Does the Glucose-Dependent Insulin Secretion Mechanism Itself Cause Oxidative Stress in Pancreatic β-Cells?

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Glucose-dependent insulin secretion (GDIS), reactive oxygen species (ROS) production, and oxidative stress in pancreatic β-cells may be tightly linked processes. Here we suggest that the same pathways used in the activation of GDIS (increased glycolytic flux, ATP-to-ADP ratio, and intracellular Ca^{2+} concentration) can dramatically enhance ROS production and manifestations of oxidative stress and, possibly, apoptosis. The increase in ROS production and oxidative stress produced by GDIS activation itself suggests a dual role for metabolic insulin secretagogues, as an initial sharp increase in insulin secretion rate can be accompanied by progressive β-cell injury. We propose that therapeutic strategies targeting enhancement of GDIS should be carefully considered in light of possible loss of β-cell function and mass. *Diabetes* 53:1942–1948, 2004

Insulin-secreting β-cells are subject to injury from oxidative stress. Formation of reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide, hydroxyl radicals, and the concomitant generation of nitric oxide have been implicated in β-cell dysfunction or cell death caused by autoimmune attack and actions of cytokines in type 1 diabetes. ROS have also been associated with the impairment of β-cell function in type 2 diabetes (1–4). Compared with many other cell types, the β-cell may be uniquely at high risk of oxidative damage and has an increased sensitivity for apoptosis (2,3,5).

Investigations (1–3,6) implicating ROS in β-cell death or damage have, for the most part, relied on the protective effects of antioxidants, scavengers, and overexpression of antioxidant enzymes in islets or transgenic mice to reduce the destructive influence of some oxyradicals. However, elevated glucose concentrations are thought to alter metabolism, create oxidative stress, and induce apoptosis in many cell types in addition to glucose-responsive β-cells (2,5). Why should ROS generation in β-cells be more dangerous than in other cell types?

We have analyzed the existing data on mechanisms of glucose-dependent insulin secretion (GDIS) in β-cells, ROS production, oxidative stress, and apoptosis and propose that the same pathways can dramatically influence oxidative stress, apoptosis, and insulin production.

GDIS and adenine nucleotide regulation. According to the most widely accepted hypothesis, glucose induces insulin release as follows (2,7–9) (Fig. 1): glucose rapidly equilibrates across the plasma membrane and is phosphorylated by glucokinase, which determines metabolic flux through glycolysis. Because the K_m of glucokinase for glucose is ~8 mmol/l, this flux climbs steeply as glucose concentration increases, underlying the dependence of the β-cell insulin secretory response to glucose in the physiological range.

Reducing equivalents are recovered by the tricarboxylic acid cycle from carbohydrates and from fats (after prior β-oxidation). Synthesized reducing equivalents [NAD(P)H and flavins] are transferred to the electron transport chain (ETC). The energy released by the ETC is used to pump protons out of the mitochondrial inner membrane, creating the transmembrane electrochemical gradient. This gradient is used to make ATP from ADP and P_i, driven by proton movement back through the ATP synthase complex. The exchange of ATP and ADP across the inner membrane is catalyzed by the adenine nucleotide translocator. These events result in increased ATP production in mitochondria and in an enhanced ratio of ATP to ADP in the cytoplasm. In the presence of glucose, the increase in intracellular ATP-to-ADP ratio closes the ATP-sensitive K^+ (K_{ATP}) channels, which in turn results in depolarization of the plasma membrane, influx of extracellular Ca^{2+}, and activation of exocytosis.

We have recently developed (10) a computer model of regulation Ca^{2+} and ATP concentrations in pancreatic β-cells. However, our modeling studies suggest that the current understanding of adenine nucleotide regulation in β-cells is incomplete (L.E.F., L.H.P., unpublished observations). In particular, the effect of substrates that markedly enhance insulin secretion, including glucose, on ATP concentration is small, but the ratio of total ATP to total ADP increases considerably in most studies (11–13).

In contrast to ATP, only a small fraction of total cellular

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$\Delta\Psi$, membrane potential; ETC, electron transport chain; GDIS, glucose-dependent insulin secretion; K_{ATP} channel, ATP-sensitive K^+ channel; NF, nuclear factor; ROS, reactive oxygen species; UCP, uncoupling protein.

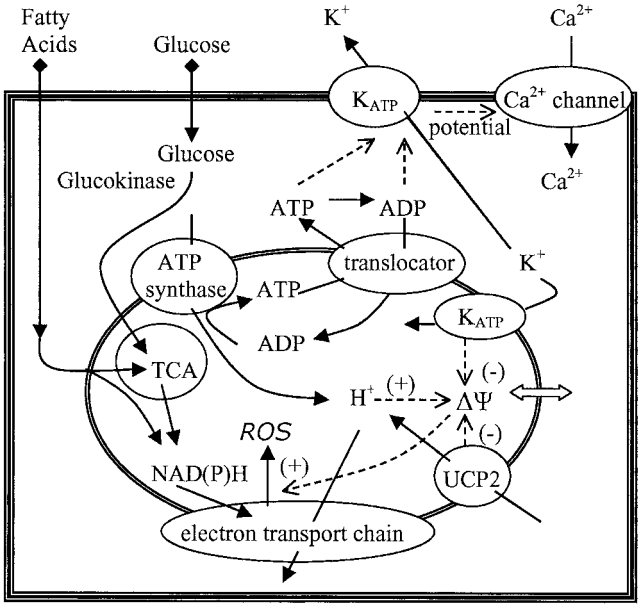

FIG. 1. The various components discussed in the text are illustrated in the cartoon of a β-cell and a mitochondrion within it. Glucose and fatty acid metabolism leads to an increase in NADH and Ca^{2+} and to a decrease in ADP. In turn, decreased ADP leads to increased mitochondrial membrane potential (ΔΨ) and a corresponding increase in ROS production. Increased glycolytic flux, decreased ADP concentration, and increased intracellular Ca^{2+} concentration could all contribute to both increased GDIS and ROS generation in the ETC. Solid lines indicate flux of substrates, and dashed lines indicate regulating effects, where (+) represents activation and (−) represents repression.

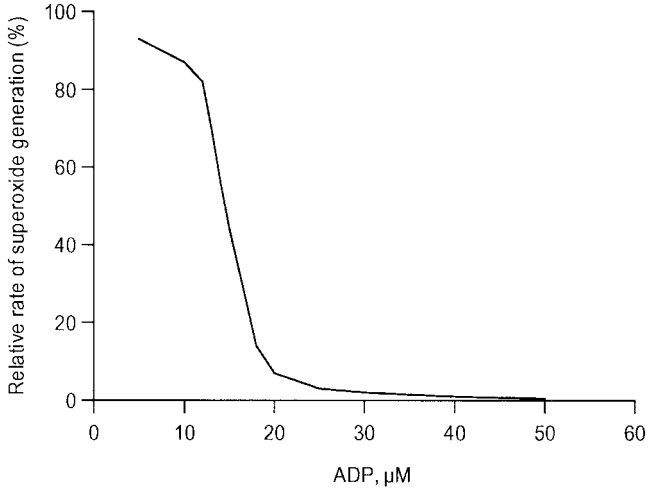

FIG. 2. Results of mathematical modeling of coupled mitochondria for relative rate of superoxide production as a function of extramitochondrial-free ADP concentration. Recalculated from Fig. 3*A* and *D* in the model of Demin, Westerhoff, and Kholodenko (27).

ADP is free (11,14). Several measurements of free ADP have been performed in β-cells. Ghosh et al. (15) found in β-cell–rich rat pancreatic islet cores that an increase of glucose from 4 to 8 mmol/l led to a decrease of free ADP from ~44 to ~31 μmol/l (pooled data from Table 5 in Ghosh et al. [15]). ATP concentration increased only insignificantly following glucose challenge in these experiments. Ronner et al. (12) found (in clonal βHC9 insulin-secreting cells) that increased glucose concentration was associated with an exponential decline in the concentration of free ADP from ~50 μmol/l at 0 mmol/l glucose to ~5 μmol/l at 30 mmol/l glucose, whereas the concentration of ATP remained nearly constant. These data suggest that a sharp decrease of free ADP with only a relatively small change in ATP concentrations is the characteristic feature of the response of β-cells to glucose stimulation. The functional necessity of this change in the ATP-to-ADP ratio can be understood from a consideration of K_{ATP} regulation (for details, see the review in ref. 9).

The activity of K_{ATP} channels decreases when pancreatic β-cells are exposed to increasing concentrations of glucose. However, as discussed above, only small changes in ATP concentrations were found following exposure to increased glucose concentrations. This cannot by itself induce sufficient closure of K_{ATP} channels. However, decreased free ADP concentrations in the physiological range can close K_{ATP} channels at constant ATP concentration (7,16,17). This means that a considerable increase in glycolytic flux and a sharp decrease in free ADP levels could be the necessary conditions leading to closure of K_{ATP} channels following glucose challenge and to insulin secretion.

Dependence of ROS production in β-cells on the GDIS mechanism. Recently we reported (18) that real-time estimations of superoxide production indicated that O_2^- generation is coupled to mitochondrial metabolism in pancreatic β-cells. A similar conclusion was reached by Sakai et al. (19).

The principal source of ROS in most mammalian tissues is the ETC itself (20–22). Superoxide anions are generated by single-electron reduction of molecular oxygen in complexes of the mitochondrial ETC. ROS production depends on the concentration of the intermediate metabolites of these complexes because the ETC carriers in a more reduced state have the property of donating electrons to oxygen (20–22). The reduced state of the ETC carriers can be achieved by increased production of reducing equivalents in mitochondria or by decreased electron transfer capability on (or after) these carriers (20–22).

β-Cells have a sensitive system, starting with glucokinase, for initiating the response to physiological changes in glucose concentration. Therefore, in contrast to most other mammalian cell types, increased glucose concentration stimulates a steeply increased glycolytic flux in β-cells, followed by a sharp stimulation in the production of reducing equivalents (7,23). This means that this part of the GDIS mechanism could lead itself to an enhancement of ROS production in pancreatic β-cells following glucose challenge. Increased fatty acid oxidation and the addition of some intermediate metabolites could also lead to additional production of reducing equivalents.

However, decreased electron transfer capability in the ETC can also be an important mechanism affecting ROS production. Because ETC is coupled to ATP synthesis through membrane potential (ΔΨ), the electron transport rate and, consequently, the rate of superoxide production will also depend on ΔΨ. The increased ΔΨ decreases electron transport capability in ETC, leading to a reduced state of the carriers and increased ROS production (20–22). It was shown experimentally (24–26) and confirmed by simulation with the corresponding mathematical model (27) that the rate of superoxide production increases

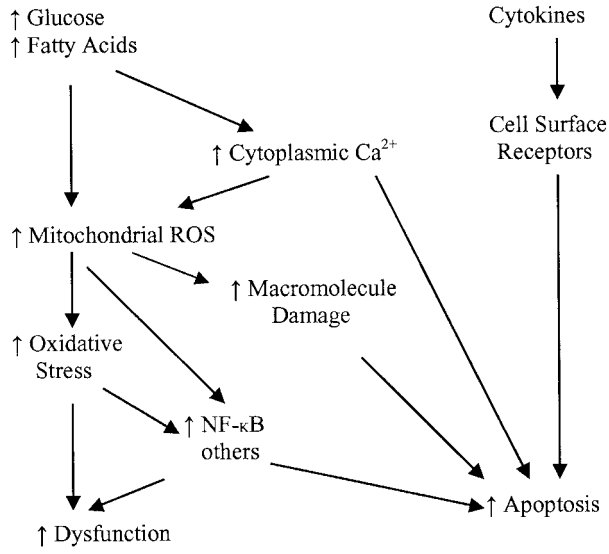

FIG. 3. Proposed causative link between elevated mitochondrial ROS generation, increased cytoplasmic Ca^{2+}, oxidative stress, β-cell dysfunction, and apoptosis (explanations are in the text).

dramatically with increased $\Delta\Psi$ >140 mV, when the rate of electron transport is restricted by increased $\Delta\Psi$.

Since $\Delta\Psi$ is used to make ATP from ADP and P_i, driven by proton movement back through the ATP synthase complex, its value also depends on the ATP production rate and, in particular, on free ADP concentration. The mitochondrial oxidative phosphorylation rate increases with increased free ADP concentration, with an apparent half-saturated concentration of ~20–45 μmol/l (28–30). Therefore, a decrease in free ADP concentration leads to decreased ATP production, which in turn increases $\Delta\Psi$ and, correspondingly, ROS production. Results of mathematical modeling of coupled mitochondria show that $\Delta\Psi$ can increase from 120 to ~200 mV as ADP decreases from 40 to 15 μmol/l (Fig. 3*A* from Demin, Westerhoff, and Kholodenko [27]). This explains the sharp increase in ROS production with decreased ADP concentration (Fig. 2). Modeling data were supported by the finding that a decrease in steady-state $\Delta\Psi$ level and a corresponding fall in H_2O_2 generation rate were both obtained after the addition of progressively increasing amounts of uncoupler (SF6847) or ADP into a mitochondrial suspension (25). These data lead to the conclusion that decreased ADP concentration can cause a considerable increase in ROS production (20,27). This idea was recently confirmed for β-cells by the demonstration that ADP inhibited ROS generation in permeabilized MIN6 cells (31).

After the addition of glucose there is a decrease in free [ADP] in β-cells (see GDIS AND ADENINE NUCLEOTIDE REGULATION). Hence, the specific stage in the GDIS mechanism leading to a decrease in free [ADP] can also be directly responsible for an overproduction of ROS. This decrease in ADP concentration is a specific property of β-cell stimulus-secretion coupling, possibly shared with other cell types that have a fuel-sensing function. In contrast, muscle work during aerobic exercise leads to increased ADP concentrations (30).

To make matters worse, β-cells have relatively low levels of free radical–detoxifying and redox-regulating enzymes such as superoxide dismutase, glutathione peroxidase, catalase (2,3,32), and thioredoxin (1). The reasons for this are unclear. Because ROS are involved in different physiological processes as mediators in signal transduction pathways (33), it was hypothesized that ROS are involved in some signaling pathways that take part in the insulin-secretion mechanism (18). In any case, the limited scavenging systems suggest that enhanced ROS concentrations in β-cells may occur due to both decreased scavenging systems and ROS overproduction.

In support of this hypothesis we recently reported an estimation of ROS using an optical method. We found that stimulation with 10 mmol/l glucose (from an initial 2 mmol/l) increased nearly twofold the O_2^- production rate in pancreatic β-cells from Zucker lean rats, confirming the possibility of abrupt increases in O_2^- production with increased glucose (18). A similar increased ROS production rate was obtained by Sakai et al. (19) at increased glucose concentrations in a pancreatic β-cell line (MIN6) and in human islets. These studies are the first to measure the production of ROS in response to glucose in the β-cell.

Manifestations of oxidative stress and apoptosis. Protective effects of antioxidants, scavengers, and overexpression of antioxidant enzymes in transgenic mouse islets suggest that ROS overproduction can lead to manifestations of oxidative stress and apoptosis in β-cells (1–3,6). Several reviews (2,3,5,33,34) have recently considered how increased ROS production (or decreased ROS consumption) can lead to oxidative stress and apoptosis in different cell types, including β-cells. We present in Fig. 3 the most common steps for this connection.

Free radicals in cells (including the β-cell) may directly damage proteins, lipids, and nucleic acids, leading to mitochondria and cell dysfunction and death (22,33–35). In our experiments, the mitochondria were generally short and swollen in islets with the highest ROS production from the Zucker diabetic fatty (ZDF) rat, in contrast to Zucker lean control (ZLC) rat islets (18).

In addition to their ability to directly damage cellular macromolecules, ROS may also activate intracellular signaling pathways that lead to cell dysfunction and apoptosis (4,5,33,34,36). Two principal apoptotic pathways exist in β-cells: the "intrinsic" pathway initiated by the mitochondria and the "extrinsic" pathway initiated by cell-surface receptors.

The "intrinsic" pathway includes the activation of nuclear factor (NF)-κB and additional stress-sensitive targets (5,34). There is some evidence that activation of NF-κB is mostly a proapoptotic event in β-cells (36). However, in vascular endothelial cells, normalizing mitochondrial superoxide production blocks several major pathways leading to hyperglycemic damage (including NF-κB activation), and it was suggested that ROS production in mitochondria is a causal link between elevated glucose and the main pathways responsible for hyperglycemic damage (37). It would appear reasonable that these pathways are also activated by ROS in the β-cell (2), but this has not been directly confirmed.

The "extrinsic" pathway includes cytokine signaling and is considered in detail in a recent review by Donath et al. (4). However, the question "What makes the β-cell so sensitive to proinflammatory cytokines?" remains open (4). It has been suggested that glucose-induced β-cell

apoptosis involves the induction of both free oxygen radicals and the synthesis of proinflammatory cytokines, especially interleukin-1, activating proapoptotic pathways (5). Apoptosis may also be induced by a combination of macromolecular and mitochondrial damage, mainly due to ROS action (35). Altered mitochondria function plays a prominent role in the induction of apoptosis in several cellular models (33,35) as well as in the β-cell line Ins-1 (38). If this is the case in the β-cell, then the specific β-cell sensitivity to proinflammatory cytokines may be explained by the combination of ROS overproduction and insufficient scavenging systems.

Interestingly, transfection of a glucagon-producing rat cell line with the pancreatic duodenal homeobox transcription factor leading to an insulin-producing β-cell phenotype resulted in a higher sensitivity to cytokine toxicity (39). In this case, the development of insulin-secretion mechanisms led to enhanced in vitro sensitivity to cytokines.

An elevation of intracellular Ca^{2+} through voltage-gated Ca^{2+} channels is an integral part of the GDIS mechanism (see GDIS AND ADENINE NUCLEOTIDE REGULATION) (Fig. 1). However, increased intracellular Ca^{2+} is also believed to stimulate mitochondrial generation of ROS (26). Voltage-gated Ca^{2+} channels are also likely to play an activating role in β-cell apoptosis, although the molecular mechanisms remain to be described (4). Hence, an increase in cytoplasmic Ca^{2+} concentration and an activation of voltage-gated Ca^{2+} channels are additional specific stages in GDIS, which may share responsibility for an increase of oxidative stress and/or for a mediation of apoptosis.

We can conclude that at least three stages of the GDIS mechanism (increased glycolytic flux, decreased ADP concentration, and increased intracellular Ca^{2+}concentration) could lead to a dramatic increase in the development of oxidative stress and apoptosis in pancreatic β-cells. We can name this connection the GDIS→ROS hypothesis. This GDIS→ROS hypothesis provides a testable framework to explain how β-cells may be uniquely at high risk for oxidative damage and apoptosis.

Dual role of the GDIS mechanism. The consequences of the GDIS→ROS hypothesis should be evaluated in light of the existing experimental data. According to this hypothesis, GDIS activation plays a dual role: the metabolic secretagogues causing increased insulin secretion can also lead to increased oxidative stress as a result of elevated ROS production.

This dual role of the GDIS mechanism might hinder investigations since an initial increase in the insulin secretion rate can at first mask eventual detrimental effects of oxidative stress on insulin production. However, there is an essential difference in the temporal development of these processes. Insulin secretion changes relatively quickly and oxidative stress seems to develop more gradually and may be revealed only after several days of exposure to metabolic secretagogues (2,40). Therefore, progressive injury of β-cell function by the same effectors that increase GDIS quickly could be considered a characteristic feature of oxidative stress activated by the GDIS mechanism itself. For example, chronic exposure to elevated glucose concentrations may cause damage to β-cells through mechanisms involving oxidative stress (2,3,34,40). This reinforces the idea that glucose initially activating insulin secretion can also injure β-cell function with time. However, the idea that glucose-induced ROS generation is responsible for β-cell glucose toxicity remains a testable speculation because glucose-induced ROS generation does occur with a brief exposure to physiologically relevant elevated glucose concentration (18), whereas glucose toxicity does not.

Lipotoxicity can also develop in β-cells in a similar fashion to oxidative stress at elevated glucose. On a short-term basis (<24 h), fatty acids stimulate GDIS in part by causing an increase in the production of reducing equivalents due to β-oxidation and additional acyl-CoA mitochondrial oxidation (7). Fatty acids may also increase Ca^{2+} mobilization from the endoplasmic reticulum (41). This can lead to decreased ADP levels, increased cytoplasmic Ca^{2+}, and increased insulin production. In contrast, chronic exposure (>24 h) of β-cells to fatty acids leads to a reduction in GDIS (2,40). Current explanations (2,42) of this lipid-induced toxicity in β-cells certainly involve the effects of oxidative stress. Hence, lipotoxicity appears to be at least partly a manifestation of supplementary ROS production induced by additional production of reducing equivalents in mitochondria pari pasu with fatty acid metabolism.

Direct data on mitochondrial O_2^- production rates obtained in our laboratory also confirms the possibility that β-cells are subject to oxidative stress at increased concentrations of fatty acids. Superoxide production in ZDF rat islets was significantly higher than in ZLC rat islets under resting conditions (with 2 mmol/l glucose), and the overproduction of superoxide was associated with perturbed mitochondrial morphology in ZDF rat islets (18). Abnormal mitochondrial morphology in ZDF rat islets and its reversal by systemic treatment with troglitazone were also observed by Higa et al. (43). Because ZDF rat islets accumulate triglycerides (43), these changes can be explained by increased ROS production as a result of increased content of free fatty acids in these β-cells.

Dual role of β-cell mitochondria uncoupling. Another consequence resulting from the GDIS→ROS hypothesis relates to the uncoupling of β-cell mitochondria. Any active or passive transport of cations or anions across the mitochondrial inner membrane will affect $\Delta\Psi$. Multiple uncoupling agents could degrade the proton gradient across the mitochondrial inner membrane and decrease the $\Delta\Psi$ level, causing a corresponding decreased ATP secretion, increased ADP concentration, and diminished ROS production rates. However, this should be accompanied by a decrease in the ATP-to-ADP ratio and, consequently, by decreased insulin production because plasma membrane K_{ATP} channels will be insufficiently closed (see GDIS AND ADENINE NUCLEOTIDES REGULATION).

This dual role of uncoupling can be illustrated by considering the principal β-cell uncoupling protein (UCP)2, which catalyzes a regulated proton leak across the mitochondrial inner membrane (Fig. 1) (see Saleh, Wheeler, and Chan [44] for review). Indeed, islets from UCP2-deficient mice have an increased ATP level and an enhanced glucose-stimulated insulin secretion compared with control animals (45). On the other hand, overexpression of UCP2 in isolated pancreatic islets results in de-

creased ATP content, reduced $\Delta\Psi$, and blunted glucose-stimulated insulin secretion (46). However, in line with the suggested dual role of mitochondrial membrane uncoupling, overexpression of UCP2 enhanced the resistance of β-cells toward H_2O_2 toxicity (47).

The inner mitochondrial membrane K_{ATP} channel is another mechanism through which $\Delta\Psi$ could be regulated. Enhanced K^+ uptake through mitochondrial K_{ATP} channels would lead to a lower $\Delta\Psi$. This effect could promote a decline in mitochondrial ROS production (48,49). There is as yet no direct data on the role of mitochondrial K_{ATP} channels in pancreatic β-cells; however, one would expect that the openers of mitochondrial K_{ATP} will act similarly to UCP2 activation.

Intracellular inhibition of ROS production can lead to a decrease in GDIS. We have suggested that the initial stages of GDIS can be responsible for increased ROS production in β-cells. Therefore, it is possible that inhibition of these stages can be used by β-cells as a defense against oxidative stress. There are several examples of such potential mechanisms. For example, studies (50,51) on the mechanism of action of the diabetogenic agent alloxan have suggested that its target, glucokinase, is sensitive to oxidation by ROS. Generation of ROS in HIT-T15 cells treated with the D-ribose caused a significant reduction in both glucokinase transcription and protein expression, leading to reduced glucokinase activity (52). Therefore, increased ROS production in β-cells might lead to glucose sensor (glucokinase) inhibition. In addition, both H_2O_2 and high glucose suppress the activity of glyceraldehyde 3-phosphate dehydrogenase, a glycolytic enzyme, in pancreatic β-cells that can lead to impaired GDIS (19).

It also seems likely that β-cells can increase UCP2 expression to decrease oxidative stress. For example, superoxide increases proton conductance in mitochondria from pancreatic β-cells, probably via activation of UCP2 (53). Increased glucose induces expression of UCP2 in isolated human islets (54). Chronic exposure of pancreatic islets to free fatty acids, blunting GDIS, is accompanied by increased synthesis of UCP2 (55). These mechanisms of protection from oxidative stress would decrease the rate of ATP production and the corresponding ATP-to-ADP ratio, leading to impaired β-cell sensitivity to glucose simulation, a characteristic feature of type 2 diabetes.

Preservation of β-cell function and islet mass versus enhancement of GDIS: mutually exclusive goals? Cellular antioxidant systems exist within cells to neutralize ROS. Oxidative stress can arise only when the endogenous antioxidant network fails to provide a sufficient compensatory response to survival or to restore a cellular function (2,35). Resent research has also demonstrated a direct link among the imbalance of oxidative stress, impaired glucose uptake, and antioxidants for both diabetic animal models and in human disease. This leads to the hypothesis that the imbalance of ROS production and antioxidants is one important factor in the etiology of diabetes (2,56). This hypothesis suggests that ROS overproduction in β-cells is only part of the process leading to the development of β-cell dysfunction. It seems likely that some additional defect as, for example, decreased antioxidant production, is required for β-cell dysfunction and/or apoptosis. However, as we have attempted to illustrate, some specific β-cell properties make them a weak link in the defense against oxyradicals.

Because the activation of initial stages of GDIS or increased $\Delta\Psi$ immediately leads to increased insulin secretion, it is not surprising that these stages are targets for therapeutic intervention. For example, glucokinase plays a key role in initial GDIS stages by catalyzing the phosphorylation of glucose in β-cells. A new class of antidiabetic agents, mixed-type glucokinase activators that increased both the affinity for glucose and the V_{max}, was shown to stimulate GDIS (8,57). A reduction in UCP2 activity was also suggested as a mechanism for significant improvement in insulin secretion (58). We can also suggest that a reduction in K_{ATP} activity in the inner mitochondrial membrane should lead to increased insulin secretion rate, as does decreased UCP2 activity. However, such a therapeutic strategy should be used with caution, since according to our proposal an increase in insulin secretion achieved by these approaches could also considerably increase ROS production, leading to oxidative stress.

The concept of "β-cell rest" as originally developed, perhaps more for amelioration of type 1 than type 2 diabetes, argued that decreased demand on β-cell function can lead to improvements in insulin secretion and β-cell viability (2,59,60). Such agents as diazoxide and calcium channel blockers, which reversibly inhibit insulin secretion, have improved β-cell function both in rodent models of diabetes (61,62) and in humans (63). This beneficial effect could be explained by the decreased ROS production during "β-cell rest" associated with decreased GDIS activity.

Inhibition of the early stages of GDIS or decreased $\Delta\Psi$ should also lead to decreased ROS production. Any inhibitor of glycolytic flux, the tricarboxylic acid cycle, fatty acid oxidation, or mitochondrial membrane uncoupling could result in decreased ROS production. For example, this could be accomplished by specific inhibitors of β-cell glucokinase, by an increase in UCP2 expression, or by openers of mitochondrial K_{ATP} channels. However, a decreased insulin secretion rate is the necessary price to pay for these approaches to increasing β-cell function and survival. For this reason, such methods can predominantly be used when the GDIS mechanism is not the main source of insulin production. This of course can occur following treatment by plasma membrane K_{ATP} channel blockers, such as sulfonylureas and meglitinides, which can compensate for the inadequate closure of these K_{ATP} channels at reduced ATP/ADP levels, or simply by insulin therapy.

However, plasma membrane K_{ATP} channel blockade is accompanied by increased Ca^{2+} levels in β-cells, which can itself increase oxidative stress (see DEPENDENCE OF ROS PRODUCTION IN β-CELLS ON THE GDIS MECHANISM). For this reason, the simplest and potentially most beneficial method to decrease oxidative stress in β-cells may be that of early use of the above-mentioned GDIS inhibitors, with insulin as necessary. This approach would decrease both glucose levels and the corresponding ROS production. Although this approach has not always been used to an advantage (59), recent studies (60,64) have suggested that early insulin treatment in type 2 diabetes indeed preserved endogenous insulin secretion. Additional intervention

with GDIS inhibitors could improve the "β-cell rest" approach to treatment.

CONCLUSIONS

We have compared metabolic pathways of GDIS and ROS production and suggest that secretagogues causing increased insulin secretion by the activation of initial steps of the GDIS mechanism can also lead to increased ROS production. This should lead to activation of oxidative stress concomitant with stimulation of the GDIS mechanism. By this reasoning, the main function of a β-cell, i.e., regulated insulin secretion, can be connected with the seeds of its own destruction. This paradoxical feature of pancreatic β-cells suggests some specific therapeutic strategies, such as reexamination of the "β-cell rest" concept in type 2 diabetes.

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