Insulin secretion and β-cell biology are the focus of the Servier-IGIS Symposia that have been held yearly since 2000 in St. Jean Cap Ferrat in southern France (1). This volume, the fourth of a series published in Diabetes (1–3), collects the proceedings of the fourth Servier-IGIS Symposium, which focused on novel factors involved in islet biology and β-cell function. Special emphasis was placed on nuclear receptors, new genes that are associated with type 2 diabetes, and the role of mitochondria and lipid modulators in islet function. This symposium offers a striking example of how better knowledge of β-cell biology and the introduction of genetic tools to explore the pathophysiology of type 2 diabetes complement each other to improve our understanding of glucose homeostasis.

Section I details the role of nuclear receptors in islet function, with special emphasis on peroxisome proliferator–activated receptors (PPARs). PPARα was cloned in 1990; related receptors were subsequently cloned, namely PPARδ (or δ) and PPARγ. They form heterodimers with retinoid X receptor (RXR); their natural ligands are fatty acids and lipid-derived substrates. These receptors are major regulators of lipid metabolism, linking availability of glucose and lipids and long-term metabolic adaptation. Most importantly, they are targeted by key drugs used in treatment of hyperlipidemias and diabetes, namely fibrates and thiazolidinediones. Thiazolidinediones are synthetic PPARγ agonists developed to improve glucose tolerance by enhancing insulin sensitivity and restoring β-cell function. Patients with dominant-negative PPARγ mutations develop severe hyperglycemia, and PPARγ gene variants have been associated with type 2 diabetes. Besides the many actions of PPARγ agonists on the transcription of genes controlling insulin sensitivity in adipose tissue, they increase glucose sensing in liver and in β-cells. In the latter, PPARγ also enhances growth and prevents apoptosis.

PPARα (the target of fibrates) controls lipid uptake and oxidation and directs lipid flux predominantly to the liver. PPARα is expressed in a wide range of tissues, especially those with free fatty acid (FFA) oxidation. In addition to indirect actions on β-cells via effects on insulin sensitivity, PPARα agonists have direct effects on islets. Exposure of β-cells to high FFA levels along with high glucose may result in depressed insulin secretion and, possibly, β-cell death. The influence of PPARα on insulin secretion includes long-term adaptation to prolonged starvation, as best exemplified in PPARα-null mice. Defective signaling via PPARα enhances insulin secretion at basal glucose concentrations, i.e., during fasting. Multiple lines of evidence suggest that PPARα activation depresses glucose-stimulated insulin release.

A third focus of section I was on glucocorticoid receptors in β-cells. Glucocorticoids have long been known to exert their diabetogenic effect through decreased glucose uptake by peripheral tissues and increased hepatic glucose production but also direct inhibition of insulin release. The development of transgenic mice overexpressing glucocorticoid receptor in β-cells under the control of the rat insulin I promoter has provided a unique model to study the long-term effects of enhanced glucocorticoid sensitivity in β-cells. Interestingly, hyperglycemia in this model is related to enhanced inhibition of insulin secretion through α2-adrenergic receptors, without evidence for increased β-cell apoptosis.

Finally, new approaches have made it possible to delineate novel activation pathways in the β-cell. Insulin release is only one facet of β-cell function; other signaling pathways are involved in integrate insulin synthesis and storage, cell proliferation, apoptosis, and differentiation. Recent findings point to receptors for novel extracellular messengers and new location of ion channels. In β-cells, the stimulus-secretion coupling is regulated by global as well as localized Ca2+ signals in the vicinity of a primed pool of secretory granules beneath the plasma membrane. Novel digital imaging methods and spot confocal microscopy have made it possible to define distinct secretory granule pools and relate them to localized Ca2+ signals. While spatial restriction of Ca2+ signals close to the
plasma membrane prevents broad activation of other Ca\(^{2+}\)-dependent functions, a prolonged rise in cytosolic Ca\(^{2+}\) may trigger such functions. Novel receptors such as a nonclassical estrogen receptor targeted by 17\(\beta\)-estradiol may also activate a pathway that involves cGMP and protein kinase G (PKG) in closing the channel. It is of interest in this context that glucose also induces an increase in islet cGMP levels. The role of nuclear Ca\(^{2+}\) exchange has been discussed as a critical link between nutrient metabolism and the expression of key genes in \(\beta\)-cells such as c-fos and c-myc.

Section II opens with calpain 10, a previously unknown player in glucose homeostasis, which has aroused interest since the discovery of association and linkage between calpain 10 variants and type 2 diabetes. Calpains are cysteine proteases that form heterodimers composed of a 80-kDa catalytic subunit and a 30-kDa regulatory subunit. At present, 14 human 80K genes and 2 30K genes have been identified. Calpains are inactive in the cytosol and are activated following translocation to membranes in response to increased cellular Ca\(^{2+}\). Calpains are involved in cell cycle regulation and differentiation and in various calcium-regulated cellular events. Their precise physiological functions are, however, still unclear. Calpain 10 is structurally distinct from other calpains and is expressed ubiquitously, especially in heart, brain, liver, kidney, and pancreas. At least eight splice variants of calpain 10 have been identified. Associations of CAPN10 single-nucleotide polymorphisms (SNPs) have been described with type 2 diabetes as well as related subphenotypes, such as fasting and 2-h plasma glucose, fasting plasma insulin, and various parameters of insulin resistance and insulin secretion. An association of variants has also been observed with calpain 10 mRNA in skeletal muscle, suggesting an influence on gene expression. The calpain 10 associations best exemplify how noncoding gene variations directly modulate susceptibility to diabetes, that different variants of the same gene play different roles in susceptibility, and that many genes are involved, each with a small impact on susceptibility.

Another gene identified by positional cloning in 1994 encodes leptin, a hormone secreted by white adipocytes. In addition to its effects on the hypothalamus to reduce food intake, increase energy expenditure, and control body weight, leptin directly acts on pancreatic \(\beta\)-cells, where it inhibits insulin biosynthesis and secretion. In turn, insulin stimulates leptin secretion from adipose tissue, thereby establishing a regulatory feedback loop. Leptin signaling represses the proinsulin and protein phosphatase-1 (PP-1) genes and induces the suppressor of cytokine signaling-3 (SOCS3) gene in \(\beta\)-cells. The \(\beta\)-cell ATP-sensitive K\(^+\) channel is another target of leptin for inhibition of insulin secretion through phosphatidylinositol 3-kinase–dependent activation of PDE3B and subsequent reduction of intracellular cAMP. In most overweight individuals, leptin resistance in \(\beta\)-cells leads to a lesser inhibition of insulin secretion and thereby possibly contributes to the hyperinsulinemia. This may initiate a vicious cycle in which hyperinsulinemia further stimulates leptin production and secretion, which may in turn enhance leptin resistance by further desensitization of leptin signal transduction pathways.

Two other hormones secreted by adipocytes are key players in regulating energy homeostasis and lipid and carbohydrate metabolism. Acylation-stimulating protein (ASP) is a C3-derived protein that increases triglyceride synthesis and storage in adipocytes. C3 knockout mice increase food intake despite reduced adipose mass and are resistant to weight gain upon high-fat feeding. They have increased energy expenditure, reduced fasting insulin levels, and improved glucose tolerance. ASP levels correlate with fat stores. Plasma ASP decreases during fasting and after weight loss. ASP secretion by adipocytes in vitro is increased by insulin, suggesting that insulin could mediate the changes in ASP production during energy restriction and after meals. Adiponectin is another complement-related protein. Its circulating levels are low in the metabolic syndrome and in obesity. Markers of insulin resistance are linked to a quantitative trait locus (QTL) on chromosome 3 in the region locating the adiponectin gene. Adiponectin knockout mice are insulin resistant. Administration of adiponectin lowers plasma glucose levels without stimulating insulin secretion in both normal and diabetic mice and reduces insulin resistance in mice with lipatrophy or obesity-induced insulin resistance. Thus, adipocyte hormones and their receptors appear as promising therapeutic targets in obesity, hyperlipidemia, and insulin resistance.

Section II ends with a review of new models of obesity and type 2 diabetes. Original models were obtained by combining independent diabetes risk–confering QTLs from unrelated parental strains, such New Zealand Obese (NZO) and Nonobese Nondiabetic (NON/Lt) strains. Heterozygous mice at all polymorphic loci that differ between parental strains led to obesity-driven diabetes. New recombinant congenic strains were further developed by introgressing NZO chromosomal loci into NON/Lt mice, one line developing diabetes. These models exemplify the interest of associating gene variants at loci controlling body weight or glycoregulation to study clinical disorders that are likely to be multigenic. The NZO model is especially interesting in that it includes variants that modify leptin physiology and immune genes controlling autoimmunity. This model may prove useful to fill the gap between type 2 and type 1 diabetes, possibly by establishing a link with latent autoimmune diabetes of the adult.

Section III is concerned with mitochondria and their physiology and potential role in \(\beta\)-cell dysfunction. Mitochondria are the site of generation of reactive oxygen species (ROS), which partake of, or interfere with, a large number of cellular functions. Again, Ca\(^{2+}\) is heavily involved in maintenance of mitochondrial membrane potential, enzyme activation, and ATP generation. Uncoupling proteins (UCPs) are general regulators of proton transfer across the mitochondrial membrane. Overexpression of UCP\(_1\) protects against obesity and diabetes, while overexpression of UCP\(_2\) protects from atherogenesis. UCP\(_2\) is the only UCP expressed in \(\beta\)-cells, where its excess, e.g., by transfection, is associated with reduced insulin secretion. This mechanism probably mediates the negative impact of a high-fat diet on insulin secretion and the enhanced production of ROS resulting from exposure of \(\beta\)-cells to high FFA levels. Conversely, UCP\(_2\) knockout is associated
with increased insulin content, β-cell mass, and neogenesis and decreased β-cell apoptosis.

Mitochondria host maternally inherited DNA. Mitochondrial diabetes (mtD) can be type 1 or type 2, is usually insulin sensitive, and bears no evidence of autoimmunity. Mitochondrial proteins are partly encoded by mtDNA and partly by nuclear DNA. mtDNA mutations reduce tRNA and send signals to nuclear DNA that result in a reduction in mt-ribosomal proteins. When heteroplasmy is high, oxygen consumption is impaired; the attendant fall in ATP production might explain the decline in insulin secretion observed in mtD. However, the degree of heteroplasmy is usually low in mtD patients, and a correlation between the level of heteroplasmy and the age of onset of diabetes has not always been observed in the different study populations. Though excessive ROS generation does not appear to be part of mtD, hyperglycemia does lead to enhanced mitochondrial ROS production. The search for lipophilic anions that can permeate the mitochondrial membrane and balance ROS excess is a very promising avenue for the pharmacological treatment of microvascular (and possibly macrovascular) complications of diabetes.

Section IV carries an in-depth discussion of lipid modulation of β-cell function. In β-cell lines, preincubation with palmitate>oleate>glucose stimulates insulin secretion but decreases insulin content. Palmitate-resistant β-cells obtained by clonal selection store cholesterol esters. Another lipid modulator is arachidonic acid: generated by activation of cytosolic phospholipase A2 (PLA2), arachidonic acid stimulates insulin secretion. Thus, underexpression of PLA2 is associated with decreased insulin content. However, overexpression of PLA2 also reduces insulin secretion because it leads to overexpression of UCP2. Once again, Ca2+ metabolism may be involved insofar as movement of PLA2 from the nucleus to the cytoplasma is driven by Ca2+. Also, PLA2β, like ceramides, has been associated with apoptosis.

Finally, chronic oxidative stress plays a major role in β-cell dysfunction. Markers of oxidative stress are increased in many tissues in diabetic animals or subjects, and increased peroxide levels can be measured in islets from diabetic animals. Oxidants impair insulin secretion both in cell lines and in vivo in ZDF rats, while glutathione peroxidase protects against oxidant-induced β-cell loss.

In summary, we believe that this volume contains a wealth of up-to-date information on novel mechanisms and hypotheses of β-cell function, generated and critically discussed by the leading investigators in the field. The interested reader will find concepts of fundamental cellular physiology (e.g., mitochondrial function) revisited in the light of the most recent (and exciting) acquisitions of cellular biology. The attentive reader will notice that apparently disparate and distant concepts and blocks of knowledge actually converge toward a few pathogenic mechanisms that may eventually provide satisfactory explanations for both the modulation of β-cell function in response to changes in the environment, e.g., a high-fat diet, and the decline in β-cell function that marks the appearance of diabetes. The creative reader may derive ideas for further experimentation and testing. Any reader, we hope, will appreciate the effort that went into the preparation of this volume.

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