

Editorial

Impact of Treatment on Islet Function in Type 2 Diabetes

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Five years already! We, the members of the International Group on Insulin Secretion (IGIS), are very happy that the Servier-IGIS Symposium on insulin secretion and islet pathophysiology, which we first organized in 2000, has, beyond our expectations, become a traditional, awaited, and much appraised event. The pleasant venue and climate of the French Riviera in early spring undoubtedly contribute to the attractiveness of the symposium. However, we trust that the major reason for its success is the high quality of the presentations and scientific exchanges. These standards extend to the rapid publication of most contributions as refereed and edited review or original articles in a series of supplements to *Diabetes* (1–4). Both the symposium and the publication are made possible by a generous, unrestricted educational grant from Les Laboratoires Servier (Paris).

This fifth edition focused on therapeutic approaches of type 2 diabetes and their impact on islet function. Although their hypoglycemic properties had already been recognized in 1942, sulfonamides (carbutamide) started to be used in the treatment of type 2 diabetes in 1955 (5). If millions of diabetic patients have successfully been treated with sulfonylureas for 50 years, it is because these compounds luckily hit a key regulatory site of insulin

secretion, the ATP-sensitive K⁺ channel (K_{ATP} channel) that was to be discovered about 30 years later (6,7) and whose structure was identified only 10 years ago (8,9). Several sessions of the symposium were dedicated to this target, its role in various tissues, and the perspective of designing novel drugs with high selectivity. Alternative ways to stimulate deficient insulin secretion were discussed as well as the impact that treatment, whether directly targeting β -cells or not, may have on islet function and progression of diabetes. Whenever possible and relevant, the contributors bridged our increasingly sophisticated knowledge of β -cell pathophysiology to more clinical aspects of diabetes.

Section I emphasizes that type 2 diabetes is a major manifestation of the metabolic syndrome. Since this syndrome is a systemic disease, affecting multiple organs, a systems-level analysis is important both for understanding the pathophysiology of the disease and for optimization of its treatment. In patients with the metabolic syndrome, the body's inherent dynamics that ensure robustness against unstable food supply are disturbed. The metabolic and hormonal perturbations leading to diabetes develop slowly. Thus, a model is proposed that defines five stages of evolving β -cell dysfunction during the progression to diabetes. In stage 1, insulin secretion is increased to maintain normoglycemia in the face of insulin resistance. In stage 2, glucose levels start to rise, due to reduction in the β -cell mass and disruption of function. Stage 3 is a transient unstable period of early decompensation, while stage 4 is characterized by a significant reduction in β -cell mass, eventually leading to the severe decompensation of stage 5. Some morphometric studies on postmortem pancreata from patients with type 2 diabetes have measured a 50% reduction in the β -cell mass, but it should be pointed out that the extent of decrease is clearly smaller in other series. Although it is often assumed that apoptosis is responsible for β -cell loss in type 2 diabetes, limitations in β -cell replication and/or neogenesis could be just as important. Whatever the magnitude of the changes in β -cell mass, there is little doubt that a major β -cell dysfunction contributes to the insufficient secretion of insulin in type 2 diabetes. For example, fasting and stimulated proinsulin levels are disproportionately elevated relative to insulin levels. It is reported that reducing β -cell secretory load with somatostatin decreased (but did not normalize) the proinsulin-to-insulin ratio in type 2 diabetes. These findings suggest that an intrinsic β -cell abnormality could increase the proinsulin-to-insulin ratio and that the anomaly is exacerbated when the demand of

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AMPK, AMP-activated protein kinase; [Ca²⁺]_i, cytosolic Ca²⁺; DPP-IV, dipeptidyl-peptidase IV; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IGIS, International Group on Insulin Secretion; K_{ATP} channel, ATP-sensitive K⁺ channel; LXR, liver X receptor; NBD, nucleotide-binding domain; PPAR, peroxisome proliferator-activated receptor; SOCS, suppressors of cytokine signaling; SREBP, sterol regulatory binding protein; SUR1, sulfonylurea receptor 1.

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insulin secretion is so important that it leads to reduction of insulin stores.

An important focus of section I is on β -cell function in obesity, with special emphasis on the impact of weight loss. In nondiabetic subjects, obesity is associated with a modest expansion of β -cell mass, possibly amounting to 10–30% for each 10 kg of overweight. Both fasting insulin secretion and the total insulin response to orally administered glucose increase with BMI in an almost linear fashion. BMI exerts a positive effect that is distinct from that of insulin resistance, i.e., obesity may be a state of primary insulin hypersecretion. In contrast, dynamic β -cell functions—glucose sensitivity, i.e., dose-response function, rate sensitivity, and potentiation—do not seem to be altered by obesity, body fat distribution, or insulin resistance, provided that glucose tolerance is preserved. In subjects with normal glucose tolerance and a genetic predisposition to the development of diabetes, β -cell dysfunction appears before insulin resistance is detectable. When present, insulin resistance can be mostly attributed to obesity and/or reduced physical fitness. Accordingly, three prospective intervention studies have demonstrated the efficacy of lifestyle modification, including diet and exercise, in reducing the probability of type 2 diabetes in a high-risk population with impaired glucose tolerance. When diabetes is established, particularly in subjects with vascular damage, behavior treatment is usually combined with multidrug therapy. In the Steno-2 study, patients were randomized to conventional treatment with their general practitioner, following national guidelines, or to intensified multifactorial intervention by a diabetes team at the Steno Diabetes Center, Copenhagen, integrating both behavior modification and multidrug therapy. Eight years of intensified multifactorial intervention resulted in the significant relative risk reduction of 53% (absolute risk reduction 20%) for the composite endpoint of cardiovascular disease.

Section II starts with a brief review of stimulus-secretion coupling in β -cells and identifies six major pathways or sites that could be targets of potential insulin-secreting drugs. Strategies to reach these targets are described, and their feasibility is discussed. Much emphasis is put on approaches that, unlike all currently used insulin-releasing drugs, would not be aimed at the K_{ATP} channel. All drugs closing K_{ATP} channels eventually produce a similar increase in the concentration of cytosolic Ca^{2+} ($[Ca^{2+}]_i$), which serves as a triggering signal. It is becoming increasingly evident that regulation of exocytosis is also exerted at sites distal to this $[Ca^{2+}]_i$ rise (amplifying pathway). Although the molecular mechanisms are still incompletely understood, the effector proteins and their interactions with metabolic and second messenger signals are progressively being identified. ATP is one of these partners and could thus play a dual regulatory role at the K_{ATP} channel (triggering pathway) and at the sites of exocytosis (amplifying pathway). In addition, extracellular ATP can also potentiate insulin secretion by binding to membrane purinergic receptors of the P2Y type. Previous studies showed that this action was accompanied and possibly caused by changes in β -cell $[Ca^{2+}]_i$. However, the coupling is probably more complex as suggested by the persistence of a potentiating action under conditions where $[Ca^{2+}]_i$ can be expected to be clamped.

It has recently been proposed that the AMP-activated protein kinase (AMPK) could participate in the amplifying pathway by facilitating the recruitment of insulin granules. In many tissues, AMPK is a key sensor of cellular energy, and the suggestion that it participates in fuel regulation of insulin secretion is attractive. Previous supportive evidence is reviewed and novel data on correlations between amino acid effects on AMPK activity and insulin secretion are presented. Not all pieces of the puzzle neatly fit, however. It is particularly puzzling that knockout (KO) of neither the $\alpha 1$ isoform (predominant in islets) nor the $\alpha 2$ isoform of the enzyme altered insulin secretion from isolated islets. Double KO in β -cells should provide the answer. Regardless of the exact physiological role of AMPK in β -cells, it is important to bear in mind that its pharmacological activation, a potentially novel therapeutic approach of type 2 diabetes, inhibits insulin secretion. This unwanted effect must be balanced with the beneficial actions that are expected from activation of the enzyme in the liver (inhibition of gluconeogenesis) and skeletal muscles (increase in glucose uptake).

A second focus of section II is on the role of nuclear receptors in islet function, with a special emphasis on liver X receptors (LXRs) and peroxisome proliferator-activated receptor (PPAR)- γ . Activation of LXRs decreases blood glucose in rodent models of type 2 diabetes by decreasing gluconeogenesis and increasing glucose uptake in adipose tissue. LXR is expressed in islet cells, and LXR agonists are reported to stimulate insulin biosynthesis and secretion. PPAR- γ is also expressed in β -cells, and in vivo treatment with thiazolidinediones improves glucose-stimulated insulin secretion in animals and humans with type 2 diabetes. However, it remains disputed whether this improvement results from a direct action of thiazolidinediones in β -cells. Arguments supporting this hypothesis are presented together with data showing that PPAR- γ ligands (agonist and antagonist) prevent the effects of fatty acids on insulin secretion. Finally, section II discusses the roles of HNF-1 α (hepatocyte nuclear factor-1 α), sterol regulatory binding proteins (SREBPs), and suppressors of cytokine signaling (SOCS), in the regulation of β -cell function and gene expression. Mice with targeted suppression of β -cell HNF-1 α demonstrate marked glucose intolerance due to impaired insulin response, whereas nonglucose secretagogues remain effective. Forced overexpression of the 1c isoform of SREBP leads to accumulation of triglycerides in and inhibition of glucose-stimulated insulin secretion from pancreatic islets and β -cell lines. SREBP1c-induced genes include those involved in cholesterol, fatty acid, and eicosanoid synthesis. Proinflammatory cytokines induce β -cell death by acting alone or in combination. Interleukin-1 β provokes desensitization of insulin signaling in β -cells, which potentially alters β -cell survival. The interconnection between interleukin-1 β and insulin signaling pathways in β -cells is not dependent on NO production but is mediated by induction of SOCS-3 expression, which belongs to the SOCS family of inhibitory proteins.

β -cell K_{ATP} channels are heterooctameric complexes of the sulfonylurea receptor 1 (SUR1), acting as a regulatory subunit, and of an inwardly rectifying K^+ channel Kir 6.2, forming the pore. Section III reports recent progress in our understanding of the β -cell K_{ATP} channel architecture and

regulation. SUR1 belongs to the family of ATP-binding cassette transporters. They all possess two conserved transmembrane domains and two nucleotide-binding domains (NBDs), but SUR1 is characterized by the presence of an additional N-terminal *trans* membrane domain (TMD0) that ensures the interaction with Kir 6.2. Interactions between the two NBDs and with other parts of the molecule are shown to form pockets for Mg-nucleotides. This novel information helps understanding distinct properties of the two NBDs. Most current models ascribe the control of K_{ATP} channels to changes in the cytosolic ATP-to-ADP ratio, with ATP closing the channel by acting on Kir 6.2 and MgADP opening the channel by acting on SUR1. This view is an oversimplification that ignores the importance of the absolute concentrations of both nucleotides (not only their ratio) and the modulation of their action by agents such as phosphoinositides and long-chain acyl CoA esters. Slow hydrolysis of MgATP into MgADP at NBD2 might also play a role in the opening of the channel. A novel suggestion is that Kir 6.2 itself might sense changes in the concentration of ATP. This refined structural picture of the channel is expected to facilitate interpretation of the impact of mutations or polymorphisms associated with decreased (hyperinsulinism) or increased (type 2 diabetes) activity of the channel. Identification of the different sites of drug binding to SUR1 also opens the prospect of designing optimal compounds to open or close K_{ATP} channels.

However, extrapolation of modeling information or experimental observations to the clinical situation is not always straightforward. The therapeutic usefulness (efficacy and safety) of even very active compounds depends on their pharmacokinetic properties. It is also a common practice observation that the therapeutic efficacy of oral agents stimulating insulin secretion tends to decrease with time in a number of patients. Importantly, escalation in the administered doses is often counterproductive, whereas temporary drug withdrawal may prove useful. Desensitization of β -cells by prolonged exposure to sulfonylureas or other agents stimulating insulin secretion by closure of K_{ATP} channels has long been produced *in vitro*, but its cellular and molecular mechanisms are only incompletely elucidated. A first pitfall is the confusion of desensitization with functional alterations caused by the persistence of lipophilic secretagogues that accumulate in β -cells. Lack of distinction between desensitization and exhaustion of insulin stores also often confuses the issue. Finally, the low specificity of the desensitization induced by depolarizing drugs is striking. Thus, cross-desensitization occurs between sulfonylureas and imidazolines, two families of structurally distinct agents closing K_{ATP} channels by binding to SUR1 and Kir 6.2 subunits, respectively. Most importantly, a decrease in drug efficacy during chronic treatment may also result from the progression of the disease rather than desensitization to the drug.

K_{ATP} channels are not the exclusive hallmark of pancreatic β -cells. Several isoforms of SUR (SUR1, SUR2A, and SUR2B) and Kir 6.x (Kir 6.1 and Kir 6.2) exist and variably combine to form K_{ATP} channels with properties distinct from those of the SUR1-Kir 6.2 channels. This variety of structure and broad distribution of K_{ATP} channels permits selective metabolic sensing in various tissues. However,

whereas β -cell K_{ATP} channels play a clear regulatory role under physiological conditions (their activity changes with physiological variations in blood glucose), it is much less evident whether K_{ATP} channels are constantly operative or are recruited only under stress conditions in other tissues. Several of these questions are addressed in section IV.

Distinct sulfonylureas and glinides show markedly different affinity and selectivity for the β -cell K_{ATP} channel. How these various molecules interact with SUR1 has largely been identified by concordant electrophysiological and binding studies, so that consensus models can now be proposed. Similar experimental approaches also quantify the tissue selectivity of drugs, a major issue in therapeutics. Careful characterization of the action of insulin-releasing drugs on different K_{ATP} channels (different isoforms of SUR) is important to assess possible untoward effects on nontargeted tissues, particularly the heart. Although the UKPDS (U.K. Prospective Diabetes Study) clearly showed that there was no difference in cardiovascular outcome between patients on sulfonylureas or insulin, some concern remains that sulfonylureas might interfere with cardiac preconditioning and coronary blood flow. These questions are critically discussed, with emphasis on the limited direct clinical evidence supporting these concerns and absence of evaluation taking into account the tissue specificity of action of different drugs. A further degree of complexity is linked to the incomplete, when not controversial, understanding of the role of the K_{ATP} channels in coronary arteries and cardiomyocytes. Mouse models with a KO of Kir 6.2 were instrumental to progress in this area.

In the heart, the major recognized function of K_{ATP} channels is a protective role during ischemic insult. Recent experiments using a moderate swimming protocol in mice indicate that metabolic and cardiovascular benefits of exercise require K_{ATP} channels. In contrast to wild-type animals, Kir 6.2-KO mice do not show loss of body weight (white fat mass), decrease in fasting plasma glucose, decrease in resting heart rate, and superior physical performance. On the other hand, their muscle damage after exercise and mortality by impaired cardiac contractile function are increased. Related studies show that K_{ATP} channels are necessary for normal cardiac repolarization under adrenergic stress. Alterations of the functioning of cardiac K_{ATP} channels constitute a previously unrecognized risk factor for the development of catecholamine-triggered arrhythmia.

K_{ATP} channels of the β -cell type are present in several tissues, including the hypothalamus and the other cells of the islet. This implies that, in addition to its direct impact on the control of insulin secretion, a KO of Sur1 or Kir 6.2 may also influence glucose homeostasis through an impact on the central nervous system. Moreover, because the distributions of SUR1 and Kir 6.2 are not identical, KO of one of the two subunits may exert effects different from the KO of the other. Inactivation of Kir 6.2 ablates K_{ATP} channels (SUR2A-Kir 6.2) in skeletal muscle, resulting in increased insulin-mediated glucose uptake, which does not occur after KO of Sur1.

The cellular and molecular basis of the derangements of glucagon secretion in type 2 diabetes are not known, largely because stimulus-secretion coupling in α -cells is

difficult to study. In spite of much recent progress, the many novel pieces of information obtained do not seem to fit into a coherent picture. The inhibitory action of glucose has been attributed to paracrine effects exerted by products released by β - or δ -cells and to direct effects on α -cells. One mechanism of the latter involves K_{ATP} channels. Within a narrow window of activity they serve a much more subtle function than in β -cells. Mild closure by high glucose and mild opening by low glucose would result in $[Ca^{2+}]_i$ changes that are paradoxically opposite to those in β -cells due to the characteristics of the other channels present in α -cells. The absence of inhibition of glucagon secretion by glucose in Sur1 KO islets is put forward to support the model, but discrepancies with the effect of glucose on $[Ca^{2+}]_i$ in these α -cells and with the preservation of the glucose effect in α -cells from Kir 6.2 KO mice remain unexplained. In other words, the α -cell has not told all its secrets yet.

Section V describes the incretin hormones and explores the possibility of using the incretin approach for diabetes treatment. An incretin is defined as an endocrine transmitter produced in the gastrointestinal tract, which is released by nutrients, especially carbohydrates, and stimulates insulin secretion in the presence of glucose when exogenously infused in amounts not exceeding blood levels attained after food ingestion. Presently, there is a general consensus that glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the main incretins and thus synchronize functions of the gut and the β -cells. GIP and the two forms of GLP-1, GLP-1(7-37) and GLP-1(7-36) amide, are indeed secreted after oral glucose administration or a meal, and increase glucose-induced insulin secretion in normal man at concentrations measured in the plasma after meal. The incretin effect is reduced in patients with type 2 diabetes. GIP is secreted normally or even hypersecreted, but the insulin-releasing action of exogenous GIP is altered for reasons that remain unclear. On the one hand, the insulin response to an acute intravenous bolus of GIP is barely diminished in patients with type 2 diabetes, which seems to exclude major defects in postreceptor signaling. On the other hand, the insulin response to a prolonged GIP infusion is blunted, which points to abnormally rapid desensitization of the receptor. Importantly, nearly 50% of first-degree relatives of type 2 diabetic patients also exhibit a reduced insulin response to exogenous GIP, which suggests that impaired responsiveness to GIP might play an important role in the pathogenesis of the disease. In contrast to GIP, GLP-1 retains normal insulinotropic action in type 2 diabetic patients. Its secretion in response to a meal is only moderately reduced. As this defect is small and not observed in first-degree relatives of the patients, it is viewed as a consequence of poor metabolic control rather than a primary abnormality. In addition to its insulinotropic effect, GLP-1 stimulates β -cell growth and insulin synthesis, inhibits glucagon release, and prolongs gastric emptying. This multiplicity of actions confers to exogenous GLP-1 a marked antidiabetogenic effect in man and animal models of diabetes. However, GLP-1 is rapidly degraded by the ubiquitous enzyme dipeptidyl-peptidase IV (DPP-IV), which cleaves two amino acid residues from the N-terminus and yields an inactive metabolite. Pres-

ently, both DPP-IV-resistant and long-acting GLP-1 analogs are under clinical development. Inhibitors of DPP-IV are also being tested. Orally active compounds increase insulin secretion and virtually correct the markedly impaired glucose tolerance induced in mice by a high-fat diet. Their efficacy has also been shown in patients with type 2 diabetes. A major issue is whether inhibition of the degradation of the many other substrates of DPP-IV will or will not cause unacceptable side effects. Desensitization is another potential problem that might limit the usefulness of exogenous GLP-1 in the treatment of diabetes. Fortunately, although stable and long-acting GLP-1 derivatives do produce desensitization in insulin-secreting cell lines, no significant reduction of its efficacy appears to occur after chronic administration in vivo. Regarding the mechanism of action, it is well documented that GLP-1 agonists increase β -cell cAMP, which then enhances glucose-dependent insulin secretion and synthesis as well as cellular growth and proliferation. The hypothesis is presented that incretin-derived cAMP also participates in the metabolic activation of the mTOR (mammalian target of rapamycin) by mobilizing intracellular Ca^{2+} stores. mTOR is a protein kinase that integrates signals from mitogens and nutrients to regulate cellular growth and proliferation.

GLP-1 is not the sole novel hormonal approach in the treatment of diabetes. Islet amyloid polypeptide (or amylin) is normally cosecreted with insulin. It inhibits glucagon secretion, delays gastric emptying, and promotes satiety. Several large-scale studies have evaluated the interest of pramlintide, a synthetic amylin analog, as a potential adjunctive therapy in patients with type 1 or type 2 diabetes. Pramlintide was found to exert a modest beneficial effect on glycemic control without an increase in hypoglycemic events and weight gain.

The wealth of information contained in this supplement attests to the intensity and dynamism of present-day islet research. Therefore, we feel guardedly optimistic regarding the imminent elucidation of the defects in β -cell function in type 2 diabetes and the prospects of development of more optimal insulin-releasing agents to treat the disease.

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