

Regulation of Insulin Production

In Search of Therapeutic Targets

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This is the Third Servier-IGIS Symposium *Diabetes* Supplement. As members of the International Group on Insulin Secretion (IGIS), we have done the utmost to fulfill the promise made at the First Servier-IGIS Symposium (1): the provision of yearly symposia of the highest scientific significance dealing with insulin secretion, β -cell biology, and islet pathophysiology. These symposia are made possible by a generous unrestricted educational grant from Servier (Paris). This company also manages the complex logistical tasks involved in the organization of these symposia, which are held in a pleasant venue that provides excellent opportunities for scientific exchange.

The Third Servier-IGIS Symposium focused on the 'Regulation of Insulin Production' and explored both genetic and molecular mechanisms behind the control of stimulus-secretion coupling in β -cells. A special emphasis was placed on the regulation of K^+ ATP channels in β -cells and the understanding of cellular mechanisms transducing nutrient and hormonal signals into insulin release. Importantly, this symposium illustrated how a better knowledge of islet biology and pathology has in turn generated novel concepts and compounds for the treatment of type 2 diabetes. The contributions by leading scientists active in these areas constitute the basis of the articles of this supplement; these have been reviewed and carefully edited by the IGIS board and selected referees.

Section 1 is concerned with efforts to discover genes associated with the susceptibility to diabetes mellitus. One identified locus has a major effect on type 1 diabetes susceptibility (IDDM 1), while other loci also have small but significant effects (IDDM 2, IDDM 15). One limitation in studies to date has been the lack of statistical power due

to the inadequate size of collections of affected families. With regard to type 2 diabetes, genome scans in families with multiple affected individuals from several racial/ethnic groups have been performed. In one such study presented at the symposium, a number of potential loci were identified, but the evidence for linkage was not strong and the regions identified were broad. It appears that the approach of linkage disequilibrium mapping, which narrows regions of linkage for complex multigenic disorders such as type 2 diabetes, will be a difficult one. The importance of selecting traits to be subjected to quantitative genetic analysis was emphasized. It was also suggested that type 2 diabetes and obesity could be subjected to a pregenetic dissection of complexity into simpler quantitative traits (QT) based on pathogenetic mechanisms, the time course of the trait, and the individual's age within the predisease period, rather than on descriptive parameters of postdisease diagnosis.

In section 2, the fact is emphasized that of the six monogenic forms of maturity onset diabetes of the young (MODY) identified, all except MODY 2 (glucokinase defects) are associated with transcription factors (HNF-1 α , HNF-4 α , HNF-1 β , Neuro D1). MODY 4 is linked to heterozygosity for mutations in the developmentally important pancreatic duodenal homeobox-1 (PDX-1) gene. In the adult β -cell, PDX-1 has a dose-dependent effect on the expression of the insulin gene and therefore contributes to the maintenance of adequate insulin production and euglycemia. Importantly, a number of transcription factors binding to and modulating the transcriptional activity of regulators of PDX-1 expression have been identified, including HNF-3 β , HNF-1 α , SP1/3, USF, and PDX-1 itself. Since several of these are MODY genes, it is possible that mutations in, or the functional impairment of, other transcription factors that control the expression of PDX-1 in the β -cell could result in additional subtypes of MODY. Understanding the cellular mechanisms that underlie hyperglycemia in MODYs has been facilitated by the study of animal models. A MODY 3 transgenic model was developed through β -cell-targeted constitutive or conditional expression of dominant-negative HNF-1 α . These animals developed hyperglycemia, decreased insulin secretion, and reduced pancreatic insulin content. The conditional suppression of HNF-4 α , the MODY 1 gene, in INS-1 cells showed a phenotype similar to that of HNF-1 α suppression, confirming the existence of a regulatory circuit

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between these two transcription factors. Interestingly, HNF-1 α -deficient mice demonstrated an increased half-life of the sulfonylurea compound glibenclamide, due to a defect in its hepatic uptake, which might explain the hypersensitivity to sulfonylureas in MODY 3 patients.

The K⁺_{ATP} channel is a hetero-octameric complex of two different types of protein subunits: the inwardly rectifying K⁺-channel Kir 6.2 and the sulfonylurea receptor SUR. Section 3 discusses how the presence of SUR 1 in β -cells and of SUR 2 in cardiac and smooth muscle cells confers different drug sensitivities to these cell types. It is suggested that, like other ATP-binding cassette transporters, SUR possesses a large multifaceted drug-binding pocket that can accommodate several structurally distinct compounds that bind to distinct residues. Three common missense single nucleotide polymorphisms in KIR 6.2 have been observed in population studies. While two polymorphisms were without effect on the properties of reconstituted human SUR 1/KIR 6.2 channels, E23K markedly affected channel gating and increased spontaneous open probability. Studies in man and transgenic mice indicate that both the homozygous and the heterozygous states for this mutation are associated with reduced glucose-induced insulin secretion. It is suggested that the relatively common E 23 K polymorphism in KIR 6.2 might be responsible for a significant proportion of type 2 diabetes cases.

Sections 3 through 5 provide a summary of stimulus-secretion coupling in β -cells and review the roles of metabolic and hormonal factors, as well as of pharmacological compounds that regulate insulin secretion. The key role of glucose in sustaining the β -cell and globally regulating its gene expression has been revealed in elegant detail by representational difference analysis or gene microarray analysis. A comprehensive recent study presented herein demonstrates that an optimal glucose concentration is required for maintenance of the glucose-responsive insulin secretory state. β -cells continuously regulate expression of many proteins involved in glucose metabolism, insulin biosynthesis, membrane transport, and signal transduction. In response to glucose stimulation, they also activate pathways for conversion of certain mitochondrial metabolites into lipid intermediates (cataplerosis). The latter may influence β -cell function by affecting lipid and membrane biogenesis and other signaling pathways responsive to lipids, such as protein kinase C and/or nuclear receptors such as HNF family members.

It is generally accepted that glucokinase serves as the glucose sensor in the β -cells. Structure-function studies suggest the presence of a thus far unknown allosteric activator site for this enzyme. A recently discovered glucokinase activator drug is an excellent tool for exploring the role of glucokinase in type 2 diabetes and constitutes a new approach to the treatment of the disease. The complexity in the mechanisms regulating transduction of the glucose signal is illustrated by challenging and original observations that the pyruvate carboxylase-mediated pyruvate cycling pathway is involved in the control of glucose-stimulated insulin secretion. There is a general consensus that long-term elevations in glucose concentrations are progressively deleterious to the islet ('glucotoxicity'). It also has been proposed that elevated levels of circulating and intracellular lipids play an important role

in β -cell failure ('lipotoxicity'). An interesting model of β -cell 'glucolipotoxicity' is presented and suggests that the most important cellular events are alterations in β -cell malonyl-CoA concentration, PPAR α , and sterol regulatory element binding protein (SREBP-1C) expression, and lipid partitioning. In agreement with this hypothesis, overexpression of SREBP-1C in the insulin-secreting cell line INS-1 increased expression of lipogenic enzymes and decreased glucose-stimulated insulin release.

Insulin secretion in response to glucose and other nutrients (amino acids) is potentiated by several neurotransmitters and hormones. At present, glucagon-like peptide-1 (GLP-1) is a major focus of interest because of its strong antidiabetic action, which includes the inhibition of glucagon release, as demonstrated in clinical studies. As discussed in Section V, GLP-1 potentiates glucose-induced insulin secretion by multiple actions, such as antagonism of K_{ATP} channels, potentiation of voltage-dependent Ca²⁺ channels, release of Ca²⁺ from intracellular stores, activation of nonspecific cation channels, and direct effects on exocytosis. However, the most important pathway of GLP-1 action involves G protein-mediated elevation of cAMP and activation of protein kinase A (PKA). Type I PKA is predominantly cytoplasmic, whereas type II PKA is associated with cellular structures and organelles. Evidence presented at this meeting indicates that the localization of type II PKA within the cell is preserved by its association with nonenzymatic scaffolding proteins called A-kinase anchoring proteins (AKAPs). There are more than 50 cloned AKAPs, and these explain the diversity in the cellular effects of cAMP: AKAPs may coordinate signaling pathways by recruiting multiple signaling enzymes near potential substrates, effectively coupling upstream activators with downstream targets. GLP-1 research has stimulated the search for compounds that enhance glucose-induced insulin release and inhibit glucagon secretion. Imidazoline compounds not only inhibit K⁺_{ATP} channels but also enhance the insulinotropic effect of glucose in depolarized β -cells with clamped cytosolic calcium. However, the novel imidazoline BL 11282 does not block K⁺_{ATP} channels but markedly stimulates insulin secretion in depolarized and permeabilized islets. The compound restores impaired insulin response to glucose in islets from spontaneously diabetic GK rats. These studies suggest the possibility of developing new compounds that demonstrate a strong glucose-dependent effect on insulin release and suppress glucagon secretion while minimizing the risk of hypoglycemia.

It is well documented that glucose-stimulated insulin secretion is dependent on Ca²⁺ entry through voltage-gated Ca²⁺ channels. There is also evidence for an important role of intracellular Ca²⁺ stores and their modulation in the β -cell through signal transduction pathways involving the insulin receptor, IRS1 and ER calcium pump (SERCA). These aspects are discussed in sections 4 and 6, which also include a report that cADPR (a metabolite of NAD⁺) and nicotinic acid adenine dinucleotide phosphate (NAADP, a metabolite of NADP⁺) modulate the sensitivity of the endoplasmic reticulum for Ca²⁺ release in pancreatic acinar cells. Since β -cells express the ADP-ribosyl cyclase, which is responsible for production of both cADPR and NAADP, it is proposed that both of these

metabolites are physiologically important in β -cells. It has been suggested that cADPR-mediated mobilization of Ca^{2+} from the ER may play an ancillary role in glucose-induced insulin secretion. The hypothesis is presented that the diabetogenic agents alloxan and streptozotocin induce DNA strand breaks. This increases poly(ADP-ribose) polymerase (PARP) activity and results in the consumption of β -cell NAD^+ , decreases in cADPR, and lethal necrotic injury to β -cells. Accordingly, the inhibition of PARP activity may be a possible therapeutic target in the treatment of diabetes. The leukocyte surface antigen CD38 has ADP-ribosyl cyclase activity. Autoantibodies against CD38 have been described in 10–12% patients with type 2 diabetes. The data presented demonstrate that exposure of human islets to sera positive for anti-CD38 autoantibodies results in increased, and subsequently decreased, insulin release and decreased islet viability. This finding illustrates not only the possible role of cADPR in the regulation of insulin release, but also the impact of ER stress on β -cell function. Evidence is also presented in this section that mutations affecting the ER stress-activated

PERK kinase and its downstream effector, the translation initiation complex eIF2, have a profound effect on islet cell development, function, and survival.

We hope that this supplement has succeeded in condensing forefront research on the genetics of diabetes and the cell biology of stimulus-secretion coupling in β -cells. This approach has facilitated presentation of novel concepts concerning the pathogenesis of impaired insulin secretion in type 2 diabetes and novel strategies for the development of new drugs for treatment of the disease.

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