation amplifies insulin secretion by mobilizing calcium from internal stores to increase  $\text{Ca}^{2+}$  levels<sup>57,58</sup> and by controlling the granule density in proximity to the plasma membrane,<sup>59</sup> activated PKA exerts its effects by modulating  $\text{K}_{\text{ATP}}$ -channel<sup>60,61</sup> and calcium channel<sup>62,63</sup> activity through phosphorylation, thereby enhancing the number of highly  $\text{Ca}^{2+}$ -sensitive insulin-containing granules<sup>64</sup> and the probability of releasing secretory vesicles from the readily releasable pool,<sup>65</sup> respectively.

### The incretins GLP-1 and GIP

The gut-derived hormones GLP-1 and GIP, which are secreted from enteroendocrine L-cells<sup>66</sup> and K-cells,<sup>67</sup> respectively, upon glucose,<sup>66,68</sup> fructose,<sup>69</sup> amino acid<sup>70</sup> and free fatty acid (FFA)<sup>71,72</sup> ingestion, also potentiate insulin release through the so-called incretin effect. This effect describes the observation that orally, but not intravenously, administered glucose enhances insulin secretion by triggering GLP-1 and GIP secretion;<sup>73–75</sup> the resulting potentiation of insulin secretion may account for up to 50% of the total release. The underlying mechanism includes GLP-1 and GIP binding to their GPCRs (GLP-1R and GIPR), both of which are expressed in pancreatic  $\beta$ -cells.<sup>76</sup> The binding induces a conformational change in the receptors' structure, followed by the exchange of guanosine diphosphate for guanosine triphosphate and the subsequent dissociation of the  $\text{G}_s\alpha$ -subunit from the receptors. This subunit, in turn, activates adenylate cyclase to convert ATP into cAMP, thereby stimulating the cAMP signaling pathway described above.<sup>77–82</sup> Furthermore, GLP-1 increases intracellular calcium concentrations by mobilizing  $\text{Ca}^{2+}$  from ryanodine-sensitive stores<sup>83,84</sup> or, similar to GIP, by acting

on voltage-dependent  $\text{Ca}^{2+}$ -channels,<sup>85</sup> thereby potentiating insulin release.<sup>85–87</sup> Recent studies have also shown that GLP-1R agonists, such as exendin-4<sup>88</sup>, induce the PKA-mediated phosphorylation of Snapin or Synaptotagmin-7, which in turn enhances GSIS by Snapin interacting with SNAP-25<sup>89</sup> or by directly enhancing glucose- and  $\text{Ca}^{2+}$ -triggered insulin release.<sup>90</sup>

### Free Fatty Acids

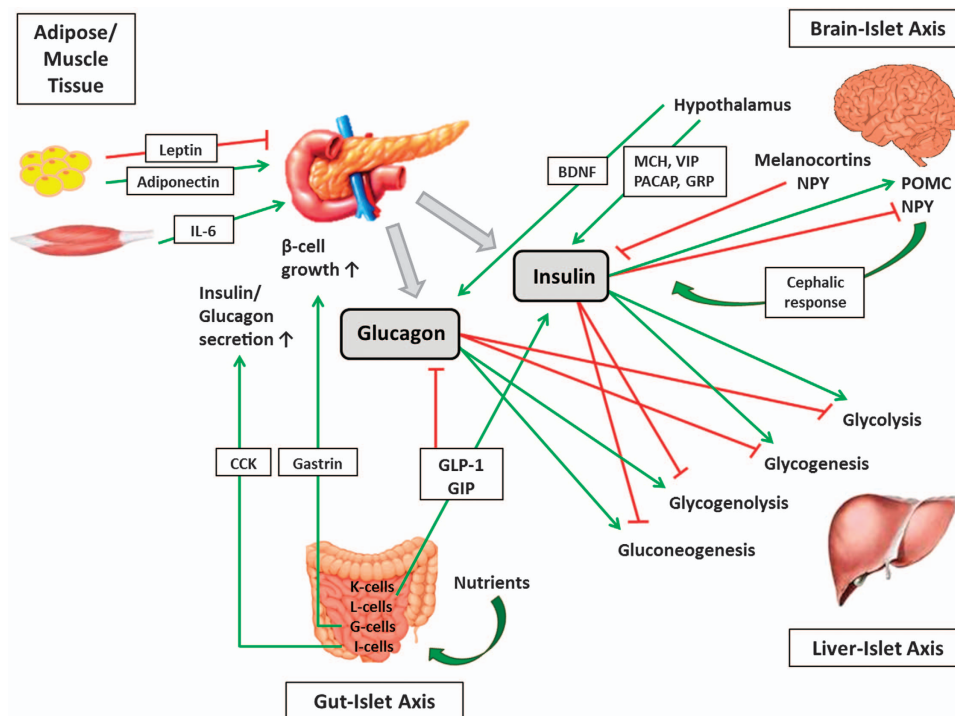
FFAs not only stimulate incretin secretion but are also known to modulate insulin release through fatty acid metabolism. Although long-chain FFAs augment insulin secretion, short-chain FFAs inhibit it. The binding and subsequent interaction of long-chain FFAs with the G-protein-coupled free fatty acid receptor (FFAR) 1 in the pancreatic  $\beta$ -cells leads to the activation of phospholipase C. PLC then hydrolyzes phosphatidylinositol-4,5-bisphosphate ( $\text{PIP}_2$ ) to diacylglycerol and inositol-1,4,5-triphosphate ( $\text{IP}_3$ ), with the latter docking on a calcium channel in the endoplasmic reticulum. The subsequent release of  $\text{Ca}^{2+}$  into the cytosol increases the intracellular  $\text{Ca}^{2+}$  concentration, which eventually triggers insulin secretion.<sup>91–94</sup> In contrast, short-chain FFAs inhibit glucose-stimulated insulin secretion due to decreased glucose oxidation and the subsequently decreased ATP/ADP ratio.<sup>95</sup> Another inhibitor of insulin release is stress, specifically norepinephrine (norepinephrine) produced in response to stress.<sup>96</sup> Norepinephrine binds to its  $\alpha_2$ -adrenergic receptors, which are linked to GPCRs, resulting in the inhibition of AC as well as in hyperpolarization. This prevents an increase in the cytosolic  $\text{Ca}^{2+}$  concentration and, subsequently, insulin secretion.<sup>97,98</sup>

## INTERPLAY BETWEEN THE PANCREATIC ISLETS AND OTHER ORGANS

### The brain–islet axis

Just as insulin exerts its effects on other organs and tissues, other organs interact with the pancreas to modulate insulin secretion (Figure 4). One of these interacting organs is the brain, which comprises the mutual brain–islet axis that interacts with the pancreas and vice versa. The pancreas is highly innervated with both, parasympathetic<sup>99,100</sup> and sympathetic<sup>100,101</sup> nerve fibers from the autonomic nervous system. At the same time, insulin receptors are widely distributed within the brain, including the hypothalamus, cerebral cortex, cerebellum<sup>102</sup> and hippocampal formation<sup>103</sup> in humans, as well as the olfactory and limbic areas,<sup>104,105</sup> hypothalamus<sup>106</sup>—particularly the periventricular nucleus<sup>107</sup> and the arcuate nucleus<sup>108,109</sup>—hippocampus and the choroid plexus<sup>105</sup> in rat brains. Lesions in various brain regions were shown to affect pancreatic hormone secretion. The destruction of the ventromedial hypothalamus results not only in insulin hypersecretion<sup>110–112</sup> due to loss of the ventromedial hypothalamus-mediated inhibitory impact on pancreatic  $\beta$ -cells<sup>113</sup> but also in higher glucagon levels.<sup>111,112</sup> Glucagon secretion may also be modulated by the hypothalamic brain-derived neurotrophic factor<sup>114</sup> via efferent nerves,<sup>115</sup> whereas the melanocortin system directly reduces basal insulin levels by





**Figure 4** The interplay of the pancreas with the brain, liver, gut as well as adipose and muscle tissue. The pancreas interacts with the brain, liver, gut and adipose and muscle tissue in a highly sophisticated network via various hormones, neurotransmitters and cytokines. BDNF, brain-derived neurotrophic factor; CCK, cholecystokinin; GIP, glucose-dependent insulintropic peptide; GLP-1, glucagon-like peptide 1; GRP, gastrin-releasing peptide; IL-6, Interleukin 6; MCH, melanin concentrating hormone; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating polypeptide; POMC, pro-opiomelanocortin; VIP, vasoactive intestinal peptide.

stimulating sympathetic nerve fibers via  $\alpha$ -adrenoceptors.<sup>116</sup> Acting via  $\alpha$ -adrenoceptors,<sup>117</sup> norepinephrine also inhibits insulin secretion,<sup>96</sup> which is an important aspect of the fight-or-flight response. The neurotransmitter Neuropeptide Y (NPY), which is mainly expressed in the sympathetic nerve fibers of the autonomic nervous system, also blunts insulin release,<sup>118,119</sup> and the loss of NPY's inhibitory action results in elevated basal and glucose-stimulated insulin secretion as well as in increased islet mass.<sup>120</sup> NPY binding to its GPCR  $Y_1$  causes the activated  $G_i\alpha$ -subunit to block adenylate cyclase activation, which in turn inhibits the cAMP pathway.<sup>121</sup> Furthermore, the NPY-mediated inhibition was shown to be  $G_{\beta\gamma}$ - and  $Ca^{2+}$ -independent.<sup>122</sup> In addition to the well-known insulin stimulator acetylcholine, which exerts its effects via  $M_3$  muscarinic receptors,<sup>123</sup> melanin concentrating hormone, vasoactive intestinal peptide (VIP), its close relative pituitary adenylate cyclase-activating polypeptide (PACAP) and gastrin-releasing peptide also promote insulin and, in the case of VIP<sup>124</sup> and PACAP,<sup>125</sup> glucagon release. The various neuropeptides exert their effects through various pathways, including the extracellular signal-regulated kinase (ERK)/Akt pathway, and modulation of  $Ca^{2+}$ -influx (melanin concentrating hormone),<sup>126</sup> cAMP and, to a lesser extent, PI3K signaling (VIP and PACAP),<sup>127,128</sup> muscarinic/ $\beta$ -adrenoceptors signaling, PI3K/PKC signaling and  $Ca^{2+}$ -mobilization from intracellular stores (gastrin-releasing peptide).<sup>129,130</sup>

Likewise, insulin release is stimulated by the so-called cephalic phase, which represents the conditioned reflex of increased hormone secretion, referred to as cephalic phase insulin response,<sup>131</sup> even in the absence of nutrients/glucose as a trigger,<sup>132–134</sup> such as when anticipating a meal, to prepare the organism to adequately respond to incoming nutrients.<sup>135</sup> Moreover, cephalic phase insulin response is pivotal for ensuring normal postprandial glucose management.<sup>136</sup> The neural mechanism underlying cephalic phase insulin response was found to include cholinergic and non-cholinergic processes<sup>136</sup> as well as the dorsal vagal complex located in the medulla oblongata.<sup>137</sup> Conversely, insulin released in response to a meal enters the brain via the blood–brain–barrier<sup>138</sup> to decrease food intake<sup>139,140</sup> by stimulating hypothalamic pro-opiomelanocortin neurons<sup>141</sup> and initiating the PI3K signaling pathway<sup>142</sup> in these pro-opiomelanocortin neurons.<sup>143</sup> In contrast to its pro-opiomelanocortin-stimulating action, insulin inhibits NPY expression<sup>144</sup> in Agouti-related peptide (AgRP/NPY) neurons, which are known to secrete the orexigenic neuropeptides NPY<sup>145–147</sup> and AgRP.<sup>148,149</sup> Both, peripheral and central insulin signaling are impaired in obese or diabetic states.<sup>150–154</sup>

### The liver–islet axis

The second group represents the liver–islet axis. The liver has a key role in glucose homeostasis by storing (glycogenesis) or releasing (glycogenolysis/gluconeogenesis) glucose upon

interaction with insulin and glucagon, respectively. The binding of glucagon to its hepatic GPCR evokes the signaling cascade described under 'External factors affecting pancreatic hormone secretion', eventually resulting in the activation of PKA, which in turn stimulates two processes; one promotes glycogenolysis/gluconeogenesis and the other inhibits glycolysis/glycogenesis.<sup>155,156</sup> Glycogenolysis is a multistep process that includes the PKA-mediated phosphorylation of phosphorylase kinase,<sup>157</sup> cleavage of glucose-1-phosphate (G-1-P) from glycogen by activated glycogen phosphorylase a<sup>158</sup> and the conversion of G-1-P into G-6-P,<sup>159</sup> eventually resulting in phosphate and free glucose. Hepatic gluconeogenesis is promoted by the PKA-mediated phosphorylation of the cAMP response element-binding protein, which in turn upregulates peroxisome proliferator-activated receptor- $\gamma$  coactivator (PGC)-1.<sup>160</sup> Together with the hepatocyte nuclear factor (HNF)-4, PGC-1 induces the transcription of phosphoenolpyruvate carboxykinase,<sup>161</sup> which catalyzes the conversion of oxaloacetate into phosphoenolpyruvate, a rate-limiting step in gluconeogenesis. This is followed by reversed glycolysis, during which stimulation of the bifunctional PFK-2/FBPase-2 leads to both, enhanced gluconeogenesis through the abrogation of disabled fructose-1,6-bisphosphatase (FBPase)-1, which facilitates the successive conversion of substrates into G-6-P, and to suppressed glycolysis.<sup>162,163</sup> Glycolysis is further inhibited by the PKA-mediated inactivation of pyruvate kinase,<sup>164–166</sup> resulting in the production of glucose instead of pyruvate. In addition, glucagon was found to suppress pyruvate kinase gene expression as well as to enhance pyruvate kinase mRNA degradation.<sup>167,168</sup> Finally, the PKA-induced inactivation of hepatic glycogen synthase<sup>169–171</sup> decreases glycogen synthesis and concomitantly increases the hepatic glucose pool.

As glucagon's opponent, insulin stimulates glycolysis via enhanced expression of the hepatic glucokinase gene,<sup>14,15</sup> a key enzyme that converts glucose into G-6-P. This increase is mediated by the sterol regulatory element binding protein-1c<sup>15</sup> and requires the absence of cAMP.<sup>14</sup> Furthermore, insulin inactivates glycogen phosphorylase and glycogen synthase kinase (GSK)-3<sup>172</sup> through the PI3K pathway, which in turn activates glycogen synthase.<sup>18–20</sup> The second liver-specific effect of insulin is to repress the expression of the phosphoenolpyruvate carboxykinase and G-6-Pase genes; the first by disrupting the association of cAMP response element-binding protein and RNA polymerase II with the phosphoenolpyruvate carboxykinase gene promoter,<sup>23</sup> whereas G-6-P suppression requires PKB $\alpha$ /Akt and forkhead transcription factor (FOXO1),<sup>24,25</sup> whose expression was shown to be diminished by the inhibition of GSK-3.<sup>26</sup>

It is not only insulin and glucagon acting on the liver; hepatocyte-derived factors conversely influence the pancreas and/or insulin secretion. Although HNF3 $\beta$  was proposed to be pivotal for the transcription of the pancreatic and duodenal homeobox 1 (pdx1 or insulin promoter factor 1 (IPF-1)) gene, a transcription factor regulating pancreatic development<sup>173,174,175</sup>, it is the loss of HNF1 $\alpha$  resulting in an almost abolished insulin

secretion, likely due to a decreased response to intracellular calcium. These findings support the importance of HNF1 $\alpha$  in maintaining  $\beta$ -cell function<sup>176</sup> and its involvement in maturity-onset diabetes of the young (MODY3).<sup>177</sup>

The hepatokine betatrophin, also known as TD26, re-feeding induced fat and liver (RIFL), lipasin or angiopoietin-like (ANGPTL) 8, was first identified as a factor that drives  $\beta$ -cell proliferation and thus increases  $\beta$ -cell mass in a murine model of insulin resistance.<sup>178</sup> Subsequent studies, however, did not reveal impairments in glucose homeostasis<sup>179</sup> or  $\beta$ -cell expansion in *Angptl8* knockout mice.<sup>180</sup> Moreover, betatrophin does not have an effect on human  $\beta$ -cell replication, challenging its usefulness in diabetes therapy.<sup>181</sup> This is substantiated by the fact that betatrophin levels are higher in T2DM patients,<sup>182–184</sup> although they were lower in one study.<sup>185</sup> However, this is likely to be due to technical issues.<sup>186</sup>

### The gut–islet axis

Another important axis is the gut–islet axis. The gut releases various hormones upon nutrient ingestion, including GLP-1 and GIP, that bind to their respective receptors on pancreatic  $\beta$ -cells to potentiate insulin secretion, as described under 'External factors affecting pancreatic hormone secretion'. Furthermore, both hormones exert pancreatic effects, such as GLP-1-stimulated insulin gene expression,<sup>77,187</sup> incretin-induced  $\beta$ -cell neogenesis, proliferation<sup>188–191</sup> and survival,<sup>192</sup> the prevention of  $\beta$ -cell apoptosis in general<sup>193,194</sup> and in response to glucolipototoxicity.<sup>195</sup> The extrapancreatic actions of GLP-1 include suppression of endogenous glucose production<sup>196</sup>/glycogenolysis,<sup>197</sup> glucagon secretion,<sup>197,198</sup> appetite,<sup>199,200</sup> a delay in gastric emptying<sup>198,199</sup> and improved  $\beta$ -cell insulin sensitivity<sup>199,201,202</sup> and glucose disposal,<sup>203,204</sup> whereas GIP positively affects lipid<sup>205–207</sup> and bone metabolism.<sup>208–211</sup> Thus, GLP-1 and GIP mediate insulin secretion and concomitantly, insulin modulates GIP<sup>212</sup> and GLP-1 release; the latter occurring through the PI3K/Akt- and mitogen-activated protein kinase kinase (MAPKK or MEK)/ERK1/2 pathway.<sup>213</sup> The importance of this interplay is also demonstrated by defective insulin responses and consequent glucose intolerance in *GLP-1R<sup>-/-</sup>* and *GIPR<sup>-/-</sup>* mice<sup>214–218</sup> as well as in the pathogenesis of T2DM.<sup>219–223</sup>

In addition to incretins, there are the so-called dectetins, namely limostatin and Neuromedin U (NmU), which are secreted during fasting to suppress insulin release. NmU, a (neuro)peptide that mediates the contraction of smooth muscles in the uterus (hence the 'U') among others, was first isolated from the pig spinal cord.<sup>224</sup> Further mRNA expression studies, however, revealed NmU to be highly expressed in the gastrointestinal (GI) tract with the highest levels found in the upper GI, that is, duodenum and jejunum.<sup>225,226</sup> Within the GI structure, NmU is mainly located in submucosal and myenteric cells,<sup>227,228</sup> indicating its possible involvement in the neuronal control of GI function.<sup>229</sup> In addition to this, NmU is likely to regulate insulin secretion; the G-protein-coupled NmU receptor 1 (NmUR1) is expressed in pancreatic islets and its stimulation dose dependently decreased insulin release.<sup>230,231</sup>

The underlying mechanism involves the simultaneous release of somatostatin—a known modulator of insulin secretion<sup>6</sup>—upon NmUR1 activation.<sup>232</sup> A very recent study showed<sup>231</sup> that the peptide hormone limostatin, which is expressed in *Drosophila melanogaster*, also reduces insulin secretion and its absence caused hyperinsulinemia, hypoglycemia and obesity. Moreover, knockdown of the fly NmUR orthologue not only reproduced the consequences of limostatin deficiency but also diminished its insulin-suppressing ability. Limostatin release is initiated by food depletion and hence may represent a novel mechanism for modulating insulin secretion during fasting.

Other gastrointestinal hormones that interact with the pancreas are gastrin and cholecystokinin (CCK). Gastrin, which is secreted from G-cells in the stomach and duodenum, acts as an islet growth factor, together with transforming growth factor- $\alpha$ , by promoting differentiation of ductular precursor cells<sup>233</sup> and  $\beta$ -cell neogenesis as well as by enhancing the islet mass from transdifferentiated exocrine pancreatic tissue.<sup>234</sup> Furthermore, it induces the expression of glucagon genes in  $\alpha$ -cells.<sup>235</sup> Along the same lines, CCK, which is synthesized and released from duodenal I-cells, potentiates basal, glucose-<sup>236,237</sup> and amino acid-induced insulin secretion,<sup>238</sup> and augments glucagon secretion.<sup>237,239</sup> The pivotal role of CCK in modulating glucose homeostasis is reflected in postprandial hyperglycemia, which is due to reduced CCK plasma levels in noninsulin-dependent diabetes mellitus.<sup>240</sup>

Another important factor that is related to metabolic disorders such as obesity, T2DM and type 1 DM (T1DM) is the gut microbiota. Obesity, T2DM and T1DM patients display alterations in the composition of their microbiota that may initiate and/or promote the respective disorder. Recent findings linked an aberrant microbiome, which is generally represented by diminished diversity, including fewer butyrate-producing (butyrate was shown to trigger mucin production and hence gut integrity) and mucin-degrading bacteria,<sup>241</sup> to the development of autoimmunity in T1DM.<sup>242</sup> An altered microbiota composition may also contribute to obesity<sup>243,244</sup> as well as to T2DM<sup>245–247</sup> and ‘correction’ by antibiotics,<sup>248</sup> probiotics<sup>249</sup> or prebiotics, the last of which causing a short-chain FFA-stimulated increase in GLP-1,<sup>250</sup> may improve the disease condition.<sup>251</sup>

### The adipocytes/myocytes–islet axis

On one hand, insulin’s interplay with adipose and muscle tissues is broadly based on facilitating insulin-dependent glucose uptake through the glucose transporter 4 (GLUT4).<sup>11–13</sup> On the other hand, adipokines and myokines secreted from the adipose and muscle tissue, respectively, modulate insulin release. As part of the so-called adipoinsular axis,<sup>252</sup> leptin, the most famous adipokine, mainly acts on its receptors in the hypothalamic arcuate nucleus to inhibit food intake and control whole body homeostasis.<sup>253</sup> However, leptin receptor (Ob-R) mRNA expression was also observed in pancreatic islets<sup>254</sup> and its stimulation caused a reduction in insulin secretion<sup>255–257</sup> due to the activation of  $K_{ATP}$ -channels,

which in turn prevented  $Ca^{2+}$ -influx<sup>258</sup> and the subsequent signaling pathway. Furthermore, leptin was shown to suppress insulin gene expression,<sup>259,260</sup> representing a negative feedback loop. Conversely, insulin enhances *ob* gene expression and leptin secretion.<sup>261–264</sup> Likewise, insulin modulates the expression of adiponectin, another well-known adipokine, the abundance of its receptor in adipose and muscle tissue<sup>265,266</sup> as well as its secretion.<sup>267,268</sup> Adiponectin is not only involved in glucose and fatty acid metabolism<sup>269</sup> but it also forestalls  $\beta$ -cell apoptosis and induces insulin gene expression and release,<sup>270</sup> the latter was mediated by the ERK/Akt pathway in one study<sup>270</sup> and by the AMPK pathway in another study.<sup>271</sup> Other adipokines, such as apelin,<sup>272,273</sup> chemerin,<sup>274–276</sup> omentin,<sup>277,278</sup> resistin<sup>279</sup> and visfatin,<sup>280,281</sup> were also shown to directly interact with insulin, whereas retinol-binding protein 4, tumor necrosis factor- $\alpha$  and vaspin are related to insulin in an indirect manner.<sup>282</sup> In addition to adipokine secretion by adipocytes, myocytes release cytokines, which are referred to as myokines. Fibroblast growth factor-21 is a widely expressed protein with a broad mode of action, including the regulation of carbohydrate and fatty acid metabolism<sup>283</sup> and may be considered as a myokine due to its secretion from muscle cells.<sup>284</sup> Fibroblast growth factor-21 is regulated by insulin<sup>285</sup> through the PI3K/Akt1 signaling pathway.<sup>286</sup> Interleukin

(IL)-6, which is both an adipokine and myokine,<sup>287</sup> was shown to influence the pancreas by controlling the expression of pro-glucagon mRNA as well as glucagon secretion. It also increases  $\alpha$ -cell proliferation and islet mass while protecting the pancreas from metabolic stress-induced apoptosis.<sup>288</sup> Furthermore, IL-6 increased GLP-1 production from proglucagon in pancreatic  $\alpha$ -cells and its secretion from  $\alpha$ -cells and intestinal L-cells, eventually resulting in a GLP-1-mediated increase in insulin secretion.<sup>289</sup>

### MODULATING INSULIN SECRETION AS A MEANS OF DIABETES THERAPY

Due to the worldwide, still spreading epidemic of T2DM, there is an urgent need for (new) anti-diabetic drugs and therapies that are more effective and have fewer side effects. Currently, the most commonly used drugs can be classified into agents that enhance insulin secretion (secretagogues such as sulfonylureas (SUs) and incretin mimetics), sensitize the target organs of insulin (for example, metformin from the class of biguanides or thiazolidinediones), or reduce glucose absorption from the gastrointestinal tract (inhibitors of gastrointestinal  $\alpha$ -glucosidase). Different therapies address different problems and stages of T2DM and may be prescribed in combination to exert synergistic effects.

#### Sulfonylureas

$\alpha$ -glucosidase inhibitors and sensitizers do not target the pancreas or insulin secretion itself but instead target the upstream (slowed intestinal glucose absorption) or downstream (improved insulin sensitivity) processes. In contrast, insulin secretagogues directly modulate insulin release. The SUs are the



first broadly applied oral anti-hyperglycemic drugs. To date, there are two generations of agents: acetohexamide, chlorpropamide, tolazamide and tolbutamide, which constitute the first generation and glibenclamide/glyburide, gliclazide, glimepiride, glipizide and gliquidone, which comprise the second generation. First-generation SUs are rarely used these days since tolbutamide intake was associated with an increase in lethal cardiac events.<sup>290,291</sup> More importantly, the second-generation SUs are more potent due to modifications in their side chains' structure, resulting in improved SUR-affinity, accompanied by lower effective plasma levels, which in turn may reduce undesirable drug-protein interactions.

All SUs share a central SU backbone but differ in their side chains. Despite having different pharmacokinetics, they work in the same way, namely by triggering endogenous insulin release by blocking  $K_{ATP}$ -channels and hence activating the insulin signaling pathway. More precisely, SUs bind to the sulfonylurea receptor (SUR) subunit of the  $K_{ATP}$ -channel with high affinity.<sup>292,293</sup> SUR, together with the pore-forming subunit Kir6.x, forms a hetero-octameric complex consisting of four inner Kir6.x subunits surrounded by four SUR subunits (4:4 stoichiometry).<sup>294,295</sup> Moreover, different isoforms of the two subunits are expressed, depending on the tissue-specific expression of the  $K_{ATP}$ -channels: SUR1 and Kir6.2 are expressed in the pancreas and brain,<sup>296</sup> Kir6.2 and SUR2A are expressed in the heart and skeletal muscle,<sup>297</sup> while SUR2B is expressed in the brain and smooth muscle,<sup>298</sup> and Kir6.1 and SUR2B are expressed in vascular smooth muscle.<sup>299</sup> Although SUs bind to both, SURs and Kir6.2, the interactions with the latter are of low affinity<sup>300,301</sup> and hence only SUR-interacting agents are used for diabetes treatment. In addition to their mode of action as inhibitors of  $K_{ATP}$ -channels, SUs were shown to improve glucose uptake into insulin-dependent tissues and glucose disposal as well as to reduce hepatic glycogenolysis/gluconeogenesis.<sup>302–304</sup>

In contrast to SUs inactivating the  $K_{ATP}$ -channels by binding to the SUR1 subunit, ATP closes them by interacting with Kir6.2.<sup>305</sup> Moreover, while the binding of only one ATP molecule is sufficient to completely close the channel,<sup>306</sup> inhibition by SUs is incomplete as the channel might still open even when SUs are bound to SUR1.<sup>299</sup> Nonetheless, second-generation SUs reduce the glycated hemoglobin or HbA<sub>1c</sub>, which represent the average plasma glucose concentrations over time and thus serve as a diagnostic measure for diabetes mellitus, by 1.0–2.0%. In addition to the weight gain attributed to the anabolic effects of increased insulin secretion, the main side effect of SUs is hypoglycemia<sup>307,308</sup> due to excess circulating insulin levels and due to the fact that SUs evoke insulin secretion in a glucose-independent manner.<sup>309</sup>

Although they are not SUs *per se*, meglitinides, that is, repaglinide and nateglinide, share their mode of action of inhibiting  $K_{ATP}$ -channels.<sup>310</sup> However, meglitinides and some of the second-generation SUs, for example, glibenclamide, interact with both, the SUR1 and the SUR2A or B isoforms.<sup>311</sup> Despite the possible disadvantage of this generalized binding that may cause undesirable effects on other  $K_{ATP}$ -channel types, for

example, those in the heart,<sup>312</sup> meglitinides, namely nateglinide, have an earlier onset of action and a faster dissociation rate from the sulfonylurea receptor,<sup>313–315</sup> resulting in a diminished risk of hypoglycemia.<sup>316</sup> Like SUs, meglitinides also cause weight gain.<sup>317,318</sup>

### Incretin mimetics

Another group of insulin secretagogues is comprised of the incretins GLP-1 and GIP. As both incretins are rapidly inactivated by the enzyme dipeptidyl peptidase IV (DPP-IV),<sup>319</sup> their application in T2DM treatment focuses on modified analogues<sup>320–325</sup> or receptor agonists, including the well-known, short-acting exenatide.<sup>326–328</sup> The long-lasting agonists exenatide LAR,<sup>329,330</sup> liraglutide<sup>331,332</sup> and lixisenatide<sup>333–335</sup> are currently under investigation. However, based on the lipogenic properties<sup>205–207</sup> of GIP, insufficient insulin-potentiating effects in T2DM patients<sup>220,336</sup> and a possible worsening effect by GIP,<sup>337,338</sup> the focus is on GLP-1 analogues/receptor agonists for T2DM treatment. By acting on its receptor, GLP-1 induces the signaling cascade described under 'External factors affecting pancreatic hormone secretion', resulting in its main effect: potentiating insulin secretion. In addition to reducing the HbA<sub>1c</sub> levels, GLP-1 analogues/receptor agonists promote weight loss and, more importantly, do not evoke hypoglycemia, as do SUs,<sup>326–334</sup> due to the glucose-dependent mode of action and the self-regulating mechanism of GLP-1.<sup>68,336,339</sup> When blood glucose levels are lowered to physiological levels, GLP-1 is incapable of enhancing insulin secretion, thereby preventing hypoglycemia.<sup>79,340</sup> In addition, GLP-1 (analogues/receptor agonists) exerts further pancreatic and extrapancreatic actions, as mentioned under 'Interplay between the pancreatic islets and other organs'. Although GLP-1 (analogues/receptor agonists) exhibits some minor side effects, including nausea, vomiting or gastrointestinal impairments,<sup>326–335</sup> the beneficial properties outweigh the negative effects, and thus, GLP-1 is a promising anti-diabetic agent.

### Insulin sensitizers

Metformin, which is generally the most widely used first-line anti-diabetic medication,<sup>341</sup> is a so-called (insulin) sensitizer. It not only diminishes hepatic glucose output due to glycogenolysis/gluconeogenesis<sup>342</sup> but it also enhances glucose uptake into peripheral tissues, such as skeletal muscle, by activating 5'-adenosine monophosphate-activated protein kinase (AMPK- $\alpha$ 2).<sup>343</sup> Furthermore, it supports weight loss<sup>344</sup> by reducing food consumption.<sup>345</sup> With respect to its effects on  $\beta$ -cell function, metformin was shown to increase insulin gene expression,<sup>346</sup> possibly by nuclear accumulation of pdx1 and its subsequently improved DNA-binding activity.<sup>347</sup> Interestingly, metformin exerts opposing effects on  $\beta$ -cell proliferation and/or apoptosis; on the one hand, it suppresses  $\beta$ -cell proliferation and enhances apoptosis through an AMPK-dependent and autophagy-mediated mechanism<sup>348</sup> following the metformin-induced activation of c-Jun-N-terminal kinase and caspase-3.<sup>349</sup> On the other hand, metformin reduces



caspase-3- and -8-mediated apoptosis in isolated islets from T2DM patients<sup>350</sup> and protects against lipotoxicity-induced  $\beta$ -cell defects.<sup>348,351</sup>

The other members of the sensitizer group include the thiazolidinediones (or glitazones). Currently, only pioglitazone is available; troglitazone was withdrawn from the market in 2000 and rosiglitazone was withdrawn in 2010 due to liver toxicity, drug-induced hepatitis<sup>352–354</sup> and the increased risk of cardiovascular events, respectively.<sup>355</sup> Their mode of action involves activation of the peroxisome proliferator-activated receptor (PPAR $\gamma$ ), a nuclear transcription factor that is highly expressed in adipose tissue, and the subsequent regulation of genes that are involved in glucose and fat metabolism.<sup>356–358</sup> By promoting lipogenesis, FFAs are removed from the blood stream, whereupon cells become dependent on glucose as an energy substrate. However, enhanced lipogenesis also leads to the weight gain observed in thiazolidinedione-treated T2DM patients.<sup>359</sup> In contrast to metformin, pioglitazone prevents (oxidative stress-induced) apoptosis<sup>360,361</sup> by decreasing the expression of apoptosis-promoting genes, while increasing anti-apoptotic and anti-oxidative gene expression. However, this may depend on the disease state.<sup>362,363</sup> Furthermore, pioglitazone increases  $\beta$ -cell mass by upregulating cell differentiation/proliferation genes.<sup>364</sup> Although they have partially different modes of action, both groups of sensitizers cause a reduction in the HbA<sub>1c</sub> level by 1.5–2.0%.

### A-glucosidase inhibitors

A-glucosidase inhibitors, such as acarbose, miglitol and voglibose, not only decelerate the breakdown of starch into glucose in the small intestine but also decrease its bioavailability, resulting in reduced levels of glucose entering the blood stream and hence attenuated postprandial glucose excursions.<sup>365–370</sup> In addition, they support weight loss<sup>371,372</sup> and ameliorate blood pressure,<sup>373</sup> insulin sensitivity<sup>367,368</sup> and triglyceride levels.<sup>369,370</sup> Similar to pioglitazone,  $\alpha$ -glucosidase inhibitors attenuate reductions in  $\beta$ -cell mass, which may delay the onset of diabetes.<sup>374–376</sup> As  $\alpha$ -glucosidase inhibitors only mildly reduce HbA<sub>1c</sub> levels (0.5–1.0%), they are usually only used in the early stage of T2DM, that is, impaired glucose tolerance or in combination with other drugs.<sup>377</sup>

### CONCLUSIONS AND OUTLOOK

The pancreas has key roles in maintaining normal blood glucose levels by producing and releasing insulin and glucagon. These opponents interact not only with each other through the intra-islet insulin axis<sup>378–381</sup> but also with other organs/tissues, that is, the brain, liver, gut as well as insulin-dependent adipose and muscle tissues. Altogether, the islet–organ/tissues axes described here form a highly sophisticated network that includes, but is not limited to, various signaling molecules, that is, neuropeptides (brain-derived neurotrophic factor, NPY, melanin concentrating hormone, gastrin-releasing peptide, VIP and PACAP), hepatokines (betatrophin and HNFs), enteroendocrine hormones (the incretins GLP-1 and GIP, the decretins NmU and limostatin, gastrin and CCK) as well as

adipokines (leptin and adiponectin) and myokines (fibroblast growth factor-21 and IL-6) that mainly interact through GPCR signaling pathways, such as the cAMP cascade. In good health, the well-functioning interactions between all of the organs and tissues involved ensure glucose homeostasis. However, impairments in the secretion of and/or sensitivity to insulin may result in metabolic diseases, such as T2DM. Referring to the American Diabetes Association, T2DM and noninsulin-dependent diabetes mellitus are characterized by insulin resistance, hyperglycemia and a relative insulin deficiency. Furthermore, T2DM is associated with low-grade inflammation,<sup>382,383</sup> cardiovascular disease,<sup>384,385</sup> nephropathy<sup>386,387</sup> and alterations in the secretion of various hormones, including IL-6, IL-18, tumor necrosis factor- $\alpha$ ,<sup>388</sup> adiponectin and leptin,<sup>389</sup> neuropeptides,<sup>390</sup> ghrelin<sup>391,392</sup> and the incretins GLP-1 and GIP.<sup>219–223</sup> Although lifestyle interventions<sup>393</sup> and weight loss<sup>394</sup> reverse T2DM in early stages, when insulin is still secreted, T2DM patients may become dependent on anti-diabetic drugs in later stages. Currently, there are three classes of agents: insulin secretagogues, insulin sensitizers and  $\alpha$ -glucosidase inhibitors, all of which have different modes of action and hence target different stages and symptoms of T2DM. Treatments that modulate insulin release—on condition of an appropriate insulin sensitivity of the target organs—appear to be promising approaches. Current research is unveiling new molecules, enzymes and interactions that are involved in the signaling pathways underlying insulin secretion, among others, and is likely to introduce new therapeutic approaches. Strategies that target these mediating molecules may include, but are not limited to, the calcium sensor Syt-7,<sup>90,395</sup> the SNARE-associated protein Snapin,<sup>89</sup> the *t*-SNARE SNAP-25,<sup>396</sup> cyclin-dependent kinase (Cdk) 5,<sup>397</sup> ryanodine receptor (RyR) 2,<sup>398</sup> the nucleotide exchange factor and intracellular cAMP sensor Epac2,<sup>57–59,399</sup> mammalian uncoordinated proteins (munc)13<sup>400,401</sup> and munc18<sup>40,41</sup> as well as the Ras-related proteins (Rab) 3A<sup>402</sup> and 27A.<sup>403</sup>

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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