Physiologically, insulin secretion is subject to a dual, hierarchical control by triggering and amplifying pathways. By closing ATP-sensitive K\(^+\) channels (K\(_{ATP}\) channels) in the plasma membrane, glucose and other metabolized nutrients depolarize β-cells, stimulate Ca\(^{2+}\) influx, and increase the cytosolic concentration of free Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)), which constitutes the indispensable triggering signal to induce exocytosis of insulin granules. The increase in β-cell metabolism also generates amplifying signals that augment the efficacy of Ca\(^{2+}\) on the exocytotic machinery. Stimulatory hormones and neurotransmitters modestly increase the triggering signal and strongly activate amplifying pathways biochemically distinct from that set into operation by nutrients.

Many drugs can increase insulin secretion in vitro, but only few have a therapeutic potential. This review identifies six major pathways or sites of stimulus-secretion coupling that could be aimed by potential insulin-secreting drugs and describes several strategies to reach these targets. It also discusses whether these perspectives are realistic or theoretical only. These six possible β-cell targets are 1) stimulation of metabolism, 2) increase of [Ca\(^{2+}\)]\(_i\) by closure of K\(_{ATP}\) channels, 3) increase of [Ca\(^{2+}\)]\(_i\) by other means, 4) stimulation of amplifying pathways, 5) action on membrane receptors, and 6) action on nuclear receptors. The theoretical risk of inappropriate insulin secretion and, hence, of hypoglycemia linked to these different approaches is also envisaged. *Diabetes* 53 (Suppl. 3):S48–S58, 2004

Optimal treatment of type 2 diabetes is difficult because of the complex pathogenesis of the disease (1,2). Pharmacological agents improving the action of insulin on its target tissues and agents correcting the deficient secretion of insulin by β-cells both have a place in our armamentarium. Many chemicals can increase insulin secretion in vitro and are useful tools to unravel the mechanisms of stimulus-secretion coupling. Until recently, however, hypoglycemic sulfonylureas were the only drugs used to stimulate insulin secretion in patients with type 2 diabetes.

The story of sulfonylureas started in 1942, in Montpellier (rev. in 3). Marcel Janbon and his colleagues recognized that some patients receiving a sulfonamide (2254RP) for the treatment of typhoid fever were experiencing severe hypoglycemia. Auguste Loubatières rapidly confirmed experimentally that the drug was causing hypoglycemia and, in 1946, at the end of a remarkable series of experiments for the time, concluded that the underlying mechanism was a direct stimulation of insulin secretion by 2254RP. In 1955, in Berlin, Franke and Fuchs reported that another antibacterial sulfonamide, carbutamide, also caused hypoglycemia. The drug was rapidly used to treat diabetic patients who did not require insulin, and was followed 1 year later by tolbutamide (3). The discovery of hypoglycemic sulfonylureas was thus serendipitous. Although many drugs have since been reported to exert hypoglycemic side effects, none has had such a prolific progeny. The reason of the success of sulfonylureas is obvious. They all, including the mother compound 2254RP (3), act on ATP-sensitive K\(^+\) channels (K\(_{ATP}\) channels), which play a central role in the regulation of insulin secretion by glucose itself.

Nowadays, search for novel insulin secretagogues is guided by our knowledge of stimulus-secretion coupling in β-cells. In this review, I shall first outline the major mechanisms regulating insulin secretion before discussing how distinct pathways or sites of action could serve as therapeutic targets.

**THE PHYSIOLOGICAL CONTROL OF INSULIN SECRETION**

Insulin secretion is subject to tight control by glucose, other nutrients, neurotransmitters, and hormones. Although numerous and complex, the mechanisms underlying this multifactorial regulation can be schematized by a hierarchical interaction between triggering and amplifying pathways (Fig. 1) (4,5).

When the concentration of glucose increases, β-cell metabolism accelerates, leading to closure of K\(_{ATP}\) channels in the plasma membrane. These channels are composed of the pore-forming K\(^+\)\(_{IR6.2}\) and the regulatory sulfonylurea receptor 1 (SUR1). Binding of intracellular ATP to K\(^+\)\(_{IR6.2}\) closes the channel, whereas binding of MgADP to SUR1 opens the channel. The increase in the ATP/ADP ratio resulting from the metabolism of glucose thus closes the channel (Fig. 1). The consequence is a

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[Ca\(^{2+}\)]\(_i\), free cytosolic Ca\(^{2+}\) concentration; Epac, exchange protein activated by cAMP; GEF, guanine nucleotide exchange factor; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; GTP, guanosine triphosphate; PKA, protein kinase A; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; SUR1, sulfonylurea receptor 1.

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depolarization of the plasma membrane, with opening of voltage-dependent Ca\(^{2+}\) channels, acceleration of Ca\(^{2+}\) influx, and increase in the concentration of cytosolic free Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) that is necessary and sufficient to trigger insulin secretion (6–10). However, this triggering signal alone is poorly effective. Its efficacy is augmented by an amplifying pathway also using signals issued from glucose metabolism. The nature of these signals and their intracellular targets are still unclear, but a role of ATP and ADP is plausible (11–13). The same dual regulation applies to all nutrients that are actively metabolized and increase the ATP/ADP ratio in \(\beta\)-cells (5). Although no direct evidence is as yet available, both clinical investigation of diabetic patients and experimental studies of animal models suggest that the two pathways, triggering and amplifying, may be impaired in \(\beta\)-cells affected by type 2 diabetes (4).

Importantly, the triggering signal (rise in [Ca\(^{2+}\)]\(_i\)) can also be produced or augmented by mechanisms that are independent of K\(_{ATP}\) channels (Fig. 1). Some hormones and neurotransmitters mobilize Ca\(^{2+}\) from intracellular stores (14–16). Agents acting on various ionic channels (e.g., inhibitors of voltage-dependent K\(^+\) channels) can augment glucose-induced depolarization, thereby potentiating the [Ca\(^{2+}\)]\(_i\) rise (17). Cationic amino acids, like arginine, are poorly metabolized but depolarize \(\beta\)-cells because of their entry in a positively charged form, thus without direct interaction with an ionic channel (5).

Stimulatory hormones and neurotransmitters, such as glucagon-like peptide 1 (GLP-1) and acetylcholine, usually potentiate insulin secretion by a dual action. They moderately increase the triggering signal (rise in [Ca\(^{2+}\)]\(_i\)) through complex, variable, but largely glucose-dependent mechanisms. They also produce major amplifying signals, mainly through activation of protein kinases, in particular protein kinase A (PKA) and protein kinase C (PKC) (14–16,18–19). In addition to PKA, cAMP-regulated guanine nucleotide exchange factors (GEFs, or Epac) might mediate part of the effects of cAMP on insulin secretion (16,20). Activation of PKA or PKC, or of GEF/Epac, augments the efficacy of Ca\(^{2+}\) on exocytosis. The biochemical mechanisms of this type of amplification are, however, distinct from those implicated in the amplifying pathway of glucose and other nutrients (5).

Finally, inhibitory hormones and neurotransmitters also act via two pathways. They depress insulin secretion partly by decreasing the triggering signal (via membrane repolarization) and mainly by reducing the efficacy of Ca\(^{2+}\) on exocytosis (attenuating pathway operating via kinases or small guanosine triphosphate [GTP]-binding proteins) (21).

**HOW CAN DRUGS FOOL \(\beta\)-CELLS TO SECRETE EXCESSIVE AMOUNTS OF INSULIN?**

Hypoglycemia induced by excessive insulin secretion is a major complication of current pharmacological treatments of type 2 diabetes. Under physiological conditions, it is the triggering pathway that determines whether insulin is secreted. The amplifying pathway serves to optimize the secretory response induced by the triggering signal but does not induce secretion if [Ca\(^{2+}\)]\(_i\) is not increased (5). This strict hierarchy between the two pathways can be perturbed by pathological defects or by drugs. Theoretically, actions on either the triggering or amplifying path-
Site 1: Stimulation of β-cell metabolism
- Activation of glucokinase
- Inhibition of glucose-6-phosphatase
- Alternative fuels
- Inhibition of mitochondrial Na+/Ca2+ exchanger

Site 2: Increase of β-cell [Ca2+]i, by blockade of KATP channels
- Interaction with SUR1
- Interaction with K+,-6,2

Site 3: Increase of [Ca2+]i, by action at sites other than KATP channels
- Blockade of other K+ channels
- Activation of Ca2+ channels
- Action on other ionic channels
- Inhibition of [Ca2+]i-lowering processes

Site 4: Stimulation of amplifying pathways in β-cells
- Activation of the nutrient-mediated amplification
- Inhibition of AMP kinase
- Inhibition of 11β-hydroxysteroid dehydrogenase type 1 sensitization to Ca2+
- Inhibition of cAMP degradation
- Activation of the PKC pathway

Site 5: Action on β-cell membrane receptors
- Antagonists of inhibitory receptors
- Agonists of stimulatory receptors

Site 6: Action on β-cell nuclear receptors

Table 1: Potential sites of action of insulin-secreting drugs

Site 1: Improvement of β-cell metabolism
Alterations of glucose metabolism often characterize β-cells from animal models of type 2 diabetes and are likely β-cells from type 2 diabetic patients. Correction of specific defects would obviously be optimal, but a global improvement of glucose/fuel metabolism in β-cells could also have several advantages: restoration of normal triggering and amplifying signals, stimulation of proinsulin biosynthesis with lesser danger of store exhaustion, and little risk of hypoglycemia if the control steps are not offset.

Theoretically, β-cell metabolism might be boosted by a selective action on an identified perturbed site (e.g., hyperglycemia due to a mutation or a change in expression), by supply with alternative fuels that bypass a defective enzyme or pathway, and by less specific, indirect measures to accelerate global metabolism. Some of these approaches have already been tested experimentally.

Allosteric activation of glucokinase. At the entry of glycolysis, glucokinase plays a primary regulatory role in the control of glucose metabolism in β-cells (10). Loss of function and gain of function of glucokinase respectively cause deficient and excessive insulin secretion in type 2 maturity-onset diabetes of the young (MODY2) patients and in certain infants with persistent hyperinsulinemic hypoglycemia (24,25). An allosteric activator of glucokinase (compound RO-28-1675) has been developed recently (26). It activates glucose metabolism and lowers the threshold concentration for glucose-induced insulin secretion in rat islets. It also increases plasma insulin and decreases plasma glucose levels in normal mice and promotes glucose usage in the liver (26). This novel family of drugs opens interesting perspectives for the development of original antidiabetic agents. One should, however, be aware that patients taking excessive doses of such a compound will mimic the activating mutations of glucokinase and thus incur a risk of hypoglycemia.

Inhibition of glucose-6-phosphatase. Glucose-6-phosphatase is essential for glucose production by the liver, but its operation in β-cells creates a futile cycle of glucose phosphorylation and dephosphorylation, with reduction of glucose usage and insulin secretion as consequences (27). The activity of the enzyme is insignificant in normal β-cells, and the possibility that an increase in activity contributes to abnormal insulin secretion in animal models of diabetes remains controversial (28–30). Available drugs inhibiting hepatic glucose-6-phosphatase are inactive on the high enzyme activity in ob/ob mouse islets (31). For this approach to be successful, it should first be established that glucose-6-phosphatase is so overactive in β-cells from type 2 diabetic patients that it eventually impairs oxidative glucose metabolism and that the enzyme can be inhibited independently from its hepatic congener.

Alternative fuels. Esters of carboxylic metabolites are effective insulin secretagogues in vivo. In contrast to succinic, glutamic, and pyruvic acid, their methyl or ethyl esters enter β-cells, where the carboxylic moiety is metabolized in the Krebs cycle and thus bypasses the early and possibly defective steps of glucose metabolism. Whereas problems linked to the mode of administration in vivo (route, amount) and to the production of methanol from the methylesters can be circumvented (32), it remains to be established that undesirable

Theoretical and realistic β-cell targets for therapeutic drugs

Figure 1 schematizes the control of insulin secretion in a normal β-cell and indicates six possible pathways or sites that could be targeted by drugs designed to increase secretion. Several strategies can theoretically be followed to reach these targets (Table 1), but we will see that they are not equivalent and that a number of shortcomings often preclude transposition of theory into practice.
effects on glucose usage/production by the liver and peripheral tissues do not outweigh favorable effects on diabetic β-cells.

Monosaccharide esters, such as the pentaacetate ester of α-D-glucose, enter β-cells independently of the glucose transporters and stimulate insulin secretion (33). Because it is unlikely that glucose transport ever becomes rate-limiting in human β-cells, the potential interest of these compounds is elsewhere. Only minute amounts (much less than of glucose itself) are indeed sufficient to increase plasma insulin levels in normal rats. Unexpectedly, in vitro stimulation of insulin secretion is also observed with low concentrations of pentaacetate esters of nonmetabolized hexoses such as L-glucose or 2-deoxyglucose. The effects of these ester compounds are not attributed to an increase in β-cell metabolism, which is rather inhibited, but to an interaction with a still unidentified receptor (33). Developments in this area await further studies on the mode of action and potential negative effects of these compounds, as well as demonstration that their insulin-releasing activity is retained in models of type 2 diabetes.

**Inhibition of the mitochondrial Na⁺/Ca²⁺ exchanger.** The rise in β-cell [Ca²⁺]i produced by glucose and other secretagogues is followed by an influx of Ca²⁺ into mitochondria. It has been proposed that, by activating matrix dehydrogenases, Ca²⁺ augments the production of ATP and other putative messenger molecules (34,35). The exit of Ca²⁺ from mitochondria is mediated by a Na⁺/Ca²⁺ exchanger, whose blockade might thus be expected to promote metabolism. Compound CGP 37157 was developed for this purpose and was indeed found to increase mitochondrial [Ca²⁺], ATP production, cytosolic [Ca²⁺]i, and insulin secretion in INS-1 cells incubated in the presence of glucose (36). It also increased glucose-induced insulin secretion by rat islets and augmented plasma insulin levels at certain time points of a hyperglycemic clamp. Although encouraging, these preliminary results should be confirmed with related compounds, and the consequences of their application for longer periods should be evaluated in primary β-cells, before deciding whether this new approach is worth pursuing. The issue of tissue selectivity may also be difficult to solve.

**Site 2: Increasing the triggering signal by acting on KATP channels**

**Sulfonylureas.** The binding of sulfonylureas to SUR1 leads to closure of KATP channels with subsequent depolarization of the plasma membrane, activation of Ca²⁺ influx, and rise of [Ca²⁺]i. These drugs mimic the effect of glucose in the generation of the triggering signal, but do so independently of changes in β-cell metabolism. The different sites of sulfonylurea binding to SUR1 and the molecular mechanisms of its transduction into closure of KATP6.2 have been reviewed recently (37–40). In brief, SUR1 possesses an “A” site to which binds tolbutamide and the half of the glibenclamide molecule containing the sulfonylurea group, and a “B” site to which binds the nonsulfonylurea half of glibenclamide. Binding to one of these two sites is sufficient to produce the same final effects: closure of KATP channels, membrane depolarization, and rise in [Ca²⁺]i.

Whereas it is widely accepted that, unlike glucose, sulfonylureas do not promote proinsulin biosynthesis, it has been suggested that, like glucose, they also stimulate insulin secretion through an amplifying pathway (41,42). This is based on electrophysiological studies in which single β-cells are usually patch-clamped in the whole cell mode (permitting unrestricted exchange between cytoplasm and pipette milieu), and exocytosis of insulin granules is monitored as changes in membrane capacitance. In this study, the presence of fixed [Ca²⁺]i, intracellularly applied sulfonylureas increase exocytosis (41,42). It is therefore proposed that sulfonylureas penetrate β-cells and interact with SUR1 or a related protein in the membrane of the insulin granules to confer them release competence by facilitating their acidification. Surprisingly, this effect persists in Sur1 KO β-cells, implying that the intracellular binding protein is not SUR1 (43). It would be expected therefore that sulfonylureas retain an effect on insulin secretion—via the intracellular binding sites—in intact β-cells without KATP channels.

Figure 2 shows that unstimulated (1 mmol/l glucose) and stimulated (15 mmol/l glucose) insulin secretion was increased by 10 and 50 nmol/l glibenclamide in control mouse islets. In Sur1 KO islets, insulin secretion was already high in 1 mmol/l glucose and was further increased by 15 mmol/l glucose, which reflects the operation of the triggering pathway and the effectiveness of the amplifying pathway of glucose in the absence of SUR1 (44). Glibenclamide, however, was completely ineffective, even at 1 µmol/l, a concentration that largely exceeds those reached in vivo (Fig. 2). Extracellular tolbutamide was also without effect on insulin secretion in islets without KATP channels due to a knockout of Sur1 (44,45) or KATP6.2 (46) at both low and high glucose levels.

In conclusion, intracellularly applied sulfonylureas interact with a binding site probably on the insulin granule membrane (42). It is plausible that this site is somehow implicated in the response to physiological secretagogues, but it does not seem relevant to the therapeutic action of sulfonylureas.

**Glinides.** The nonsulfonylurea (benzamido) part of glibenclamide, known as meglitinide or HB-609, possesses blood glucose-lowering properties that have been attributed to stimulation of insulin secretion (47). Twenty years ago it was shown that meglitinide mimics the sequence of events by which tolbutamide and glibenclamide trigger insulin secretion (48). A number of other nonsulfonylurea compounds have been developed more recently, some of which are already in clinical use (49). The best known are repaglinide (AGEE-623), nateglinide (A-4166), and mitiglinide (KAD-1229 or S-21403). They are functionally related but structurally different. The common family name of “glinides” derives from their trade name but does not refer to any specific chemical structure. Except for repaglinide, it is incorrect to call them “meglitinide analogs” or “benzamido compounds.” Meglitinide and repaglinide bind to the “B” site in SUR1, whereas nateglinide and mitiglinide bind to the “A” site (39,40). Whatever the binding site, their eventual effect is similar to that of sulfonylureas: depolarization of β-cells, with subsequent rise in [Ca²⁺]i, and triggering of insulin secretion.

Measurements of β-cell capacitance have also been used to assess whether glinides amplify insulin secretion at an
Intracellular site. Nateglinide, but not repaglinide, produces such an effect (50), but, again, this conclusion is not supported by experiments using intact islets. Thus, nateglinide, repaglinide, and mitiglinide do not increase insulin secretion from islets depolarized with KCl (51). As for sulfonylureas, I conclude that the intracellular effect of some glinides, observed in dialysed single β-cells, is not therapeutically important.

In conclusion, the value of the glinides as insulin secretagogues does not reside in an original mode of action but in pharmacokinetic properties associated with a rapid onset of action and a rapid reversibility of action, at least for mitiglinide and nateglinide. Both also display an advantageous greater selectivity for SUR1 than SUR2A or SUR2B (38) and, hence, for K<sub>ATP</sub> channels of the β-cell. In this context, it has been speculated that drugs closing K<sub>ATP</sub> channels in β-cells (SUR1/K<sup>ir6.2</sup>) and opening K<sub>ATP</sub> channels in vascular muscles (SUR2B/K<sup>ir6.2</sup>) would be interesting to treat type 2 diabetic patients with hypertension. Agents like minoxidil sulfate (52) and MCC-134 (53) have such a profile, but their opposite effects on the two isoforms of the channel do not occur within the same concentration range. Blockade of the β-cell K<sub>ATP</sub> channel requires concentrations that largely exceed those needed to produce vasorelaxation.

**Drugs interacting with K<sup>ir6.2</sup>.** Many structurally different drugs used for the treatment of diseases other than diabetes occasionally produce hypoglycemia. In vitro studies have shown that they increase insulin secretion by closing β-cell K<sub>ATP</sub> channels through a direct interaction with K<sup>ir6.2</sup>. Among these drugs are antimalarial quinolines such as quinine and mefloquine (54,55), antibacterial fluoroquinolones such as norfloxacin and lomefloxacin (56), antiarrhythmic agents such as disopyramide and cibenzoline (57–59), and others (60).

More interest has been paid to drugs with an imidazoline structure (phentolamine, antazoline, midaglizole), which also inhibit K<sub>ATP</sub> channels in β-cells (61–63) by interacting with K<sup>ir6.2</sup> (64). This effect largely explains their stimulation of insulin secretion. However, efaroxan and novel imidazoline compounds also or exclusively act on an amplifying pathway, as will be discussed below.

From a mechanistic point of view, it is unimportant whether drugs close K<sub>ATP</sub> channels directly by an interaction with the pore formed by K<sup>ir6.2</sup> or indirectly by an interaction with the regulatory subunit SUR1. The net result is the same: membrane depolarization, influx of Ca<sup>2+</sup>, and generation of the triggering signal. The major difference, however, is the distribution of the two targets. The much more restricted distribution of SUR1 than K<sup>ir6.2</sup> considerably increases the tissue specificity of the drugs acting through it. Moreover, drugs closing K<sup>ir6.2</sup> directly usually also affect other channels (61).

**Site 3: Increasing the triggering signal without acting on K<sub>ATP</sub> channels.** In vitro, all depolarizing agents increase insulin secretion regardless of the mechanism underlying the depolarization. However, this approach has limited clinical applications notably because of the difficulty to achieve tissue selectivity. A further underestimated problem is that the depolarizing action of a substance may be markedly dependent on the electrical resistance of the β-cell membrane, which is essentially determined by the extent of K<sub>ATP</sub> channel closure. One instructive example is that of arginine and other cationic amino acids that depolarize β-cells because of their entry in a positively charged form (Fig. 1). The effects of arginine on membrane potential and [Ca<sup>2+</sup>]<sub>i</sub> increase with the glucose concentration (65), which partially accounts for the glucose dependency of arginine-induced insulin secretion. Defective closure of K<sub>ATP</sub> channels in diabetic β-cells may be expected to impair the production of a triggering signal by agents that depolarize by activating an inward current. The second mechanism explaining the glucose dependency of insulin secretion induced by arginine and related agents is the amplifying action of glucose (5).
Blockers of K\textsuperscript{+} channels other than \(K_{\text{ATP}}\) channels. The major voltage-dependent K\textsuperscript{+} channel in β-cells, \(K_{v} 2.1\), participates in the repolarization of the membrane during spike generation (17). Its blockade augments the influx of Ca\textsuperscript{2+} and has long been shown to increase insulin secretion (66). The wide tissue distribution of these channels is a major limitation in their use as a target for insulin-secreting drugs in vivo. However, because the activity of these channels may be subject to modulation by different subunits and to regulation by hormonal or metabolic signals, it has been speculated that the development of selective blockers of β-cell voltage-dependent K\textsuperscript{+} channels is not unrealistic (17).

Agonists of Ca\textsuperscript{2+} channels. The main voltage-dependent Ca\textsuperscript{2+} channels in β-cells are of the L-type and are inhibited by dihydropyridines, which decrease insulin secretion by lowering the triggering signal [Ca\textsuperscript{2+}], (67). Some dihydropyridines (Bay K8644 or CGP2832) instead act as agonists. Almost 20 years ago they were shown to increase Ca\textsuperscript{2+} influx in β-cells and insulin secretion in vitro (60). Since then, no progress has been made in the field and, because of insufficient tissue selectivity, the development of this class of compounds for clinical use has been discontinued.

Other ionic channels. β-cells are equipped with many other ionic channels that may subtly modulate the changes in membrane potential induced by glucose and other secretagogues. None appears to have a sufficiently important role in stimulus-secretion coupling or a sufficiently restricted tissue distribution to qualify as a target for insulin-secreting drugs.

Inhibition of [Ca\textsuperscript{2+}]-lowering processes. The [Ca\textsuperscript{2+}]\textsubscript{i} rise induced by glucose and other secretagogues must be counterbalanced by Ca\textsuperscript{2+} sequestration in intracellular organelles and by Ca\textsuperscript{2+} extrusion to the extracellular space. These tasks are achieved by several Ca\textsuperscript{2+}-ATPases and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchangers (68–70). Inhibition of one or several of these processes is followed by a variable rise of [Ca\textsuperscript{2+}]\textsubscript{i} and increase in insulin secretion (70,71). However, should effective blockers of the plasma membrane Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger become available, tissue selectivity will still remain a serious issue. Moreover, the endoplasmic reticulum stress provoked by blockade of intracellular Ca\textsuperscript{2+}-ATPases is likely to have deleterious consequences (72) that preclude this approach.

Site 4: Activation of amplifying pathways
Nutrient-mediated amplification. All measures resulting in acceleration of β-cell metabolism (see above) increase insulin secretion by a dual action on the triggering and amplifying pathways. It is accepted that the ATP/ADP ratio serves as a second messenger in the generation of the triggering signal not only because its changes occur over a wide physiological range of glucose concentrations (73), but also because the transducing molecules (SUR1 and K\textsubscript{ATP}(6,2) are known. In contrast, the molecular mechanisms of the amplifying pathway remain unclear, and the possibility that several factors are involved cannot be ruled out (Fig. 1). Our proposal (4,73) that variations in the ATP/ADP ratio (or associated changes in AMP or the GTP/GDP ratio) are implicated suffers from one shortcoming: the effector molecules have not been identified, so that suggestions for the development of insulin-releasing drugs specifically acting through that pathway are premature. One candidate effector is the SUR1-like protein of the insulin granule membrane (42). Whereas this protein is unlikely to contribute to the insulin-releasing action of sulfonylureas (see above), it might participate in the amplifying action of glucose and could thus become the target of novel drugs. SUR1 itself is also present in the granule membrane (74), but the amplification of insulin secretion by nutrients is not altered in its absence (44). Another possible target is AMP-activated protein kinase.

Inhibitors of AMP activated protein kinase. Islet AMP kinase activity is inhibited by glucose, which raises the possibility that the enzyme participates in a long- or short-term control of β-cell function, including insulin secretion (75). Activation of AMP kinase by AICAR or overexpression of a constitutively active form of AMP kinase-o1 inhibited glucose-stimulated insulin secretion but also virtually abolished glucose metabolism and the [Ca\textsuperscript{2+}]\textsubscript{i} rise in β-cells (75), making it difficult to identify the site(s) of action. Conversely, a dominant negative form of AMP kinase increased basal insulin secretion, but this increase was Ca\textsuperscript{2+}-independent (75), which is in sharp contrast with all effects of glucose (4). Thus, it is premature to suggest that β-cell selective inhibitors of AMP kinase might become insulin secretagogues.

On the other hand, because the widely used metformin can activate AMP kinase (76), it is important to discuss whether the drug exerts deleterious effects in β-cells. Culture of human islets for 16 h in the presence of 1 mmol/l metformin (~100-fold the plasma concentration in patients) (77) virtually abolished glucose-induced insulin secretion (78), whereas no effect was observed after culture with 20 μmol/l metformin (79). Permeation of metformin into cells is admittedly slow, but the key unanswered question is whether in vitro exposure to a very high or to a therapeutic concentration of metformin for a few hours only is equivalent to chronic exposure to the drug in vivo. When patients are treated with metformin, their plasma insulin level often decreases. This is attributed to improvement of the metabolic state rather than to inhibition of insulin secretion by the drug (77). This interpretation is supported by a study showing that 2 weeks of metformin administration to subjects without glucose intolerance did not change their insulin response to a hyperglycemic clamp (80).

Inhibition of 11β-hydroxysteroid dehydrogenase type 1. Glucocorticoids impair glucose homeostasis mainly by opposing the effects of insulin on hepatic glucose production and peripheral glucose uptake. Although plasma insulin is elevated in glucocorticoid-induced insulin resistance, the direct effect of glucocorticoids on insulin secretion is inhibitory (81). Overexpression of the glucocorticoid receptor in β-cells leads to hyperglycemia and hypoinsulinemia after several months (82). The mechanisms of this inhibition of insulin secretion may be multifactorial (82), but interference with the amplifying action of glucose is probable (81). Local transformation of inactive cortisone into active cortisol by 11β-hydroxysteroid dehydrogenase type 1 could play a pathogenic role in the metabolic syndrome, and inhibition of the enzyme is envisaged as a potential therapy in type 2
diabetes and obesity (83,84). 11β-Hydroxysteroid dehydrogenase type 1 is present in mouse and human islets (85), and its activity is increased in proportion to hyperglycemia in the islets from diabetic ZDF fa/fa rats (86). Inhibition of the enzyme in mouse islets prevents precursors of active corticosteroids from decreasing insulin secretion in vitro (85). This approach deserves further investigation.

**Calcium sensitizers.** Drugs are being developed with the aim of increasing the action of Ca$^{2+}$ on contractile proteins, in particular to improve myocardial performance (87). One of these, pimobendan, increases insulin secretion by isolated rat islets. Because it remains effective when β-cell [Ca$^{2+}$],i is clamped by high KCl, the drug indeed behaves like a Ca$^{2+}$ sensitiser (88). However, this study did not investigate whether the effect could be mediated by the phosphodiesterase inhibition that pimobendan can also cause (87). Effects of levosimendan, another available Ca$^{2+}$ sensitiser, on the endocrine pancreas have not been reported.

**Agents acting on the cAMP pathway.** Cyclic AMP is a potent amplifier of insulin secretion (14,18,89). Inhibition of its degradation by methylxanthines has long been known to increase plasma insulin concentrations (90). In β-cells, this degradation is mainly achieved by phosphodiesterase 3B, the inhibition of which strongly augments insulin secretion in vitro (89). Conversely, overexpression of phosphodiesterase 3B in β-cells reduces in vivo and in vitro insulin secretion in response to glucose and glucagon-like peptide-1 (GLP-1) and impairs glucose tolerance (91). Unfortunately, phosphodiesterase 3B is present in many other tissues, including hepatocytes and adipocytes where its blockade exerts anti-insulin effects. There is presently no evidence that cAMP degradation can be selectively prevented in β-cells by pharmacological agents (89). Hormones acting on β-cell membrane receptors linked to adenylyl cyclase are more promising insulin secretagogues (see below).

As already mentioned, the increase in insulin secretion produced by cAMP is not exclusively mediated by PKA but also involves GEF/Epac (20). Novel cAMP analogs activate GEF/Epac selectively and increase insulin secretion in vitro (16,43). However GEF/Epac is operative in other tissues, and it is uncertain whether some of these analogs will show enough tissue selectivity to be of therapeutic interest.

**Imidazolines and the PKC pathway.** The interest in imidazoline compounds (such as phentolamine) as potential insulin secretagogues started with the idea that their ability to block α$_2$-adrenergic receptors could relieve diabetic β-cells from a tonic sympathetic inhibition (92,93). Subsequently, it was shown that the increase in insulin secretion that phentolamine, efaxoan, and other imidazolines produce in vitro and in vivo is largely mediated by a blockade of K$_{ATP}$ channels in β-cells (see above) rather than of α$_2$-adrenergic receptors (61–63,94).

However, different experimental approaches and the development of novel compounds have shown that the mode of action of imidazolines is complex. Compound KU14R, the imidazol analog of efaxoan, does not interfere with the action of the latter on K$_{ATP}$ channels, yet it inhibits its action on insulin secretion (95). Compound RX871024 increases insulin release from permeabilized β-cells and from islets depolarized with KCl, two preparations in which [Ca$^{2+}$],i is clamped (96). A novel imidazoline (BL11282) has no effects on K$_{ATP}$ channels but increases insulin secretion in vitro (97). Three compounds, RX871024 (98), efaxoan (N.G. Morgan, personal communication), and BL11282 (S. Efendic, personal communication) increase insulin secretion from β-cells deficient in K$_{ATP}$ channels. All these observations point to an intracellular site of action of imidazolines. This site is still unidentified and has tentatively been designated “I$_1$-imidazoline binding site” (62). A number of experimental arguments suggest that it is involved in the amplification of insulin secretion by PKA and mainly PKC (62,97) and, thus, not in the amplifying pathway of glucose, which is independent of these kinases (4). Although structurally unrelated to imidazolines, β-carbolines might be endogenous ligands of the “imidazoline binding sites.” Interestingly, two β-carbolines, harmine and pinoline, possess insulin-releasing properties in vitro (99).

In summary, the family of insulin-releasing imidazolines is functionally heterogeneous. Older members (phentolamine, antazoline) owe their property to blockade of K$_{ATP}$ channels (triggering pathway), whereas newer ones (BL11282) increase the action of Ca$^{2+}$ on exocytosis (amplifying action). Because these novel compounds do not produce a triggering signal, their effect on insulin secretion is more strongly glucose dependent (97). However, the advantage of this gain in safety could be cancelled by loss of tissue specificity. Identification of their intracellular site of action (I$_1$-site) and investigation of their possible effects on kinases in other tissues are necessary before deciding whether imidazolines have a future as insulin secretagogues.

**Site 5: Inhibitory and stimulatory membrane receptors**

**Antagonists of inhibitory receptors.** β-Cells are equipped with a number of inhibitory receptors whose activation decreases insulin secretion. The major ones are the α$_2$-adrenergic, galanin, and somatostatin receptors (100). Their inhibitory effects are mediated by complex mechanisms that include partial repolarization with a small decrease in [Ca$^{2+}$],i, inhibition of adenylyl cyclase with suppression of the amplifying effects of cAMP, as well as a poorly explained but major interference with the action of Ca$^{2+}$ on exocytosis (21).

The use of antagonists of inhibitory receptors could be justified if tonic activation of these receptors contributed to the impairment of insulin secretion in pathological states. Studies in the 1970s suggested that excessive adrenergic activity could impede insulin secretion in type 2 diabetic patients (92,93), but this was not confirmed (101). Moreover, the plasma insulin increase that some α$_2$-adrenergic blocker (e.g., phentolamine) produce is now ascribed to a direct inhibition of K$_{ATP}$ channels (see above). Finally, because adrenergic inhibition of insulin secretion is a safeguard against hypoglycemia during exercise, blockade of this action may not be without risk.

Effects of antagonists of somatostatin or galanin receptors on insulin secretion have not been reported. Mice with a general knock-out of the somatostatin receptor type 5, the major type in β-cells, do not show significant
alterations of in vivo insulin secretion and glucose homeostasis. However, when their pancreas is perfused in vitro, insulin secretion is increased compared with controls, particularly in the basal state (102). Inactivation of the galanin gene unexpectedly reduces insulin secretion in vivo and impairs glucose tolerance. The inhibition of insulin secretion persists in vitro (103). Overall, there are no convincing arguments suggesting that blockade of somatostatin or galanin receptors in β-cells could be useful for increasing insulin secretion in type 2 diabetic patients.

**Agonists of stimulatory receptors.** β-Cells possess numerous membrane receptors for stimulatory hormones or neurotransmitters, and several have already been considered as potential therapeutic targets, but only few may qualify.

Acetylcholine is a physiologically important and potent amplifier of insulin secretion, but its effects in β-cells are mediated by muscarinic receptors of the M3 type, whose characteristics and tissue distribution are such that selectivity of an agonist for insulin secretion is most unlikely (15).

Extracellular glutamate increases insulin secretion from the perfused pancreas by acting on AMPA receptors, and improves insulin secretion and glucose tolerance in vivo in rats (104). However, this approach is unlikely to be useful because of rapid desensitization of the receptor and undesirable extrapancreatic effects of glutamate or analogs.

Activation of purinergic receptors of the P2Y type increases insulin secretion from rodent and human islets. It is unclear, however, whether agonists with sufficient β-cell selectivity can be designed (105).

Pituitary adenylate cyclase–activating polypeptide (PACAP) exerts its effects through a PACAP-prefering receptor (PAC1) and two receptors shared by vasoactive intestinal peptide (VPAC1 and VPAC2). VPAC1 mediates stimulation of glucose production by the liver, whereas VPAC2 in β-cells mediates stimulation of insulin secretion (106). A synthetic agonist selective for VPAC2 increased plasma insulin and improved glucose disposal in normal rats (107). Confirmation of these effects in models of type 2 diabetes and demonstration that no side effects occur remain to be seen.

Glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 are the two major intestinal hormones released during meals that strongly potentiate nutrient-induced insulin secretion (incretin effect) (108). In type 2 diabetic patients, secretion of GIP is normal or slightly increased, whereas GLP-1 is impaired but its action is preserved in type 2 diabetic patients. Therapeutic substitution may thus be justified (109,110). The situation is very different with GLP-1. Its secretion is impaired but its action is preserved in type 2 diabetic patients. Therapeutic substitution may thereby be justified and has indeed already proved successful, in particular when the short half-life of the peptide is extended (109–111). Marked glucose dependency is an important feature of GLP-1 effects on insulin secretion. The increase in cAMP produced by GLP-1 activates a PKA- and GEF/Epac-dependent amplifying pathway (14,16,19) that augments the efficacy of [Ca^{2+}]i, the rise of which depends on glucose. Second, despite some inhibitory action on K_{ATP} channels, GLP-1 hardly produces a triggering signal at low glucose for two reasons. It mainly acts on ionic channels (voltage-dependent Ca^{2+} and K^{+} channels) that become operative only when the β-cell membrane has been depolarized by glucose (19). Moreover, the mobilization of intracellular Ca^{2+} that GLP-1 promotes results from a Ca^{2+}-induced Ca^{2+} release, which itself depends on glucose-induced influx of Ca^{2+} from the extracellular medium (14,16). This glucose dependency of GLP-1 stimulation of insulin secretion might however be lost if the hormone were combined with a potent and long-acting sulfonylurea.

**Site 6: Nuclear receptors.** Several nuclear receptors, including the liver X receptor (LXR) and the peroxisome proliferator–activated receptors (PPARs), play a central role in lipid and carbohydrate metabolism in liver, muscles, and adipose tissue (112,113). Their importance for β-cell function is less clear. However, preliminary evidence suggests that activation of LXR in β-cells may influence insulin secretion (114).

PPAR-α and -γ are moderately expressed in rodent and human islets (30,115–117) and may exert a long-term influence on insulin secretion by controlling the expression of enzymes involved in fuel metabolism (118). Their role in β-cell adaptation to metabolic perturbations (e.g., insulin resistance) is under investigation, but it is known that PPAR-α is downregulated and PPAR-γ upregulated in islets from Zucker diabetic fatty rats (116). Similar changes are induced by exposure to hyperglycemia in vivo or in vitro (30,117). Two families of clinically useful compounds, fribates and thiazolidinediones, are synthetic ligands of PPAR-α and -γ, respectively. Although they are not prescribed with the specific aim of changing insulin secretion, such changes do occur. It is not always easy to determine if these effects are indirect or direct.

**PPAR-α and fibrates.** An acute (<1 h) increase of insulin secretion by fribates has been observed in vitro, but neither the mechanisms nor the specificity of the effect has been assessed (119). Culture of normal rat islets with fribates increased PPAR-α expression and exerted variable effects on enzymes of pyruvate metabolism and fatty acid oxidation, as well as on insulin secretion (116,119). The interpretation of these experiments is problematic because supratherapeutic drug concentrations were used.

In vivo treatment of normal rats with a PPAR-α agonist for 24 h increased PPAR-α and pyruvate dehydrogenase kinase 4 expression in islets, but did not affect glucose-induced insulin secretion ex vivo (120). A similar treatment exerted distinct effects in two models of insulin resistance. Hypersecretion of insulin by islets from high-fat-fed rats was reversed (120), whereas that from pregnant rats was unaffected (121), suggesting an extra-islet site of action of the drug.

**PPAR-γ and thiazolidinediones.** Treatment with thiazolidinediones is often accompanied by improved glucose-stimulated insulin secretion in type 2 diabetic patients and various animal models of the disease (118). In vitro studies are contradictory. Troglitazone and rosiglitazone have been reported to induce a glucose-dependent increase in insulin secretion by HIT cells, rat islets, or the perfused rat pancreas (122–124). These acute effects were too rapid
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