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ROS signaling, oxidative stress and Nrf2 in pancreatic beta-cell function

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Abstract

This review focuses on the emerging evidence that reactive oxygen species (ROS) derived from glucose metabolism, such as H₂O₂, act as metabolic signaling molecules for glucose-stimulated insulin secretion (GSIS) in pancreatic beta-cells. Particular emphasis is placed on the potential inhibitory role of endogenous antioxidants, which rise in response to oxidative stress, in glucose-triggered ROS and GSIS. We propose that cellular adaptive response to oxidative stress challenge, such as nuclear factor E2-related factor 2 (Nrf2)-mediated antioxidant induction, plays paradoxical roles in pancreatic beta-cell function. On the one hand, induction of antioxidant enzymes protects beta-cells from oxidative damage and possible cell death, thus minimizing oxidative damage-related impairment of insulin secretion. On the other hand, the induction of antioxidant enzymes by Nrf2 activation blunts glucose-triggered ROS signaling, thus resulting in reduced GSIS. These two premises are potentially relevant to impairment of beta-cells occurring in the late and early stage of Type 2 diabetes, respectively. In addition, we summarized our recent findings that persistent oxidative stress due to absence of uncoupling protein 2 activates cellular adaptive response which is associated with impaired pancreatic beta-cell function.

Keywords

ROS; oxidative stress; antioxidant; Nrf2; pancreatic beta-cells; insulin secretion; Ucp2

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1. Introduction

Type 2 diabetes (T2D) has become a serious public health problem in the world. Approximately 150 million people worldwide had T2D in the year 2000, with the prediction that this number could double by 2025 (Zimmet *et al.*, 2001). The medical and socioeconomic burdens of the disease caused by its associated complications, impose enormous strains on health-care systems and economic wealth (Stumvoll *et al.*, 2005). Although the primary causes of T2D are unknown, insulin resistance plays an early role in its pathogenesis and defective glucose-stimulated insulin secretion (GSIS) from pancreatic beta-cells is instrumental in the progression to hyperglycemia (Fridlyand and Philipson, 2004; Krauss *et al.*, 2005). GSIS is regulated by the rate of glucose metabolism within beta-cells. Following glucose uptake and phosphorylation, glucose oxidation involves both cytosolic and mitochondrial processes that generate signals leading to insulin secretion (Newgard and McGarry, 1995; Henquin, 2004; Jensen *et al.*, 2008). Nevertheless, the precise spectrum of signals that couples glucose catabolism to insulin secretion are still incompletely understood. Previous studies (Bindokas *et al.*, 2003; Armann *et al.*, 2007; Leloup *et al.*, 2009; Morgan *et al.*, 2009) including our own (Pi *et al.*, 2007) suggest that reactive oxygen species (ROS), such as H₂O₂, derived from glucose metabolism serve as one of the metabolic signals for GSIS. However, endogenous antioxidant enzymes that can be robustly induced in response to exposure to oxidative stressor have the potential to blunt such a glucose-triggered ROS signal and thus inhibit GSIS (Pi *et al.*, 2007). This review focuses on the emerging evidence that ROS derived from glucose metabolism may function as metabolic signaling molecules for GSIS. Particular emphasis is placed on the potential inhibitory role of endogenous antioxidants, which rise in response to oxidative challenges, in glucose-triggered ROS and GSIS.

2. Metabolic signaling in GSIS

GSIS is regulated by the rate of glucose metabolism within beta-cells. Following its initial uptake and phosphorylation, glucose metabolism involves both cytosolic and mitochondrial processes and generates signals leading to insulin secretion (Newgard and McGarry, 1995; Jensen *et al.*, 2008). It has become established in the field that glycolytic and oxidative processes leading to an increased ATP/ADP ratio are key transduction events in beta-cell signaling (Krauss *et al.*, 2005). However, the precise signals that couple glucose catabolism to insulin secretion are still incompletely understood. The consensus model explaining how glucose generates a triggering signal in beta-cells involves the following sequence of events: entry of glucose by facilitated diffusion, metabolism by oxidative glycolysis, rise in ATP/ADP ratio, closure of K_{ATP} channels, depolarization of the plasma membrane potential, opening of voltage-operated Ca²⁺ channels, influx of Ca²⁺, rise in cytosolic Ca²⁺ and activation of exocytotic machinery (Henquin, 2000; Wollheim and Maechler, 2002). Over the past few years, many novel players have been implicated in the control of insulin secretion. This extensive collection includes glutamate (Maechler and Wollheim, 1999; Lehtihet *et al.*, 2005), protein phosphatases (Ostenson *et al.*, 2002; Sjöholm *et al.*, 2002; Lehtihet *et al.*, 2005; Pagliarini *et al.*, 2005), GTP (Sjöholm *et al.*, 2002), GTP-binding proteins (Kowluru, 2003), cGMP (Soria *et al.*, 2004), cAMP-guanosine nucleotide exchange factor II (Ozaki *et al.*, 2000), diadenosine polyphosphates (Soria *et al.*, 2004), NADH shuttle (Eto *et al.*, 1999), transcription factor NF-κB (Norlin *et al.*, 2005), protein kinases including cyclin-dependent kinase 5 (Wei *et al.*, 2005), cAMP-dependent protein kinase (Wollheim and Maechler, 2002), PKC (Jones and Persaud, 1998) and cyclic pathways of pyruvate metabolism (Jensen *et al.*, 2008). Thus, there is no shortage of potential players, although the precise nature by which glucose controls insulin secretion remains elusive.

3. ROS: overlooked signaling molecules in pancreatic beta-cells?

ROS such as superoxide anion ($O_2^{\cdot-}$) and H_2O_2 are produced in aerobic cells either during mitochondrial electron transport or by several oxidoreductases and metal-catalyzed oxidation of metabolites (Forman and Torres, 2002). For many years ROS have been exclusively thought of as the unfortunate byproducts of respiratory energy production in mitochondria and believed to be deleterious to biological systems (Finkel, 1998). However, ROS generation is not always a useless or harmful process but, rather, may be an essential element required for many biological responses (Finkel, 2003; Buetler *et al.*, 2004). Small variations of intracellular ROS have been shown to modulate many physiological processes including redox-dependent transcriptional regulation (Arrigo, 1999; Liu *et al.*, 2005), ion transport (Kourie, 1998), as well as protein phosphorylation (Finkel, 1998; Rhee *et al.*, 2005).

In most cells, ROS can be produced in the presence of high concentrations of glucose via multiple mechanisms. These include oxidative phosphorylation, glucose auto-oxidation, the Schiff reaction during glycation, PKC activation, methylglyoxal formation, and hexosamine metabolism (Brownlee, 2001; Kowluru, 2003). Mitochondria, under non-stress conditions, are one of the major sources of ROS in most cells (Turrens, 2003; Brand *et al.*, 2004). The main sites of $\cdot O_2^-$ generation are in the inner mitochondrial membrane: NADH dehydrogenase at complex I, and the interface between ubiquinone and complex III (Nishikawa *et al.*, 2000; Brand *et al.*, 2004). During the process of cellular respiration, there are at least three events that can lead to dramatically increased $\cdot O_2^-$ generation in beta-cells (Fridlyand and Philipson, 2004) - increased glycolytic flux, decreased ADP concentration, and increased intracellular Ca^{2+} concentration.

$\cdot O_2^-$, the parental form of intracellular ROS, is a very reactive molecule but it can be converted to H_2O_2 by superoxide dismutase (SOD) isoenzymes, and then to oxygen and water by several enzymes including catalase (CAT), glutathione peroxidase (GPx) and peroxiredoxin (Prx). Pancreatic beta-cells are equipped with $\cdot O_2^-$ -inactivating SODs in the cytosol and mitochondria at levels of about 50% of those in the liver (Lenzen *et al.*, 1996; Tiedge *et al.*, 1997). However, the expression levels of the H_2O_2 -inactivating enzymes GPx and CAT are extremely low in islets, at levels of only about 1% of those in the liver (Lenzen *et al.*, 1996; Tiedge *et al.*, 1997). This reduced antioxidant capacity potentially makes pancreatic beta-cells sensitive to ROS-mediated signal transduction and cellular response.

H_2O_2 is a small, uncharged, freely diffusible molecule that can be synthesized and destroyed rapidly in response to external stimuli (Rhee *et al.*, 2003; Rhee, 2006). These features meet all of the important criteria for an intracellular messenger. Indeed, a growing body of evidence supports the notion that H_2O_2 is a ubiquitous intracellular messenger (Rhee, 2006). To date, many important signal transduction molecules or processes have been recognized as downstream targets of ROS, and H_2O_2 in particular. These molecules and processes include Ca^{2+} -dependent protein phosphatases (Kamsler and Segal, 2004), protein tyrosine phosphatases (Denu and Tanner, 1998; Pagliarini *et al.*, 2005), voltage-gated K^+ channels (Archer *et al.*, 2004), Ca^{2+} influx and release (Krippeit-Drews *et al.*, 1995; Todt *et al.*, 2001; Kraft *et al.*, 2004; Tabet *et al.*, 2004), tumor suppressor phosphatase PTEN (Rhee *et al.*, 2005), c-Jun N-terminal kinase (Nemoto *et al.*, 2000), extracellular signal-regulated kinases (Aikawa *et al.*, 1997), NF- κ B (Schmidt *et al.*, 1996), and SIRT1 deacetylase (Brunet *et al.*, 2004; Moynihan *et al.*, 2005). Many of these molecules may potentially regulate insulin secretion. It should be noted that the gradient of H_2O_2 in cells is very steep, as evidenced by microscopic measurements in human keratinocytes (Pi *et al.*, 2003) and mouse islets (Pi *et al.*, 2007; Pi, 2009), so that all signaling by this molecule would require the target to be in very close proximity to the source.

ROS generation occurs in glucose metabolism and correlates with insulin secretion. Nevertheless, relatively little attention has been paid to the potential role of ROS in glucose signaling and insulin secretion. In 1999, Wollheim's group found, that H_2O_2 and alloxan, which acutely increased intracellular H_2O_2 , caused a rapid elevation of intracellular Ca^{2+} and briefly increased insulin release in rat islets at basal, nonstimulatory glucose concentrations (Janjic *et al.*, 1999; Maechler *et al.*, 1999). Consistent with these observations, short-term exposure of isolated rat islets to alloxan and xanthine oxidase/hypoxanthine, a well known $\cdot\text{O}_2^-$ generation system, led to a temporarily elevated insulin release (Ebelt *et al.*, 2000). In addition, attenuation of expression of glutamate-cysteine ligase (GCL), which reduced GSH synthesis and thus potentially raised intracellular ROS, enhanced insulin secretion in MIN6 cells (Kondo *et al.*, 2000). In our recent studies (Pi *et al.*, 2007), exogenous H_2O_2 as well as diethyl maleate, which enhanced intracellular H_2O_2 levels, increased insulin secretion in INS-1 (832/13) cells and isolated mouse islets, whereas high concentration of exogenous antioxidants inhibited the GSIS in these *in vitro* models. Support for the notion that ROS mediate glucose action also comes from Leloup *et al.* who reported that mitochondrial ROS are required for hypothalamic glucose sensing (Leloup *et al.*, 2006) and GSIS (Leloup *et al.*, 2009). More recently, we determined the acute effects (30-min challenge) of various chemicals, including oxidants and antioxidants, on insulin secretion using INS-1 (832/13) cells (Table 1). Low concentrations (without significant cytotoxicity by 24-hr exposure) of oxidants, which increase intracellular H_2O_2 level, such as H_2O_2 solution and diethyl maleate (Pi *et al.*, 2007), hypochlorous acid (Pi *et al.*, 2008) and alloxan (Janjic *et al.*, 1999; Maechler *et al.*, 1999), facilitated insulin secretion, whereas antioxidants, including N-acetyl-cysteine, catalase, cell permeable glutathione, DL-dithiothreitol and reduced α -lipoic acid, blunted insulin secretion (Table 1). Interestingly, inhibiting the mitochondrial electron transport chain by various agents such as rotenone, antimycin A, oligomycin, which increase mitochondria-derived ROS but inhibit ATP generation, led to decreased GSIS. These results show that in beta-cells the glucose-induced rise in ROS alone cannot promote insulin release, and are consistent with the wealth of literature showing that glucose-induced elevations in ATP are necessary for GSIS (Henquin, 2000; Wollheim and Maechler, 2002). Since influx of Ca^{2+} is a major event to activate the insulin exocytotic machinery in the early phase of GSIS, the Ca^{2+} -dependence of H_2O_2 -stimulated insulin secretion was investigated in our previous studies (Pi *et al.*, 2007). Our results indicate that H_2O_2 -stimulated insulin secretion is an extracellular Ca^{2+} -dependent process, suggesting H_2O_2 may be involved in Ca^{2+} influx. In contrast, the evidence that exogenous antioxidants marginally inhibit KCl-induced insulin release could represent either a small nonspecific effect on secretion, or indicate that H_2O_2 is also involved in some aspect of secretion after Ca^{2+} influx (Pi *et al.*, 2007). Taken together, these studies suggest that ROS derived from glucose metabolism are potential metabolic signals that facilitate insulin secretion.

4. Oxidative stress, Nrf2-mediated antioxidant response and ROS signaling

ROS clearly possess the capacity to behave in a sporadic and destructive fashion (Finkel, 2003). Persistent elevation of ROS resulted from an imbalance between ROS production and scavenging by endogenous antioxidants can directly or indirectly disturb physiological functions of many cellular macromolecules such as DNA, protein, and lipids, and activate cellular stress-sensitive signaling pathways (Droge, 2002; Evans *et al.*, 2003). Having evolved in an oxygen environment, most cells, including beta-cells, have acquired intricate mechanisms to defend against ROS toxicity. Among them, induction of a family of antioxidant/detoxification enzymes that enhance cellular ROS-scavenging capacity is a key element in the maintenance of cellular redox homeostasis and in reducing oxidative damage (Wasserman and Fahl, 1997; Nguyen *et al.*, 2003; Itoh *et al.*, 2004). These enzymes include NAD(P)H: quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO1), GCL, glutathione reductase (GR), glutathione synthetase (GS), GPx, CAT and many others (Wasserman and Fahl, 1997; Nguyen *et al.*, 2003; Itoh *et al.*, 2004). These antioxidant genes are coordinately regulated through

consensus *cis*-elements called antioxidant response elements (AREs) in their 5'-flanking promoter regions (Nguyen *et al.*, 2003). Transcription factor Nrf2, a member of the Cap 'n' Collar family of bZIP proteins, is a central regulator in both constitutive and inducible ARE-related gene expression (Itoh *et al.*, 2004) and innate immune response (Thimmulappa *et al.*, 2006). Nrf2 knockout mice show a deficiency in this coordinated gene regulatory program and have a higher susceptibility to oxidative damage and chemical carcinogenesis (Chan and Kan, 1999; Chan *et al.*, 2001; Ramos-Gomez *et al.*, 2001).

Thus, the Nrf2-mediated antioxidant response represents a critically important cellular defense mechanism that serves to maintain intracellular redox homeostasis and limit oxidative damage (Nguyen *et al.*, 2003; Cullinan and Diehl, 2004; Itoh *et al.*, 2004). In spite of this protective role, antioxidant enzymes may function as an off switch for the signal transduction pathways mediated by ROS (Forman and Torres, 2002). Thus the Nrf2-mediated antioxidant response has a potential to be "too much of a good thing", producing undesirable effects. It could dampen normal ROS/redox signaling that could be triggered, for instance, by glucose oxidation. Therefore, we propose that Nrf2-mediated antioxidant response plays a paradoxical role in insulin secretion (Fig. 1). On the one hand, it protects beta-cells from oxidative damage and possible cell death, thus minimizing oxidative damage-related impairment of insulin secretion. On the other hand, situations leading to chronic induction of endogenous antioxidants due to oxidative stress may blunt endogenous ROS signaling, resulting in reduced GSIS.

To understand the quantitative nature of ROS signaling, we resorted to an ordinary differential equation-based model to describe the Nrf2-mediated adaptive response to oxidative stress (Zhang and Andersen, 2007; Zhang *et al.*, 2008). We investigated *in silico* whether pre-existing conditions of oxidative stressor might interfere with subsequent ROS signaling, as triggered, for instance, by glucose. Our simulation indicates that in the absence of oxidative challenge, a stimulus mimicking glucose treatment causes a large spike in intracellular H₂O₂ concentration. However, when the cell is under continuous oxidative challenge, the same glucose-mimicking stimulus triggers a much smaller increase in H₂O₂ (Pi *et al.*, 2007). These *in silico* results demonstrate that by adaptively upregulating overall antioxidant capacity chronic oxidative stress is expected to blunt acute ROS signaling.

Thus, we envisage the following scenarios for chronic oxidative stress-induced impairment of beta-cell function. Under low-level oxidative stress (without cytotoxicity), beta-cells will adapt to the condition adequately, by activating cellular adaptive response, such as the Nrf2-ARE system, thereby keeping oxidative damage/cell death-related impairment of GSIS at a minimum. However, persistent induction of endogenous antioxidants would attenuate glucose-dependent ROS signaling leading to reduced GSIS. In contrast, under high-level oxidative challenge, the Nrf2 system may be overwhelmed and unable to protect the cells from oxidative damage and/or death. In this situation, excessive oxidative damage and possible cell death become the primary cause for the impaired GSIS. These two premises are potentially relevant to impairment of beta-cells occurring in the early and late stage of Type 2 diabetes, respectively.

5. New findings on Ucp2 and endogenous ROS production in beta-cell function

Mitochondria are one of the primary sources of ROS under basal conditions (Turrens, 2003; Brand *et al.*, 2004). The $\cdot\text{O}_2^-$ production from the mitochondrial matrix is very sensitive to the proton motive force (Jezek *et al.*, 2004), so mild uncoupling can substantially decrease mitochondria-derived ROS and is believed to aid in preventing oxidative damage (Miwa and Brand, 2003; Brand *et al.*, 2004; Jezek *et al.*, 2004). Ucp2 is a widely expressed mitochondrial inner membrane carrier protein that was discovered through its homology to the brown fat Ucp1, which dissipates caloric energy in the form of heat by uncoupling mitochondrial

respiration from ATP production (Ricquier and Bouillaud, 2000). Ucp2 is not a physiologically relevant “uncoupling protein” as is Ucp1 and does not contribute to adaptive thermogenesis (Krauss *et al.*, 2002; Rousset *et al.*, 2004; Brand and Esteves, 2005). However, there is evidence that UCP2 increases the proton conductance of the mitochondrial inner membrane when it is activated by $\cdot\text{O}_2^-$ (Echtay *et al.*, 2002a; Echtay *et al.*, 2002b) and/or free radical-derived alkenals such as 4-hydroxy-2-nonenal (HNE) (Echtay *et al.*, 2003; Brand and Esteves, 2005).

Since mitochondria are one of the major sources of ROS in cells and the production of $\cdot\text{O}_2^-$ is sensitive to the uncoupling of respiration, the Ucp2-mediated feedback regulation on ROS generation may be considered as a compensatory mechanism alleviating oxidative stress (Ruzicka *et al.*, 2005). Consistent with this idea, Ucp2-ablated mice exhibit increased ROS production in macrophages and have a higher susceptibility to ROS-induced damage (Arsenijevic *et al.*, 2000; Blanc *et al.*, 2003; Krauss *et al.*, 2003; Horimoto *et al.*, 2004; Bai *et al.*, 2005). In contrast, overexpression of Ucp2 protects cells from oxidative damage (Li *et al.*, 2001; Kizaki *et al.*, 2002; Diano *et al.*, 2003; Ryu *et al.*, 2004). Furthermore, as with most antioxidant responses, the transcription of the Ucp2 gene is highly inducible under oxidative stress conditions. For instance, the treatments by H_2O_2 (Li *et al.*, 2001), lipopolysaccharide (Pecqueur *et al.*, 2001; Ruzicka *et al.*, 2005), TNF α (Lee *et al.*, 1999), free fatty acids (Chevillotte *et al.*, 2001; Medvedev *et al.*, 2001; Medvedev *et al.*, 2002), and irradiation (Voehringer *et al.*, 2000) increase Ucp2 expression *in vitro* and *in vivo*. Thus, there is good evidence that a major function of Ucp2 is to attenuate mitochondrial production of ROS and activation/induction of Ucp2 may function as an alternate adaptive response to mitochondria-derived ROS generation and oxidative damage.

The first report of Ucp2 $^{-/-}$ mice from Collins and colleagues described a phenotype of exaggerated ROS and phagocytosis in macrophages (Arsenijevic *et al.*, 2000). Another report of Ucp2 $^{-/-}$ mice showed dramatically enhanced GSIS in Ucp2 $^{-/-}$ mice (Zhang *et al.*, 2001). This finding in beta-cells of Ucp2 $^{-/-}$ mice was replicated by Collins and Corkey (Pi, 2009). Of note, all studies on Ucp2 $^{-/-}$ mice during that time were conducted with mice that were a C57BL/6J and 129 mixed background. However, after the mice were backcrossed onto one of three strains of inbred mice (C57BL/6J, A/J, or 129/SvImJ) for 8–20 generations to eliminate the potential influence of genetic background on their phenotypes, a significantly decreased GSIS in islets was observed in the Ucp2 $^{-/-}$ mice (Pi, 2009). The consistent observation of diminished GSIS from three highly congenic lines are highly suggestive that decreased GSIS of islets is the phenotype that results from absence of Ucp2.

Extensive studies have indicated that Ucp2 negatively regulates mitochondria-derived ROS generation (Arsenijevic *et al.*, 2000; Bai *et al.*, 2005). Thus, elimination of Ucp2 *in vivo* by targeted gene “knockout” has a potential to promote oxidative stress and activate adaptive antioxidant response. Consistent with this hypothesis, the expression of a broad array of antioxidant enzymes including SOD2, GPx2, GCLC, GS, CAT and HO-1 was significantly elevated in the islets of Ucp2 $^{-/-}$ mice (Pi, 2009). Importantly, despite enhanced basal H_2O_2 levels in Ucp2 $^{-/-}$ islets, glucose-stimulated ROS production was markedly lower than in Ucp2 $^{+/+}$ mice. Together with our previous results (Arsenijevic *et al.*, 2000; Bai *et al.*, 2005), these findings provide evidence to suggest that chronic absence of Ucp2 results in persistent oxidative stress and thereby activation of the antioxidant defense system, which likely blunt GSIS in islets.

6. Antioxidants as therapeutics in treating T2D: beneficial or harmful?

In contrast to the prevailing detrimental view of ROS in many cellular responses and disease states, including T2D, elevated levels of antioxidant molecules have been observed in *ob/ob* mice (Nakao *et al.*, 2000), subjects at risk of T2D (Costa *et al.*, 2002) and diabetic patients

(Chen *et al.*, 2003; Chen *et al.*, 2004). Thus, induction of endogenous antioxidant enzymes may have a role during the development of T2D. More importantly, overexpression of H₂O₂-scavenging enzymes, such as CAT and Gpx1, actually increased the likelihood of developing diabetes (McClung *et al.*, 2004; Li *et al.*, 2006). In humans, the clinical outcomes of antioxidant therapy in the treatment of T2D remain very inconclusive (Wiernsperger, 2003; Scott and King, 2004). A recent report even showed that use of antioxidants actually increased the incidence of all-cause death (Bjelakovic *et al.*, 2007). These results have cast doubt on the usefulness of antioxidant supplements, and possibly on the fundamental concept that enhancing antioxidant capacity to counteract ROS benefits T2D patients.

In the general population, many people take antioxidant supplements, such as vitamin C and E, and beta-carotene, believing these agents will be beneficial to their general health and prevent diseases. In fact, there are a number of clinical studies that have failed to find any benefit of such antioxidant vitamin supplement (Bjelakovic *et al.*, 2007). Our hypotheses raise a serious concern about antioxidant supplements, at least in the context of T2D. Given that ROS signaling is attenuated by antioxidants, we cannot rule out the possibility that the increasing incidence of T2D over the decades could be due, at least in part, to our self-prescribed preventive measures. For T2D patients, taking antioxidant supplements may even exacerbate their diseased conditions because of the further dampening of ROS signaling by exogenous antioxidants. For cancer prevention, phase II enzyme inducers, which activate Nrf2, are being considered as new candidates for chemoprevention. Though likely to be effective in controlling cancers, such treatments may predispose patients to T2D due to antioxidant-hampered beta-cell function.

Although antioxidants may function as negative regulators for ROS signaling, ROS-mediated signal transduction is relatively resistant to ROS scavenging. This assumption is supported by the evidence that ROS-mediated signal transduction occurs in normal cells which usually contain considerable amount of antioxidants. Thus, unless we could identify and eliminate specific oxidative stressors from our environmental and dietary exposures, antioxidants are still needed to cope with oxidative stress and its associated diseases. Given the complex role of antioxidants in T2D, the question should focus on how exogenous antioxidants can alleviate the stressed condition without interfering with physiological ROS signaling. This principle implies a new type of antioxidant supplementation and treatment paradigm. If the source of oxidative stress from environmental or dietary exposure can not be avoided, successful antioxidant supplements should be those that do not easily penetrate the cell membrane. In this way, the exogenous oxidative agents will be neutralized extracellularly before reaching the inside of cells. More importantly, since these new antioxidant supplements can not penetrate the cell membrane, they would not interfere with the intracellular ROS signal in beta-cells. Membrane-penetrating antioxidant supplements would be still useful in situations where the oxidative stress is internally originated, for instance, as a result of increased cellular metabolism or dysfunction of an intracellular antioxidant enzyme. To avoid interference with intracellular ROS signaling for insulin secretion, which is mostly required postprandially, the antioxidant supplements may be taken sometime after the meal. In addition, antioxidants with relatively short life and can be cleared at the time of the next meal would be more efficient. With this multi-stage uptake paradigm, the exogenous antioxidant will be bioavailable only between meals. In this way, oxidative stress would be combated for most of the day, keeping the adaptive induction of endogenous antioxidant at a low basal level. Thus, the physiological postprandial ROS signals would be spared from attenuation by endogenous and exogenous antioxidants.

7. Conclusions and Perspectives

If valid, the proposed mechanism for impaired GSIS by oxidative stress will generate a major paradigm shift in our understanding of the roles of ROS and antioxidants in T2D and perhaps

other diseases. Support for the hypotheses would lead to radically different strategies for the treatment of T2D and could suggest possible strategies for early intervention for metabolic syndrome and T2D. The potential paradoxical roles of ROS in beta-cell function suggest that site- and function-specific antioxidants or ROS donors may need to be considered in future therapeutic approaches.

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Abbreviations

CAT	catalase
GCLC	γ -glutamate cysteine ligase catalytic subunit
GPx	glutathione peroxidase
GSIS	glucose-stimulated insulin secretion
$\cdot\text{O}_2^-$	superoxide
GSH	reduced glutathione
GS	glutathione synthetase
GR	glutathione reductase
GSSG	oxidized glutathione
HO-1	heme oxygenase 1
NQO1, NAD(P)H	quinone oxidoreductase 1
Nrf2	nuclear factor E2-related factor 2
Prx	peroxiredoxin
ROS	reactive oxygen species
RNS	reactive nitrogen species
SOD	superoxide dismutase
Trx	thioredoxin
Ucp2	uncoupling protein 2

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FIG. 1. Proposed mechanisms for oxidative stress-induced impairment of pancreatic beta-cells
 (1) Environmental and/or dietary factors-induced oxidative stress might result in oxidative damage and directly diminish beta-cell function; (2) Oxidative stress activates the Nrf2-mediated antioxidant response and thus protects cells from oxidative damage; (3) Enhanced antioxidant capacity by Nrf2 activation could negatively affect glucose-derived ROS signaling that contributes to GSIS.

Table 1

Acute effects of oxidants, antioxidants and other agents on insulin secretion in INS-1 (832/13) cells *

Agents	Function	Concentrations	Insulin secretion	
			3 mM glucose	20 mM glucose
ROS or oxidants:				
H ₂ O ₂		1–100 μM	↑	↑
Hypochlorous acid		10–100 μM	↑	↑
SIN-1	NO/ONOO [−] donor	500 μM	–	↑
S-nitrosoglutathione	NO donor	100–500 μM	–	↑
4-hydroxy-2-nonenal	lipid peroxide	10 μM	–	ND
Diethyl maleate (DEM)	GSH depletor	0.1–5 mM	↑	↑
Antioxidants:				
N-acetyl-cysteine	thiol	100–400 μM	↓	↓
Catalase (CAT)	metabolizes H ₂ O ₂	5K–10K U/ml	↓	↓
PEG-catalase	Cell permeable CAT	1K–5K U/ml	–	↓
PEG-SOD	Cell permeable SOD	10–30 U/ml	–	–
GSH-EE	Cell permeable GSH	1 mM	↓	↓
DL-dithiothreitol	bithiols	100–500 μM	↓	↓
α-Lipoic acid (reduced)	bithiols	1 mM	↓	↓
α-Lipoic acid (oxidized)		1 mM	–	–
Others:				
Alloxan		10–100 μM	↑	–
tert-Butylhydroquinone	Nrf2 activator	10 μM	–	–
Rotenone	Complex I inhibitor	0.1–1 μM	–	↓
Antimycin A	Complex III inhibitor	2 μM	–	↓
Sodium azide	heme-containing peroxidases	0.6–1 mM	↓	↓
Mercaptosuccinic acid	GPx inhibitor	100 μM	–	–
Carboxymethoxylamine		100 μM	–	↓
Doxorubicin	·O [−] ₂ producer	2 μM	–	↓
CCCP	Mitochondria uncoupler	200 μM	–	↓
α-CHCA ^a		250 μM	–	–
Oligomycin ^b		2 μg/ml	–	↓
Phenylarsine oxide ^c		1 μM	–	↓
Tolbutamide ^d		0.5 mM	↑	–
KCl	Open voltage-gated Ca ²⁺ channels	30 mM	↑	↑

* Insulin secretion was determined as described previously (Pi *et al.*, 2007). ↑, increased; ↓, decreased; ND, not detected.

SIN-1, 3-morpholiniosydnonimine hydrochloride; PEG-Catalase, catalase-polyethylene glycol; PEG-SOD, superoxide dismutase-polyethylene glycol; GSH-EE, glutathione ethyl ester; CCCP, Carbonyl cyanide m-chlorophenylhydrazine;

^a α -cyano-4-hydroxycinnamic acid (α -CHCA), inhibits glycolysis-derived pyruvate transport into mitochondria;

^b inhibits mitochondrial ATPase and phosphoryl group transfer;

^c complexes vicinal thiols and inhibits phosphotyrosine phosphatase;

^d closes K_{ATP} channels and elicits electrical activity.