Calcium and Diabetes

Shahidul Islam

1. INTRODUCTION

Diabetes mellitus is a heterogeneous group of syndromes characterised by chronic hyperglycemia, perturbed lipid metabolism as well as many hormonal and metabolic disturbances. Poorly controlled diabetes leads to debilitating complications e.g. accelerated atherosclerosis, microangiopathy, nephropathy, neuropathy, foot diseases and blindness. Throughout the world, diabetes afflicts $\sim$ 140 million people and the number may double by the year 2025. Diabetic syndromes result from complex interactions between many predisposing genetic factors and environmental ones. Type 1 diabetes is due to specific destruction of pancreatic β-cells mainly by autoimmune mechanisms. Type 2 diabetes is phenotypic expression of a multitude of defects that perturb lipid metabolism, reduce the ability of insulin to lower plasma glucose effectively, and cause varying degrees of insulin deficiency. Type 2 diabetes is the commonest form of diabetic syndromes: in Sweden, annual incidences of type 1 and type 2 diabetes per 100,000 inhabitants in 1991–1995 being 14.7 and 265.6 respectively (Berger et al., 1999).

Pancreatic β-cells and skeletal muscle are two of the main tissues involved in glucose homeostasis. These two tissues are also the main targets for common antidiabetic medicines. Long-term complications of diabetes involve many other cells and tissues e.g. basement membranes, endothelium, vascular smooth muscle cells, platelets and monocytes. Ca^{2+} homeostasis and normal Ca^{2+} signalling are essential for optimal function of all of these cells. Because of crucial roles of $[Ca^{2+}]_i$ in secretion in many cells, as well as in cell-proliferation and cell-death, numerous investigators are exploring diverse aspects of Ca^{2+}-signalling in β-cells and roles of intracellular Ca^{2+} homeostasis in diabetes (reviewed by Levy, 1999). It is generally assumed that in type 2 diabetes, β-cell defects may reside in the pathways that link

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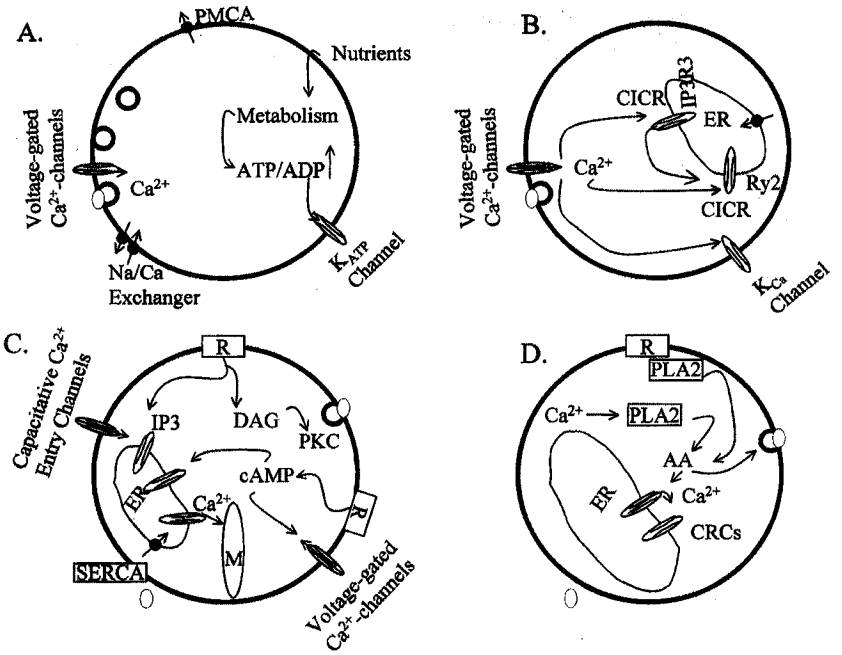

Figure 1. This figure illustrates some of the molecules and processes involved in Ca^{2+} homeostasis and Ca^{2+}-dependent stimulus secretion coupling in β-cells. (A) Nutrient metabolism increases cytoplasmic ATP/ADP ratio leading to closure of K_{ATP} Channel, membrane depolarisation and Ca^{2+} entry through voltage-gated Ca^{2+} channels. PMCA, plasma membrane Ca^{2+} ATPase. (B) Ca^{2+} signalling is amplified by Ca^{2+} induced Ca^{2+} release through intracellular Ca^{2+} channels. Calcium activated potassium channels (K_{ca} channel) mediate membrane repolarisation. IP3R3, type 3 inositol 1,4,5-trisphosphate receptor; Ry2, type 2 ryanodine receptor. (C) Depletion of intracellular Ca^{2+} stores activates capacitative Ca^{2+} entry channels in the plasma membrane. Ca^{2+} released from the ER increases mitochondrial (M) Ca^{2+} and activates mitochondrial metabolism. cAMP-dependent pathways modulate Ca^{2+} signalling by phosphorylating intracellular as well as plasma membrane Ca^{2+} channels. (D) Ca^{2+} activates PLA2 leading to formation of arachidonic acid (AA). Multiple mechanisms are involved in the formation of AA which directly or through its metabolites release further Ca^{2+} by activating the ER Ca^{2+} release channels (CRCs).

metabolism of nutrients and ligand-receptor interactions to biosynthesis and exocytosis of insulin, as well as survival of these cells. In this chapter, I shall give an overview of the roles of intracellular Ca^{2+}, placing emphasis on studies that have used diabetic patients or diabetic animal models to understand pathogenesis of the condition, its complications, and mechanisms of action of antidiabetic drugs.

2.

β-cells like most cells, share some basic mechanisms of Ca^{2+} handling which will not be elaborated in this chapter (see chapters by Guse and Nadal and

Soria in this book). Some features of Ca^{2+} signalling in electrically excitable cells, e.g. muscle and β-cells have been outlined by Macrez and Mironneau and Marsh et al. in this book. β-cells are nested in the pancreas together with other cell types, in numerous tiny islets. These cells are difficult to study since pure and functioning β-cells cannot be easily obtained in adequate numbers. These cells are specialised as fuel sensors: signals generated from nutrient metabolism increase cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) by multiple mechanisms (Nadal and Soria, 1997, and this book). It is widely accepted that an increase in the cytoplasmic ATP/ADP ratio leads to closure of ATP-sensitive potassium channels resulting in membrane-depolarisation, opening of voltage gated Ca^{2+} channels in the plasma membrane and Ca^{2+} entry (Figure 1A). Other signals that are generated from nutrient metabolism and that may be involved in metabolism-secretion coupling include malonyl CoA (Antinozzi et al., 1998), arachidonic acid (Figure 1D) (Ma et al., 1998) and cyclic ADP ribose (cADPR) (Okamoto, 1999; Guse, this book). Ca^{2+} entering through the plasma membrane Ca^{2+} channels may trigger further Ca^{2+} release through the intracellular Ca^{2+} channels i.e. type 3 and type 2 inositol 1,4,5-trisphosphate receptors (Lee et al., 1999; Parys et al., this book) and type 2 ryanodine receptor (Islam et al., 1992, 1998) (Figure 1B). Gut hormones and neurotransmitters potentiate glucose-induced insulin secretion. These agents increase $[Ca^{2+}]_i$ in β-cells by releasing the ion from endoplasmic reticulum (ER) and stimulating capacitative Ca^{2+} entry (Miura et al., 1997b). Cytoplasmic Ca^{2+} increases mitochondrial Ca^{2+} which stimulates mitochondrial metabolism resulting in increased production of not only ATP but also a putative signalling substance that may enhance exocytosis (Maechler and Wollheim, 1999). β-cells also have multiple molecular mechanisms for extrusion or sequestration of Ca^{2+}. These include several isoforms (e.g. 1b, 2b and 4b) of plasma membrane Ca^{2+} ATPase (Varadi et al., 1996a), different isoforms of thapsigargin-sensitive ER Ca^{2+} ATPases e.g. SERCA 2b and SERCA 3 (Islam and Berggren, 1993; Varadi et al., 1996b; and for a review on SERCA see Paterlini-Bréchot et al., this book) and Na^+/Ca^{2+} exchanger (Van Eylen et al., 1998). Cross-talks between Ca^{2+}- and cAMP-signalling pathways take place at multiple levels with dramatic effects on insulin secretion (Grapengeisser et al., 1991; Abdel-Halim et al., 1996; Holz et al., 1999) (Figure 1C).

Insulin secretion is associated with an increase in $[Ca^{2+}]_i$ in β-cells (Nadal and Soria, 1997). Glucose does not stimulate insulin secretion if $[Ca^{2+}]_i$ is not increased (Pertusa et al., 1999). *In vivo*, insulin is released in regular pulses ($\sim$ every 8–15 min in large mammals), superimposed on basal secretion (Goodner et al., 1977). *In vitro*, insulin pulses may be more frequent. In perfused human- and monkey-pancreata, the pulse interval is $\sim$ 5–7 min (Goodner et al., 1991). Mechanisms that generate these oscillations reside in the islets (Marchetti et al., 1994). Glucose causes oscillation of metabolism (e.g. oscillation in the intracellular ATP/ADP ratio) in β-cell by inducing oscillation in the activity of many enzymes and of concentration of their

effectors (MacDonald et al., 1997). In *in vitro* experiments, application of glucose to islets or β-cells results in conspicuous $[Ca^{2+}]_i$ oscillations. When $[Ca^{2+}]_i$ and insulin secretion is measured in single islets, the two oscillations appear to be superimposable (Bergsten, 1995). Metabolic oscillations lead to rhythmic membrane depolarisation-repolarisation and consequent opening of the voltage-gated Ca^{2+} channels (Martin et al., 1997). Metabolic oscillations can also cause periodic release of Ca^{2+} from the ER (Corkey et al., 1988). Apparently, oscillation in metabolism drives oscillation of $[Ca^{2+}]_i$, the latter being more effective in triggering insulin exocytosis (Ravier et al., 1999). The two oscillations, however, cooperate to produce pulsatile insulin release. Cellular energy and phosphorylation status determine effectiveness of Ca^{2+}-triggered exocytosis. In type 2 diabetes and obesity, regular oscillations of insulin secretion is lost even before onset of hyperglycemia, suggesting that metabolic and Ca^{2+}-oscillatory mechanisms that underlie pulsatile insulin release become deranged early in course of the pathogenesis of the disease (O'Rahilly et al., 1988; Polonsky et al., 1998).

Because of difficulty in obtaining human islets, specially human diabetic islets, most studies have used islets from many rodent models of diabetes for studying roles of Ca^{2+} in the pathogenesis of the disease. These models probably do not represent human diabetic syndromes well, but important insights have been obtained by studying them. Neonatal rats exposed to streptozotocin develop a syndrome mimicking type 2 diabetes when they become adult. Triphenyltin, an organic tin compound, produce diabetes in hamsters without causing obvious morphological changes in the islet cells. *db/db* mice and Zucker diabetic fatty (ZDF) rats lack functional leptin receptors, as a consequence of which they develop hyperphagia, obesity and diabetes resembling type 2 human diabetes. Transgenic overexpression of calmodulin, a Ca^{2+} binding protein, in β-cells results in an insulin dependent diabetes in the mice (Epstein et al., 1989). Two other models of type 2 diabetes are genetically diabetic hamsters and Goto Kakizaki (GK) rats, the latter becoming increasingly popular as a model of non-obese type 2 diabetes. β-cells from streptozotocin-induced diabetic rats (Tsuji et al., 1993), ZDF rats (Roe et al., 1996), *db/db* mice (Roe et al., 1994), genetically diabetic or triphenyltin-induced diabetic hamsters (Lindström et al., 1996; Miura et al., 1997a), all show diminished $[Ca^{2+}]_i$ response to glucose. Typically, fewer β-cells from diabetic islets respond by an elevation of $[Ca^{2+}]_i$ when challenged with stimulating concentration of glucose. $[Ca^{2+}]_i$-increase is delayed, the rate of rise of $[Ca^{2+}]_i$ is slower and magnitude of maximal increase of $[Ca^{2+}]_i$ is diminished in diabetic β-cells. More importantly, in diabetic islets $[Ca^{2+}]_i$ increase tends to be persistent rather than oscillatory as in normal islets (Figure 2) (Roe et al., 1994, 1996). When stimulated by glucose, some preparations of GK rat islets also show a delayed and slower rise of $[Ca^{2+}]_i$ (Zaitsev et al., 1997). Furthermore, maximal increase of $[Ca^{2+}]_i$ in GK rat β-cells may be lower than that in the controls (Kato et al., 1996). It should be noted that, impairment of $[Ca^{2+}]_i$ response in diabetic β-cells is generally selective for

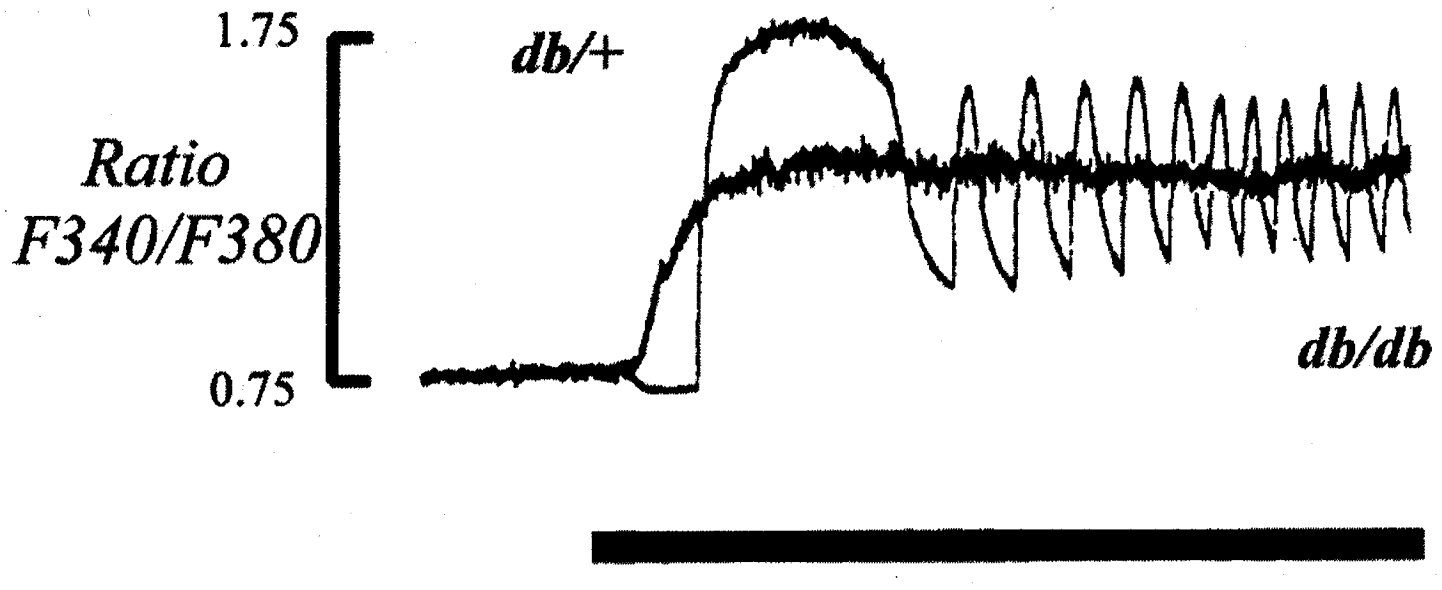

Figure 2. Cytoplasmic free Ca^{2+} concentration oscillates in normal islets of Langerhans and such oscillation is lost in diabetic islets. The figure shows changes in cytoplasmic free Ca^{2+} concentration measured by fura-2 technique, in single islets obtained from a diabetic model (*db/db* mice) or its normal counterparts (*db/+*). Initial glucose concentration was 2 mM which was increased to 12 mM during the period indicated by the thick line. (Reproduced with permission from M.W. Roe et al., 1994.)

glucose. $[Ca^{2+}]_i$ response of β-cells from neonatally streptozotocin-induced diabetic rats and GK rats, to KCl or arginine is not reduced but is rather augmented (Kato et al., 1994; Zaitsev et al., 1997). Such augmented $[Ca^{2+}]_i$ response may be due to increased L-type and T-type voltage-gated Ca^{2+} channel currents (Kato et al., 1994, 1996).

β-cell specific over-expression of calmodulin in transgenic mice reduces basal $[Ca^{2+}]_i$ and increases Ca^{2+} buffering in these cells. As a result of such alterations in Ca^{2+} handling in β-cells, these mice develop severely impaired insulin secretion and insulin-dependent diabetes (Epstein et al., 1989). Islets from these mice show markedly reduced $[Ca^{2+}]_i$ response to glucose (Ribar et al., 1995). In these islets glycolysis is impaired and β-cell mass is also grossly reduced (Ribar et al., 1995). The calmodulin-transgenic mice illustrate essential roles of Ca^{2+} in β-cells for nutrient metabolism, stimulus secretion coupling as well as proliferation of the cells. However, Ca^{2+}-independent mechanisms may also be involved in producing some of the defects observed in these mice. The reduced β-cell mass in these animals could be due to increased apoptosis. Calmodulin activates adenylyl cyclase thereby increasing intracellular cAMP and PKA activity, which are known to induce apoptosis in some cell types (Dowd and Miesfeld, 1992). $[Ca^{2+}]_i$ response of human diabetic islets has been examined only rarely and the results are difficult to interpret because of small sample size and variability of responses (Kindmark et al., 1994).

A variety of molecular mechanisms that may cause perturbations of Ca^{2+} signalling and Ca^{2+} homeostasis in diabetic β-cells have been identified. In *db/db* islets there is about five fold reduction of ER Ca^{2+} ATPase proteins (Roe et al., 1994). In GK rat islets also SERCA 3 isoform of ER Ca^{2+} ATPase is significantly reduced (Varadi et al., 1996b). In ZDF rats there is decreased expression of genes encoding the α_1-subunits of the β-cell L-type voltage-gated Ca^{2+} channel (Roe et al., 1996). In many diabetic islets, as in many

other cells in diabetes, the basal $[Ca^{2+}]_i$ is often slightly elevated (Wang et al., 1996). In β-cells of GK rats and non-obese diabetic mice (a model of type 1 diabetes), increased basal $[Ca^{2+}]_i$ may be due to increased expression or activity of voltage gated Ca^{2+}channels in the plasma membrane (Kato et al., 1996; Wang et al., 1996).

CD38 is a multifunctional protein bound to the plasma membrane of many cells including β-cells. It produces small amount ($< 2\%$) of a potent Ca^{2+} releasing second messenger, cADPR and large amount of ADP ribose from NAD^+ (Lee et al., 1999; Guse, this book). cADPR activates ryanodine receptor Ca^{2+} release channel in many cells and it has been postulated to play a role in insulin secretion from β-cells (Okamoto, 1999; Islam and Berggren, 1997). CD38 knock out mice show impaired glucose-induced increase of $[Ca^{2+}]_i$ and insulin secretion (Okamoto, 1999). In diabetic GK rats the level of CD38 (and many other proteins) is reduced (Matsuoka et al., 1995). In some diabetic patients, mutation in the CD38 gene (Yagui et al., 1998) and autoantibodies against CD38 (Pupilli et al., 1999; Ikehata et al., 1998) have been described.

When islets or β-cells are exposed to high concentrations of glucose for long period of time, they become unresponsive to subsequent challenges with stimulatory concentrations of glucose. This phenomenon called "glucose toxicity" is an important component of diabetic syndrome. In human islets exposed to high glucose for 48 hours, the basal $[Ca^{2+}]_i$ is markedly elevated and stimulation by glucose does not increase $[Ca^{2+}]_i$ further (Björklund et al., 2000).

Alterations in cytoplasmic Ca^{2+} homeostasis may play a role in causing certain forms of β-cell-death e.g. apoptosis (see chapters by Christakos et al. and Maki in this book for mechanisms of Ca^{2+} mediated apoptosis). It has been postulated that hyperactivity of voltage-gated Ca^{2+} channels in the plasma membrane and resulting Ca^{2+} overload over a long period of time, may cause progressive loss of normal β-cells in some models of diabetes (Kato et al., 1996). In type 1 diabetes, proinflammatory cytokines can mediate apoptosis by Ca^{2+}-dependent mechanisms (Suarez-Pinzon et al., 1999). According to one report, serum from patients with newly diagnosed type 1 diabetes contains factors that increase activity of voltage-activated Ca^{2+} channels and thereby induce β-cell apoptosis (Juntti-Berggren et al., 1993). Exposure to cytokines induce a low voltage-activated Ca^{2+} current in mouse β-cells causing elevation of basal $[Ca^{2+}]_i$ and apoptosis (Wang et al., 1996). In the β-cells of non-obese diabetic mice, and tumour cells derived from them (NIT-1 cells), there is increased activity of a low-voltage-activated Ca^{2+} channel in the plasma membrane, which increases basal $[Ca^{2+}]_i$ and causes cell death (Wang et al., 1996). Agents that cause prolonged increase in $[Ca^{2+}]_i$ in β-cells may cause apoptosis of these cells, a mechanism that is thought to be relevant also in the pathogenesis or progression of type 2 diabetes (Efanova et al., 1998b).

Ca^{2+} mediates actions of commonly used antidiabetic drugs on β-cells. Hypoglycemic drugs or drug-candidates belonging to both sulfonylurea (Abrahamsson et al., 1985) and non-sulfonylurea (Fujitani et al., 1997) types induce insulin secretion mainly by increasing $[Ca^{2+}]_i$. An increase in $[Ca^{2+}]_i$ in β-cells is essential also for insulin-releasing effects of imidazolines (Efanova et al., 1998a; Shepherd et al., 1996). Glucagon like peptide-1, a hormone that sensitizes β-cells to stimulation by glucose, shows great potential for treatment of type 2 diabetes. It promotes insulin secretion by orchestrating an interplay between Ca^{2+}- and cAMP-dependent pathways leading to characteristic changes in $[Ca^{2+}]_i$ (Fridolf and Ahren, 1993; Holz et al., 1995). Some thiazolidinedions also stimulate insulin secretion by stimulating Ca^{2+} entry in to the β-cells (Ohtani et al., 1996).

While physiological roles of Ca^{2+} in insulin secretion is widely known, its role in insulin action is less clear. There is some evidence that $[Ca^{2+}]_i$ may be involved in mediating some of the actions of insulin and may contribute to the pathogenesis of insulin resistance in type 2 diabetes. Insulin and insulin-like growth factor-1 increase $[Ca^{2+}]_i$ in muscle cells, the main site of glucose disposal (Bruton et al., 1999; Semsarian et al., 1999). High $[Ca^{2+}]_i$ in muscle, reduces phosphoserine phosphatase activity and thereby reduces normal dephosphorylation of glycogen synthetase and GLUT-4 which may contribute to insulin resistance (Begum et al., 1993; Sowers and Draznin, 1998). Activation of some isoforms of PKC by diacylglycerol may mediate insulin resistance in muscle (Bossenmaier et al., 1997). Elevated extracellular Ca^{2+}, as occurs in patients with hyperparathyroidism, often results in insulin resistance, hyperinsulinemia, and glucose intolerance (Richards and Thompson, 1999).

Long-term complications of diabetes are due to a vicious interplay between many secondary biochemical disturbances e.g. increased sorbitol production, non-enzymic glycosylation of proteins, oxidative stress, increased protein kinase C (PKC) and MAP kinase activity. Impaired cellular Ca^{2+} homeostasis may be another factor contributing to the pathogenesis of such complications (reviewed by Massry and Smogorzeski, 1997). Advanced glycation end products (AGE) inhibit agonist stimulated $[Ca^{2+}]_i$ increase in human glomerular mesangial cells (Mene et al., 1999). In diabetes and hypertension, basal $[Ca^{2+}]_i$ is elevated and Ca^{2+} signalling is impaired in many cells including platelets (Takaya et al., 1997; Vicari et al., 1996), endothelial cells and vascular smooth muscle cells. One action of insulin is to decrease vascular resistance by stimulating endothelial nitric oxide production and by reducing $[Ca^{2+}]_i$ in vascular smooth muscle cells (Cleland et al., 1998). Diabetic conditions alter subcellular distribution of $[Ca^{2+}]_i$ in vascular smooth muscle cells resulting in increased contractility of the cells and hypertension (Fleischhacker et al., 1999). High glucose also impairs Ca^{2+} signalling in vascular endothelial cells and may thus contribute to defective endothelium-dependent relaxation associated with type 2 diabetes (Salameh and Dhein, 1998). High $[Ca^{2+}]_i$ in the endothelial cells may

contribute to thickening of basement membrane, a hall mark of diabetic small vessel disease, by increasing secretion of basement membrane proteins. Increased $[Ca^{2+}]_i$ in endothelial cells may also promote leakiness of the capillaries possibly by stimulating contraction of the endothelial cell cytoplasm (Michel and Curry, 1999). Heart cells cultured in high glucose show a slower clearance of intracellular Ca^{2+} (Ren et al., 1999). Persistent activation of the diacylglycerol-PKC pathway under conditions of hyperglycemia is implicated in the pathogenesis of vascular complications of diabetes (Park et al., 1999). PKC activation in monocytes may contribute to atherosclerosis in diabetes (Ceolotto et al., 1999). Mechanisms by which high glucose increases basal $[Ca^{2+}]_i$ in many cells, may include non-enzymic glycosylation of Ca^{2+}-ATPase and increased PKC activity (Gonzalez Flecha et al., 1999).

3. CONCLUDING REMARKS

$[Ca^{2+}]_i$ signalling is crucial for insulin secretion and is possibly also involved in insulin action. It must be emphasised that diabetes and its complications are unlikely to be due to defect in any one single signalling molecule or pathway. It is, however, remarkable that numerous studies have consistently demonstrated impaired Ca^{2+} signalling in β-cells in nearly all animal models of type 2 diabetes. Such impairments may be symptomatic of primary metabolic perturbations or secondary complications of hyperglycemia, but may also be due to defects in molecules involved in Ca^{2+} signalling and Ca^{2+} homeostasis. Diabetic conditions alter Ca^{2+} handling in many cells thus elevating basal $[Ca^{2+}]_i$ or altering subcellular Ca^{2+} distribution, mechanisms that may be among the factors that lead to long-term complications of the disease. The role of perturbed Ca^{2+} signalling and Ca^{2+} homeostasis in the pathogenesis of diabetes needs to be appreciated in the wider context of many other metabolic and signalling defects that have been reported in these complex group of syndromes.

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