Perspectives in Diabetes

Are Oxidative Stress–Activated Signaling Pathways Mediators of Insulin Resistance and β-Cell Dysfunction?

Joseph L. Evans,[1] Ira D. Goldfine,[2] Betty A. Maddux,[2] and Gerold M. Grodsky[2]

In both type 1 and type 2 diabetes, diabetic complications in target organs arise from chronic elevations of glucose. The pathogenic effect of high glucose, possibly in concert with fatty acids, is mediated to a significant extent via increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and subsequent oxidative stress. ROS and RNS directly oxidize and damage DNA, proteins, and lipids. In addition to their ability to directly inflict damage on macromolecules, ROS and RNS indirectly induce damage to tissues by activating a number of cellular stress-sensitive pathways. These pathways include nuclear factor-κB, p38 mitogen-activated protein kinase, NH_2-terminal Jun kinases/stress-activated protein kinases, hexosamines, and others. In addition, there is evidence that in type 2 diabetes, the activation of these same pathways by elevations in glucose and free fatty acid (FFA) levels leads to both insulin resistance and impaired insulin secretion. Therefore, we propose here that the hyperglycemia-induced, and possibly FFA-induced, activation of stress pathways plays a key role in the development of not only the late complications in type 1 and type 2 diabetes, but also the insulin resistance and impaired insulin secretion seen in type 2 diabetes. ***Diabetes*** **52:1–8, 2003**

Both type 1 and type 2 diabetes possess a significant genetic component (1–3). In the case of type 2 diabetes, additional environmental factors, including hormones, increased caloric intake, decreased physical inactivity, and adiposity (1,2), have a marked influence on the disease. Now there is evidence that elevated levels of metabolic substrates contribute to the diabetic phenotype.

There are considerable amounts of data indicating that the chronic elevation of plasma glucose causes many of the major complications of diabetes, including nephropathy, retinopathy, neuropathy, and macro- and microvascular damage (1,4). A causative role for elevated free fatty acid (FFA) levels in the development of microvascular complications remains to be established, however. Increased levels of FFAs are positively correlated with both insulin resistance (5,6) and the deterioration of β-cell function in the context of concomitant hyperglycemia (7,8). These latter effects may result from oxidative stress.

There is evidence that oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and antioxidant defenses, leads to tissue damage (9). Oxidative stress results from increased content of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS). Examples of ROS include charged species such as superoxide and the hydroxyl radical, and uncharged species such as hydrogen peroxide (9). There are data indicating that ROS formation is a direct consequence of hyperglycemia (10); more recent studies have suggested that increased FFA levels may also result in ROS formation (see below).

Because of their ability to directly oxidize and damage DNA, protein, and lipid, ROS are believed to play a key direct role in the pathogenesis of late diabetic complications (9,11). In addition to their ability to directly inflict macromolecular damage, ROS can function as signaling molecules to activate a number of cellular stress-sensitive pathways that cause cellular damage, and are ultimately responsible for the late complications of diabetes. Furthermore, these same pathways are linked to insulin resistance and decreased insulin secretion. In this review, we propose that ROS and oxidative stress induced by elevations in glucose and possibly FFA levels play a key role in causing insulin resistance and β-cell dysfunction by their ability to activate stress-sensitive signaling pathways (Fig. 1).

HYPERGLYCEMIA AND STRESS-ACTIVATED PATHWAYS

In vivo studies have revealed that oxidative stress caused by hyperglycemia (and perhaps FFAs) occurs before the complications of diabetes become clinically evident

From [1]Medical Research Institute, San Francisco, California; and [2]the University of California at San Francisco, San Francisco, California.

Address correspondence and reprint requests to Dr. Joseph L. Evans, Medical Research Institute, 444 De Haro St., Suite 209, San Francisco, CA 94107-2347. E-mail: jevansphd@earthlink.net.

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AGE, advanced glycation end product; ERK, extracellular signal–related kinases; FFA, free fatty acid; IκB, inhibitory protein κB; IKK-β, IκB kinase-β; IR, insulin receptor; IRS, IR substrate; JNK, NH_2-terminal Jun kinase; LA, α-lipoic acid; NF-κB, nuclear factor-κB; MAP, mitogen-activated protein; MAPK, MAP kinase; NAC, *N*-acetyl-L-cysteine; PKC, protein kinase C; RAGE, receptor for AGEs; RNS, reactive nitrogen species; ROS, reactive oxygen species; SAPK, stress-activated protein kinases; VEGF, vascular endothelial growth factor.

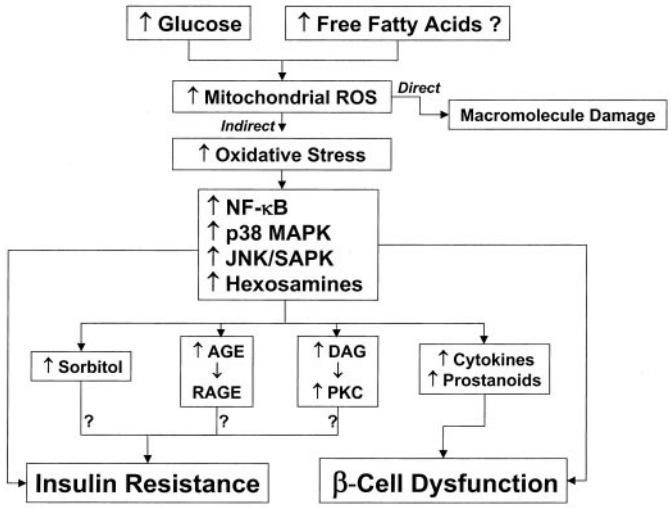

FIG. 1. Proposed general theory of how elevated glucose and possibly FFA levels contribute to the pathophysiology of diabetes via the generation of ROS and consequent activation of numerous stress-sensitive pathways. The causative link among hyperglycemia, mitochondrial ROS generation, oxidative stress, and the development of diabetic complications has been previously suggested (10,11,22). ROS (and RNS), by inflicting macromolecular damage, may play a key direct role in the pathogenesis of diabetes. ROS also function as signaling molecules (analogous to second messengers) to activate several stress-sensitive pathways (indirect role). In addition, in type 2 diabetes, there is growing evidence that activation of stress-sensitive pathways, such as NF-kB, p38 MAPK, JNK/SAPK, and hexosamine, by elevations in glucose and possibly FFA levels leads to both insulin resistance and impaired insulin secretion. Thus ROS and oxidative stress, induced by elevations in glucose and possibly FFA levels, may play a key role in causing insulin resistance and β-cell dysfunction by their ability to activate stress-sensitive signaling pathways. The proposed sequence of events may also include other stress pathways, such as the increased production of AGE, sorbitol, cytokines, and prostanoids along with PKC activation. DAG, diacylglycerol.

(9,12,13). Wolff and Dean (15) suggested that nonenzymatic protein glycation, a mechanism proposed early on to account for glucose cytotoxicity (14), was dependent on ROS (superoxide and hydroxyl) formation through transition metal-catalyzed glucose autoxidation. Research in numerous laboratories has indicated that hyperglycemia activates several major, well-characterized biochemical pathways that play a significant role in the etiology of diabetic complications. These pathways include advanced glycation end products (AGEs) and receptors for AGE (RAGE) (12), protein kinase C (PKC) (13), and the polyol pathway (16).

More recently, hyperglycemia has been implicated in the activation of additional biochemical pathways, including the stress-activated signaling pathways of nuclear factor-κB (NF-κB), NH_2-terminal Jun kinases/stress activated protein kinases (JNK/SAPK), p38 mitogen-activated protein (MAP) kinase, and hexosamine (17–19). Data now indicate that activation of these pathways is linked not only to the development of the late complications of diabetes, but also to insulin resistance and β-cell dysfunction.

NF-κB pathway. The most extensively studied intracellular pathway that is a target of hyperglycemia, ROS, and oxidative stress is the transcription factor NF-κB (17,20,21). NF-κB plays a critical role in mediating immune and inflammatory responses and apoptosis. NF-κB regulates the expression of a large number of genes, including several of those linked to the complications of diabetes (e.g., vascular endothelial growth factor [VEGF] and RAGE) (20). Many of the gene products regulated by NF-κB in turn activate NF-κB (e.g., VEGF, RAGE), leading to a vicious circle. The aberrant regulation of NF-κB is associated with a number of chronic diseases, including diabetes and atherosclerosis. Activation of NF-κB involves the phosphorylation-induced, proteasome-mediated degradation of the inhibitory subunit, inhibitory protein κB (IκB). IκB is phosphorylated by an upstream serine kinase, IκB kinase β (IKK-β), which is phosphorylated and activated by additional upstream serine kinases.

A recent study in bovine endothelial cells found that exposure to hyperglycemia initially increased the production of intracellular ROS, followed by activation of NF-κB (22). Subsequently, PKC activity and AGE and sorbitol levels increased. Disruption of mitochondrial ROS production by several distinct approaches blocked the hyperglycemia-induced increase in ROS production. As a consequence, hyperglycemia-induced effects on NF-κB, PKC, and AGE and sorbitol levels were also suppressed. The effects of hyperglycemia on ROS formation and NF-κB activation preceded the stimulation of the other systems. Therefore, these data implicated NF-κB activation as the initial signaling event. If extended to other cell types and tissues, these findings would support the idea that ROS formation is a primary event followed by activation of the other systems.

JNK/SAPK pathway. The JNKs/SAPKs are members of the complex superfamily of MAP serine/threonine protein kinases. This superfamily also includes the p38 MAP kinases (p38 MAPKs) and the extracellular signal−related kinases (ERKs) (18). In contrast to ERKs (also referred to as MAPKs), which are typically activated by mitogens, JNK/SAPK and p38 MAPK are known as stress-activated kinases, and are responsive to a variety of exogenous and endogenous stress-inducing stimuli, including hyperglycemia, ROS, oxidative stress, osmotic stress, proinflammatory cytokines, heat shock, and ultraviolet irradiation. JNK/SAPK are activated by hyperglycemia-induced oxidative stress and are likely involved in apoptosis mediated by hyperglycemia in human endothelial cell (23,24). H_2O_2 generation, JNK/SAPK activity, and subsequent apoptosis induced by hyperglycemia could be suppressed by vitamin C (23).

p38 MAPK pathway. Activation of the p38 MAPK pathway occurs in response to hyperglycemia and in diabetes. In vascular smooth muscle cells, treatment with insulin and hyperglycemia induces the activation of p38 MAPK (25). In rat aortic smooth muscle cells, high glucose causes a fourfold increase in p38 MAPK (26). In a study of glomeruli of rats rendered diabetic by streptozotocin, p38 MAPK activity was increased compared with controls, followed by increased phosphorylation of heat shock protein 25, a downstream substrate of p38 MAPK (27). These effects were mediated by increased ROS production. Increases in total levels of JNK/SAPK and p38 MAPK have been reported in nerve tissue of patients with type 1 and type 2 diabetes (28), although a causative role in the pathophysiology has not been established.

Hexosamine pathway. The excessive flux of glucose or FFAs into a variety of cell types results in the activation of the hexosamine biosynthetic pathway (19,29), which in

turn leads to insulin resistance and the development of late complications of diabetes (19,29,30). Recent data have implicated a hyperglycemia-induced increase in ROS formation in the activation of the hexosamine pathway. In bovine endothelial cells, hyperglycemia induced a significant increase in the hexosamine pathway, an effect that was blocked by an inhibitor of electron transport, a mitochondrial uncoupling agent (CCCP), and the expression of either uncoupling protein 1 or MnSOD (31).

Taken together, there is strong evidence to indicate that the NF-κB, JNK/SAPK, p38 MAPK, and hexosamine pathways are stress-sensitive signaling systems that can be activated by hyperglycemia and ROS in vitro and in vivo. Chronic activation of these pathways is associated with the late complications of diabetes. This in an area worthy of continued research activity, and one that could yield new insights into the molecular pathogenesis of hyperglycemia as well as identify pharmacological targets for the treatment and/or prevention of the late complications of diabetes. What has become equally intriguing is the growing number of reports linking the activation of these same pathways to insulin resistance and β-cell dysfunction.

OXIDATIVE STRESS AND INSULIN RESISTANCE

Both insulin resistance and decreased insulin secretion are major features of the pathophysiology of type 2 diabetes (1,32–34). Insulin resistance most often precedes the onset of type 2 diabetes by many years, is present in a large segment of the general population, and is multifactorial (1,32). It is clear that insulin resistance has a genetic component (1–3): insulin resistance is a feature of the offspring of parents with type 2 diabetes, aggregates in families, and, in longitudinal studies of families, has been implicated as a major risk factor for developing type 2 diabetes.

Insulin resistance is also caused by acquired factors, such as obesity, sedentary lifestyle, pregnancy, and the presence of excess hormones (1,33). Initially, insulin resistance is compensated for by hyperinsulinemia, through which normal glucose tolerance is preserved. Reaven (32) and others have reported that at least 25% of nondiabetic individuals exhibit insulin resistance that is in the range of that seen in patients with type 2 diabetes. Deterioration into impaired glucose tolerance occurs when either the insulin resistance increases or the compensatory insulin secretory responses decrease, or when both occur. An increase in insulin, FFA, and/or glucose levels can increase ROS production and oxidative stress as well as activate stress-sensitive pathways. This, in turn, can worsen both insulin action and secretion, thereby accelerating the progression to overt type 2 diabetes.

Antioxidants and type 2 diabetes. As discussed above, oxidative stress has long been associated with the late complications of diabetes, and has been implicated in their etiology (9,11,35). More recently, studies have linked ROS production and oxidative stress to insulin resistance (36–40). Through in vitro studies and in animal models of diabetes, it has been found that antioxidants, especially α-lipoic acid (LA), improve insulin sensitivity (40–42). Several clinical trials, albeit small and of short duration, have also demonstrated that treatment with vitamin E, vitamin C, or glutathione improves insulin sensitivity in insulin-resistant individuals and/or patients with type 2 diabetes (43,44). The effect of LA has been quantitated by the euglycemic-hyperinsulinemic clamp (Fig. 2). For LA, the magnitude of this increased insulin sensitivity compares favorably with the currently available medications metformin and rosiglitazone. These insulin sensitizers produced an ~25 and ~20% improvement in insulin-stimulated glucose metabolism, respectively (45,46). Recently it has been shown that oral administration of a controlled release formulation of LA for 6 weeks lowered plasma fructosamine levels in patients with type 2 diabetes (47). Also, noncontrolled-release LA recently has been reported to increase insulin-mediated glucose disposal in patients with type 2 diabetes (48).

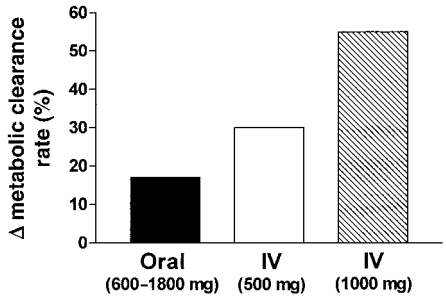

FIG. 2. LA increases insulin-stimulated glucose metabolism in patients with type 2 diabetes. Intravenous (IV) administration of LA is able to significantly increase insulin sensitivity (as judged by the percent change [Δ] in the metabolic clearance rate [MCR]) in patients with type 2 diabetes, whereas oral administration exerts a lesser effect. ■, 17% increase in MCR ($P < 0.05$) (106); □, 30% increase in MCR ($P < 0.05$) (107); ▨, 55% increase in MCR ($P < 0.05$) (108). Figure reprinted with permission from Evans and Goldfine (44).

LA's site of action has not yet been defined. Several laboratories have reported that use of LA in vitro at high concentrations (2.5 mmol/l) has a direct stimulatory effect on GLUT4 translocation or activation (49–51). However, these data should be interpreted with caution, as this concentration of LA is ~10- to ~100-fold greater than the level sufficient to protect against oxidative stress–induced insulin resistance in cells (40,41) and to increase insulin sensitivity in patients with type 2 diabetes (44,47).

Activation of stress-sensitive signaling systems, insulin receptor substrate serine phosphorylation, and insulin resistance. In vitro, ROS and oxidative stress lead to the activation of multiple serine kinase cascades (18). The insulin signaling pathway offers a number of potential targets (substrates) of these activated kinases, including the insulin receptor (IR) and the family of IR substrate (IRS) proteins. For IRS-1 and -2, an increase in serine phosphorylation decreases the extent of tyrosine phosphorylation and is consistent with the attenuation of insulin action (52,53) (Fig. 3).

In Chinese hamster ovary cells, stress activation of JNK/SAPK increased serine phosphorylation (at Ser307) and inhibited insulin-stimulated tyrosine phosphorylation of IRS-1 (54). In L6 muscle cells, H_2O_2-mediated inhibition of insulin-stimulated glucose transport was accompanied by activation of p38 MAPK by H_2O_2 (40,55). Insulin-stimulated glucose transport could be restored by LA and a specific inhibitor of p38 MAPK (40,55). To determine whether the protective effects of LA could also be observed under more physiological conditions, we have used hyperglycemia to induce oxidative stress and blunt the

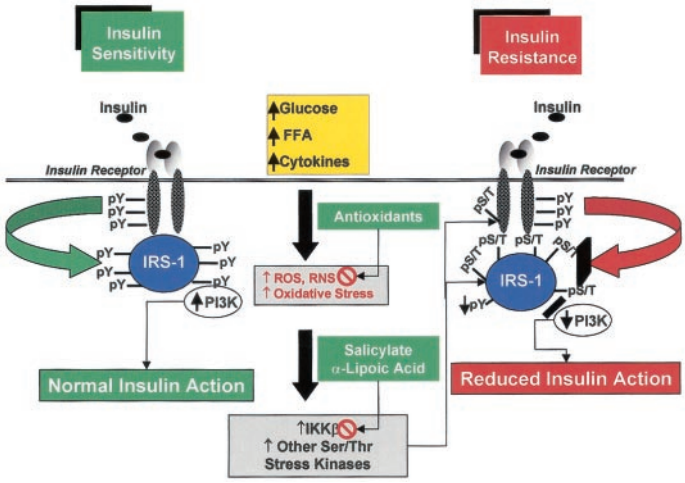

FIG. 3. The role of serine kinase activation in oxidative stress–induced insulin resistance. A variety of stimuli, including hyperglycemia, elevated FFA levels, cytokines, and others, increase ROS (and RNS) production and oxidative stress. This results in the activation of multiple stress-sensitive serine/threonine (Ser/Thr) kinase signaling cascades such as IKK-β and others (see text for details). Once activated, these kinases are able to phosphorylate multiple targets, such as the IR and IRS proteins (including IRS-1 and IRS-2). Increased phosphorylation of IR or IRS proteins on discrete serine or threonine sites (pS/T) decreases the extent of insulin-stimulated tyrosine phosphorylation (pY) (52,53). Consequently, the association and/or activities of downstream signaling molecules (e.g., phosphatidylinositol 3-kinase [PI3K]) are decreased, resulting in reduced insulin action (insulin resistance). The protective effects of antioxidants (e.g., LA) on oxidative stress–induced insulin resistance could relate to their ability to preserve the intracellular redox balance (neutralizing ROS) or, analogous to pharmacological agents (e.g., salicylates, p38 MAPK inhibitors), to block the activation of stress-sensitive kinases (40,55,56).

effects of insulin. In one study, chronic incubation of L6-GLUT4-IR cells (L6 cells that express both human GLUT4 and IR) with 20 mmol/l glucose caused a marked decrease in insulin-stimulated glucose transport (B.A.M. and I.D.G., unpublished observations). Coincubation with LA (100 μmol/l) completely protected the cells against hyperglycemia-induced insulin resistance.

Activation of IKK-β, a serine kinase that regulates the NF-κB pathway, inhibits insulin action (56). Salicylates lower blood glucose (rev. in 56), augment glucose-induced insulin secretion in normal subjects, and restore insulin secretion in patients with type 2 diabetes (57,58). In addition, salicylates inhibit IKK-β activity and restore insulin sensitivity, both in vitro and in vivo (56,59). Treatment with aspirin or salicylates alters the phosphorylation patterns of IRS proteins, resulting in decreased serine phosphorylation, increased tyrosine phosphorylation, and improved insulin action (56,59). Further support for the importance of IKK-β in insulin resistance is provided by results of recent gene knockout experiments in mice. IKK-β (+/−) heterozygotes were more insulin sensitive compared with their normal (+/+) littermates (56). Treatment of nine type 2 diabetic patients for 2 weeks with high dosages of aspirin (7 g/day) resulted in reduced hepatic glucose production and fasting hyperglycemia and increased insulin sensitivity (60). Although these latter data are preliminary and require confirmation in an expanded study, they are consistent with a role for activation of IKK-β in the pathogenesis of insulin resistance. Furthermore, they suggest that inhibition of IKK-β might be an attractive pharmacological approach to increasing insulin sensitivity.

Obesity, fatty acids, and insulin resistance. Because insulin resistance is evident before the development of chronic (fasting) hyperglycemia (1,32), it is unlikely that insulin resistance at the prediabetic stage results from oxidative stress triggered by hyperglycemia per se. However, the strong association of obesity and insulin resistance suggests that a mediator of oxidative stress–induced insulin resistance at the prediabetic stage might be an adipocyte-derived factor.

In this regard, several possible candidate molecules have been suggested including tumor necrosis factor-α (61), leptin (62), FFAs (5,6,63), and, most recently, resistin (64). However, the evidence is strongest that FFAs are the most likely link between obesity and insulin resistance (5,6,65). Several mechanisms of how elevated FFA levels decrease insulin sensitivity have been proposed, including the Randle hypothesis (63) along with a more recent alternative concerning inhibition of insulin-stimulated glucose transport (65). It also should be noted that FFAs and many of their metabolites interact directly with transcription factors to regulate gene expression, especially those involved in lipid and carbohydrate metabolism (66).

Fatty acids, redox balance, and activation of NF-κB. In patients with type 2 diabetes, there is a significant inverse correlation between the fasting plasma FFA concentration and ratio of reduced/oxidized glutathione (the major endogenous antioxidant) (36). In healthy subjects, infusion of FFAs (as intralipid) causes increased oxidative stress, as judged by increased malondialdehyde levels and a decline in the plasma reduced/oxidized glutathione ratio (36). Malondialdehyde, a highly toxic by-product generated in part by lipid oxidation and ROS, is increased in diabetes (67). In both normal individuals and in type 2 diabetic patients, restoration of redox balance by infusion of glutathione improves insulin sensitivity along with β-cell function (68).

Evidence in vitro indicates that elevated FFA levels have numerous adverse effects on mitochondrial function, including the uncoupling of oxidative phosphorylation (69) and the generation of ROS, including superoxide (70). This latter situation is exacerbated because FFAs are not only capable of inducing oxidative stress, but also impair endogenous antioxidant defenses by reducing intracellular glutathione (36,71,72). Numerous in vitro studies have reported FFA-mediated activation of NF-κB, a likely consequence of the ability of FFAs to increase ROS formation and reduce glutathione (72–75). This effect might be also linked to FFA-mediated activation of PKC-θ (76), which has the unique ability among PKC isoforms to activate NF-κB (77). FFA-induced activation of NF-κB can be prevented by vitamin E (72), suggesting that the alteration in cellular redox status is a contributory component of the proinflammatory effects of FFAs. The association of obesity, fatty acids, and oxidative stress with insulin action clearly merits further attention, with a particular focus on identifying the molecular mechanisms.

OXIDATIVE STRESS AND β-CELL DYSFUNCTION

An additional target of oxidative stress is the β-cell. β-Cells are responsible for sensing and secreting the

appropriate amount of insulin in response to a glucose stimulus (78). Although this process is complex and dependent on many factors (rev. in 34), the critical importance of mitochondrial glucose metabolism in linking stimulus to secretion is well established (78–80). Therefore, the ability of oxidative stress (H_2O_2) to damage mitochondria and markedly blunt insulin secretion is not surprising (80).

Many studies have suggested that β-cell dysfunction is the result of prolonged exposure to high glucose, elevated FFA levels, or a combination of the two. There is considerable evidence that chronic hyperglycemia in patients with type 2 diabetes contributes to impaired β-cell function (34,81). However, in vitro evidence for a direct toxic effect of glucose has been conflicted because, in large part, of variations in the definition of toxicity along with subtle differences in experimental design (34). For example, evidence of impaired secretion may simply reflect a normal decrease in β-cell insulin content caused by prior exposure to elevated glucose levels (34,82). Moreover, recent data have suggested that the combined effects of elevations in glucose and FFA levels, acting by the generation of ROS, may be particularly toxic. As discussed above, chronic exposure to these molecules can result in increased production of ROS and RNS, and activation of stress-sensitive pathways.

β-Cells are sensitive to ROS and RNS because they are low in free-radical quenching (antioxidant) enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (83). Overexpression of the antioxidant enzymes in islets or transgenic mice prevents many of the deleterious effects noted above (84,85). Oxygen stress generated by short exposure of β-cell preparations to H_2O_2 increases production of p21 (an inhibitor of cyclin-dependent kinase), decreases insulin mRNA, cytosolic ATP, and calcium flux in cytosol and mitochondria, and causes apoptosis (rev. in 80). Insulin secretion stimulated by glucose or methyl succinate can be inhibited within 30 min, whereas the response to K^+ remains normal (80). These results indicate that the mitochondrial processes involved in glucose-mediated insulin secretion are particularly affected by oxidative stress.

Inhibition of insulin secretion and glucose oxidation also occurs when islets are exposed to lipid peroxidation products (86). Conversely, antioxidants such as *N*-acetyl-L-cysteine (NAC), aminoguanidine, zinc, and the spin-trapping agent α-phenyl-tert-butylnitrone, can protect against β-cell toxicity and the generation of glycation end products and inhibit the activation of NF-κB (87–91). Recently, β-cell function was evaluated in islets after overexpression of glutamine:fructose-6-phosphate amidotransferase, the rate-limiting enzyme of hexosamine biosynthesis (92). Activation of the hexosamine pathway resulted in significant deterioration of glucose-stimulated insulin secretion along with other indexes of β-cell function, coincident with an increase in H_2O_2 (92). These effects were counteracted by treatment with the antioxidant NAC.

β-Cell glucose-induced toxicity. In patients with type 2 diabetes, reducing hyperglycemia with diet, insulin, or sulfonylureas results in improved insulin release (rev. in 34; 93). Conversely, in healthy individuals, high glucose infused as a clamp reduces insulin release (93). In vitro, long-term culture of either HIT-T15 or βTC-6 cells with elevated glucose decreases insulin release, insulin mRNA, and binding of insulin mRNA transcription factors (94,95). The antioxidants NAC and aminoguanidine markedly prevent glucotoxic effects on insulin gene activity (87). These antioxidants have been shown to partially prevent glucose-induced decreases in insulin mRNA, DNA-binding of pancreatic duodenal homeobox-1, insulin content, and glucose-stimulated insulin secretion (87).

β-Cell lipid-induced toxicity. Increased sensitivity to low glucose after prolonged high FFA levels (96–98) and coculture of normal islets with high FFA levels and moderate glucose causes increased secretory response during a test stimulus (96–99). In contrast, prolonged culture of β-cell preparations with FFAs causes decreased mitochondrial membrane potential and increased uncoupling proteins, leading to the opening of K^+-sensitive ATP channels and selective impairment of glucose-stimulated, but not K^+-stimulated, insulin secretion (100,101). Impaired insulin secretion has been associated with an FFA-induced increase in ROS (96).

Prolonged culture of β-cell preparations from animals with a predilection for type 2 diabetes, particularly those with impaired leptin production or leptin receptors, results in consistently demonstrable impaired secretion as well as other deleterious effects on β-cell function (rev. in 99). Therefore, genetic defects may amplify the toxic effects of FFAs that are not evident with normal insulin secreting cells.

β-Cell combined glucose/lipid toxicity. Because both glucose and FFA levels are elevated in type 2 diabetes, it is possible that their combination is required to maximize β-cell toxicity. This hypothesis is supported by recent studies showing that when either isolated islets or HIT cells were exposed to chronic elevated glucose and FFA levels, there was a clear decrease in both insulin mRNA and the activation of an insulin-gene reporter construct (102). In other studies, coculture of islets with high levels of glucose and palmitate resulted in almost complete impairment of glucose-stimulated insulin secretion, despite partially sustained stored insulin (96). Recent studies have suggested that β-cell lipotoxicity is an amplifying effect only if mediated by concurrent hyperglycemia (7,8).

CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH

As discussed above, there is considerable evidence from in vitro and in vivo studies that in a variety of tissues, hyperglycemia and possibly elevated FFA levels (both alone and in combination) result in the generation of ROS and RNS and consequently increased oxidative stress. In the absence of an appropriate compensatory response from the cell's endogenous antioxidant network, the system becomes overwhelmed, resulting in redox imbalance, thereby further exacerbating the situation. The reactive species not only directly damage cells by oxidizing DNA, protein, and lipids, but indirectly damage cells by activating a variety of stress-sensitive intracellular signaling pathways such as NF-κB, p38 MAPK, JNK/SAPK, hexosamine, PKC, AGE/RAGE, sorbitol, and others. Activation of these pathways results in the increased expression

of numerous gene products that also cause cellular damage and play a major role in the etiology of the late complications of diabetes.

In addition, recent data in vitro and in vivo suggest that activation of the same or similar stress pathways results in insulin resistance and impaired insulin secretion. Accordingly, we propose the existence of a link among the hyperglycemia- and FFA-induced increases in ROS and oxidative stress, activation of stress-sensitive pathways, and the eventual development of not only the late complications of diabetes, but also insulin resistance and β-cell dysfunction

Although our understanding of how hyperglycemia-induced oxidative stress ultimately leads to tissue damage has advanced considerably in recent years (7,10,13,103), effective therapeutic strategies to prevent or delay the development of this damage remain limited. We believe that research needs to be carried out on several fronts. First, antioxidant therapy needs to be improved. Either older antioxidants such as vitamin E, LA, and NAC need to be reformulated, or newer antioxidants need to be identified. At this juncture, the general use of antioxidant mixtures may not prove useful and could even interfere with other therapies and, therefore, is not advised (104). Moreover, screening tests to monitor oxidative stress need to be standardized and used in patients with diabetes.

Second, strategies to interrupt the stress pathways need to be studied more thoroughly. There has been some progress in this area. The specific inhibitor of PKC-β, LY333531, is active in cell and animal models, and is now being used in clinical trials in humans. In addition, the recent finding that salicylates, which inhibit IKK, improve insulin action in both cells and animal models is a major advance. A major challenge, however, is to obtain a more detailed understanding of the nature of the stress pathways, and to develop effective modulators that can be used clinically.

Third, additional research is clearly needed to firmly establish whether either the reduction of ROS formation activated by hyperglycemia and elevated FFA levels and/or the blockade of the ROS-induced stress pathways will result in improved insulin action and/or secretion. Analysis of the effects of hyperglycemia and hypertriglyceridemia in muscle, fat, and pancreatic islets on the development of oxidative stress and activation of stress pathways is urgently needed. Although small clinical studies with antioxidants such as vitamin E, LA, and NAC provide support for a role for oxidative stress in these conditions, several prospective clinical studies evaluating the effectiveness of vitamin E on cardiovascular outcomes have yielded disappointing results (rev. in 105). Nonetheless, the totality of available data provide support for conducting more extensive clinical studies evaluating the effectiveness of antioxidant treatment.

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