Published in final edited form as:

Curr Diabetes Rev. 2012 July 1; 8(4): 303–315.

The Good and Bad Effects of Cysteine S-nitrosylation and Tyrosine Nitration upon Insulin Exocytosis: A Balancing Act

Dean A. Wiseman^{1,*} and Debbie C. Thurmond^{1,2,3,*}

¹Department of Pediatrics, Herman B Wells Center, Basic Diabetes Group, Indian University School of Medicine, Indianapolis, IN 46202

²Department of Biochemistry and Molecular Biology, Indian University School of Medicine, Indianapolis, IN 46202

³Department of Cellular and Integrative Physiology, Indian University School of Medicine, Indianapolis, IN 46202

Abstract

As understanding of the mechanisms driving and regulating insulin secretion from pancreatic beta cells grows, there is increasing and compelling evidence that nitric oxide (•NO) and other closely-related reactive nitrogen species (RNS) play important roles in this exocytic process. •NO and associated RNS, in particular peroxynitrite, possess the capability to effect signals across both intracellular and extracellular compartments in rapid fashion, affording extraordinary signaling potential. It is well established that nitric oxide signals through activation of guanylate cyclase-mediated production of cyclic GMP. The intricate intracellular redox environment, however, lends credence to the possibility that •NO and peroxynitrite could interact with a wider variety of biological targets, with two leading mechanisms involving 1) S-nitrosylation of cysteine, and 2) nitration of tyrosine residues comprised within a variety of proteins. Efforts aimed at delineating the specific roles of •NO and peroxynitrite in regulated insulin secretion indicate that a highly-complex and nuanced system exists, with evidence that •NO and peroxynitrite can contribute in both positive and negative regulatory ways in beta cells. Furthermore, the ultimate biochemical outcome within beta cells, whether to compensate and recover from a given stress, or not, is likely a summation of contributory signals and redox status. Such seeming regulatory dichotomy provides ample opportunity for these mechanisms to serve both physiological and pathophysiologic roles in onset and progression of diabetes. This review focuses attention upon recent accumulating evidence pointing to roles for nitric oxide induced post-translational modifications in the normal regulation as well as the dysfunction of beta cell insulin exocytosis.

Keywords

Exocytosis; Islet; Insulin; Nitration; Oxidative Stress; Post-Translational Modification; S-Nitrosylation; SNARE Protein

INTRODUCTION

Type 2 diabetes ensues when beta cell function can no longer compensate for peripheral insulin resistance. Beta cell dysfunction arises either by means of loss of appropriate insulin secretion and/or by loss of beta cell mass. Early signs of beta cell dysfunction include impairment in the pulsatile secretion of insulin, which can be observed prior to onset of insulin resistance [1, 2], and reduced insulin secretion in response to normal stimuli while retaining normal glucose tolerance [3]. And while this dysfunction/dysregulation of insulin secretion has been traditionally attributed to either beta cell “exhaustion” or secretagogue-mediated cell toxicity, the mechanisms for how dysfunction ensues remain poorly understood. It is known as a consequence of glucose stimulation, that levels of both reactive oxygen species (ROS) and reactive nitrogen species (RNS) are elevated. However, beta cells generally lack robust antioxidant capacity when compared to other cell types, implying that beta cells are particularly vulnerable to oxidative and/or nitrosative stress [4-7]. Interestingly, despite this susceptibility to such stress, recent evidence indicates that ROS and RNS participate in unique, discrete modulating/facilitative roles within the beta cell insulin secretion mechanism [8], and that perturbation of these roles contributes to overall beta cell dysfunction. Moreover, with a deeper understanding of the mechanistic details of insulin secretion mechanisms in pancreatic beta cells under normal physiologic circumstances and during the earliest onset of departure from normal physiology, there is a unique opportunity to improve care and aid successful intervention of diabetes and diabetes-associated morbidity. In this review we will describe the current understanding of known nitrosative protein post-translational modifications in pancreatic beta cells, specifically as they pertain to the mechanism of regulated glucose-stimulated insulin secretion (GSIS).

Glucose-Stimulated Insulin Secretion

Glucose is the only fuel capable of triggering insulin secretion. The ability of the pancreatic beta cell to perceive increased blood glucose concentrations, transduce signals to its intracellular insulin secretory machinery and ultimately deliver insulin to the blood stream requires an extensive and highly regulated set of mechanisms. Insulin release from isolated beta cells or intact Islets of Langerhans in response to glucose under normal physiological conditions is characterized as being biphasic, with distinct first and second phases. As summarized in Fig. (1), glucose is transported into the beta cell through the glucose transporter (GLUT1/2) proteins present at the cell surface, and upon internalization glucose is metabolized to yield a net increase in the ratio of intracellular ATP to ADP. This stoichiometric shift toward ATP induces closure of ATP-dependent potassium (K^+) channels and results in membrane depolarization. In turn, L-type voltage-dependent calcium (Ca^{2+}) channels open, allowing entry of Ca^{2+} into the cell to trigger fusion of insulin secretory granules proximally located at the inner leaflet of the plasma membrane (the “readily-releasable pool”) in what is termed first-phase insulin release. Insulin release from these ‘docked and primed’ granules is accomplished through a carefully orchestrated series of steps in a process referred to as insulin granule exocytosis. These steps are predicated upon the formation of specific protein binding complexes, wherein SNARE (soluble N-ethylmaleimide-sensitive attachment receptor) proteins populating both the granular and plasma membranes combine to form a “SNAREpin” to fuse the granule with the plasma membrane, allowing formation of a fusion pore through which the insulin cargo is released into the extracellular environment [9]. At the molecular level, the insulin granule/vesicle-associated membrane v-SNARE protein (VAMP2) binds to cognate plasma/target-membrane t-SNARE receptor binary complexes, comprised of one SNAP-25 (or SNAP-23) and one Syntaxin protein. Upon binding, v-SNARE and t-SNARE proteins form a highly stable heterotrimeric SNARE “core complex,” and vesicular fusion with the plasma membrane occurs.

The initial “first phase” of insulin release is rapid and transient (5-10 min) and known to require function of the particular t-SNARE isoforms Syntaxin 1A and Syntaxin 4 [10, 11]. Immediately following this first phase, if sufficient glucose stimulation persists, a second more sustained insulin release phase ensues. This second phase of secretion derives from translocation of more distally located insulin granules and can be sustained for several hours [12], and requires additional intracellular events, including cortical F-actin reorganization [13-16]. Under more pathological circumstances, if glucose stimulation is sustained for more than 24 hours, a glucose-refractory third phase has been characterized, where a spontaneous decline in insulin secretion occurs which cannot be attributed to finite levels of insulin within the cell [17]. However, the molecular mechanism underlying this refractory phase remains undefined.

As delineation of the regulated exocytosis mechanism of insulin in beta cells has advanced, the suite of regulator and effector proteins overlaps strongly with the exocytotic apparatus present in other cell types, notably neuronal and adrenal chromaffin cells. This specifically includes N-ethylmaleimide sensitive factor (NSF), the SNARE and SNARE-associated proteins, and others [18, 19]. This parallelism underscores a compelling argument for a unifying functional mechanism, though clearly significant regulatory delineations must occur to account for massive differences in rate kinetics and responsivity. However, for purposes of this review, we will note instances where proteins undergo modification in other cell types, but given that the same proteins are present and required for insulin secretion, will presume the possibility that are similarly modified in beta cells. We will also note at least one instance where modification occurs in one cell type, but does *not* occur in beta cells, and may represent a cell-type mechanistic departure.

Part 1. THE MOLECULES INVOLVED, AND THEIR POTENTIAL FOR MEANINGFUL MODIFICATIONS

Nitric Oxide (•NO)

Since its original identification 35 years ago as the endothelial-derived relaxation factor in vascular tissue [20-22], the extraordinary role of •NO in biological systems has been brought to light. Two key properties are its specific reactivity and short lifetime in biological systems (~1 sec), making it a nearly ideal molecule for rapid and finely-tuned signal transduction. •NO has been shown to play roles in cellular processes such as regulation/modulation of kinases, phosphatases, G-proteins, mitochondrial function, ion channels, transcription factors, and serves as a mediator of apoptosis. Likewise, dysregulation of •NO has been associated with pathological processes, including cancer carcinogenesis and tumor maintenance [23], as well as neurological [24], cardiovascular [25], and diabetes-related diseases [26-28].

The primary source of •NO in cells are the family of nitric oxide synthases (NOS), which synthesize •NO through NADPH-mediated conversion of L-arginine to citrulline [29]. In mammalian cells the NOS family is comprised of three members: Type 1 “neuronal” nNOS (NOS1), Type 2 “inducible” iNOS (NOS2), and Type 3 “endothelial” eNOS (NOS3). Within this family, NOS1 and NOS3 are occasionally classified together as “constitutive” cNOS, as their expression is relatively stable in the tissues where they are found, and they are primarily regulated by phosphorylation and the presence of appropriate co-factors, including calcium. In contrast, the NOS2 proteins are tightly and continuously bound to calmodulin, and essentially calcium-independent. Moreover, NOS2 protein levels can expand several thousand-fold in response to inflammatory and/or stress-related signaling, and are thus capable of synthesizing many orders of magnitude more •NO than NOS1 and NOS3 isoforms collectively. Consequently, NOS2 is frequently associated with pathological

cellular scenarios; notably however such scenarios and time points occur beyond the acute stimulation and response timeline involved in the insulin secretory process.

The various •NO-mediated effects are summarized in Fig. (2A), and can be categorized across a spectrum ranging from beneficial and/or physiological to detrimental and/or toxic. Once released within cells, •NO is capable of mediating intracellular signals by a plethora of mechanisms, both direct and indirect. The canonical mechanism of •NO-mediated signaling is activation of soluble guanylate cyclase (sGC), resulting in increased intracellular cGMP second-messenger levels with subsequent propagation of signal, and is an example of an indirect signaling mechanism. On the other hand, •NO is directly reactive with proteins containing heme groups (sGC is one example) and other transition metal centers. In particular, •NO has been shown to inhibit mitochondrial function through reaction with iron/sulfur clusters within the electron transport chain [30, 31]. A third •NO-mediated mechanism involves post-translational modification of highly-specific thiol groups on cysteine residues forming a nitrosothiol moiety, a process termed S-nitrosylation. It is this latter mechanism which has seen tremendous expansion in the known signaling potential of •NO. And although it is significantly smaller than other posttrans-lational modification moieties, •NO groups can be added or subtracted from specific cysteine residue sites in a rapid and reversible fashion, resulting in changes in function in dynamic a manner analogous to phosphorylation. An example of this in the beta cell GSIS process is in the S-nitrosylation of Syntaxin 4 (Fig. 2B), wherein •NO modified a cysteine residue in the center of the third alpha helix, positioned in the 'closed' conformation of Syntaxin 4 in a key location to evoke change to what is considered the 'open' and active conformation [32, 33].

There are two intriguing "advantages" of the cell's use of •NO to S-nitrosylate proteins versus phosphorylation. Firstly, the modification process does not appear to require specific catalytic transfer, as opposed to the need for a kinase protein to transfer a phosphate moiety to its substrate, though it should be noted that proximity to the specific site of •NO production can be important, and adapter proteins for NOS are involved for target recruitment. Secondly, a combination of dual-solubility and small size affording •NO the ability to easily penetrate interior and hydrophobic regions of proteins and diffuse through membranes [34]. At this time, more than 1,000 proteins have been identified to be post-translationally modified and regulated by S-nitrosylation in cells and tissues [35], although only two proteins have been specifically shown to be S-nitrosylated in beta cells to date (see below). A representative list of those factors implicated in signaling and exocytosis mechanisms and their respective SNO motifs is summarized in Table 1, including many which are known to be expressed in beta cells, but remain to be examined for modification by S-nitrosylation.

Despite what may be considered a relatively short one-second lifetime in cells and tissues, this highly lipophilic molecule is capable of exerting paracrine effects in an area ranging several cell diameters. The vast majority of •NO is eliminated from cells through rapid reaction with hemoglobin in the blood stream, which establishes a steep diffusion gradient [36, 37]. For these reasons, •NO has been theorized as a signal to integrate and coordinate activity within a local group of cells [38] or as a metabolic "shock absorber", simultaneously promoting and limiting processes within cells [34]. In terms of pathological function, though many adverse and/or cytotoxic effects have been attributed to elevated levels of •NO, it may actually be a consequence of metabolism of •NO into higher oxides, including ONOO⁻, which is formed from a supercatalytic reaction of •NO with superoxide (O₂•⁻).

In beta cells, NOS1 has been found to be localized on insulin secretory granules [39]. Following onset of stimulation by glucose, elevations in •NO production can be observed almost immediately, which is temporally consistent with the participation of NO in GSIS/

insulin exocytosis [40, 41]. Similarly, •NO-associated protein modifications can be detected rapidly following onset of stimulation [32, 40], which is also consistent with •NO synthesis from constitutively-expressed NOS1 and NOS3 isoforms [42] rather than the inducible isoform, NOS2. A summary of early-onset intracellular events following glucose stimulation are summarized in Table 2. What is evident from this timeline is that induction of •NO synthesis is an extremely early event in the onset of the insulin secretion process. While NO synthesis has been detected prior to detectable elevations of intracellular Ca^{2+} [41], it remains arguable that this occurred because the calcium levels involved in NOS activation were actually subthreshold for detection via Fura-2. Indeed, this interpretation is compatible with a body of work which supports the concept of a requirement for Ca^{2+} /calmodulin binding in the activation of NOS [43]. As such, one could speculate that producing •NO at such an early point would support the idea of a positive role, rather than a negative one, and that for negative regulation to occur, a certain threshold must be crossed.

Peroxynitrite (ONOO^-)

Though not nearly as extensively studied as its precursor counterpart •NO, overwhelming evidence holds that ONOO^- is a major contributor to the role of RNS in biological systems. Due to its high reactivity and very short physiological half-life (~10 ms), the associated biochemistry of ONOO^- is complex. It functions in cells in an equilibrium with peroxynitrous acid (ONOOH), and thus may participate in the oxidation of proteins, nucleic acids and lipids [34]. Consequently, a majority of effects attributed to ONOO^- to date are negative or pathological in nature, including inhibition of mitochondrial and other metabolic proteins, such as aconitase and alcohol dehydrogenase [44]. Moreover, ONOO^- has been shown to positively activate pro-apoptotic proteins, such as poly (ADP-ribose) polymerase (PARP) [45, 46]. And though it is primarily implicated in pathologic and cell death-associated processes, there is growing evidence indicating that it also contributes during normal cellular physiology and may even serve as a positive regulator in cell signaling, and function in normal cell processes such as the cell cycle [47]. For example, low levels of ONOO^- have been shown to enhance tyrosine phosphorylation signaling through inhibition of tyrosine phosphatases *in vitro* and in neurons [48, 49], and the Src tyrosine kinase and its associated signaling pathway undergo peroxynitrite-induced activation in neurons as well as erythrocytes [50, 51]. Interestingly, in beta cells Src is emerging as a potential signaling factor between glucose-mediated insulin secretion and the secretion-enhancing effects of GPCR- and GTPase-mediated signaling events [52-54], exemplifying the potential opportunities for investigation in beta cell-specific contexts.

ONOO^- is primarily formed as the product of a supercatalytic reaction of •NO with superoxide anion ($\text{O}_2^{\bullet-}$) [55, 56]. Given the extreme rapidity of this reaction as well as the high reactivity of superoxide in cells, ONOO^- formation is believed to correspond with specific sites of superoxide formation, such as at the plasma membrane (NADPH oxidase) or mitochondria (electron transport chain oxidases). And despite its short life span at physiological pH, by virtue of its ability to diffuse through membranes, its influence may be seen as far away as 1- 2 cell diameters [57], implying that, like •NO, both autocrine and paracrine/juxtacrine signaling potentials exist. Pertaining specifically to beta cells, given that stimulation with glucose induces mitochondrial ATP synthesis, that the production of $\text{O}_2^{\bullet-}$, presumably as a byproduct of mitochondrial electron transport occurs simultaneously with •NO production during glucose-stimulated insulin secretion.

The best-characterized effect of ONOO^- upon biomolecules is the post-translational protein nitration of specific tyrosine residues in proteins to form 3-nitrotyrosine. In most cells, the abundance of 3-nitrotyrosine is estimated to occur in 1 per 10^6 tyrosine residues, making it a relatively low-abundant event [58]. Modification of tyrosine to 3-nitrotyrosine causes the

pKa of the phenol side chain to shift from 10 to ~7.3, making the modification akin to phosphorylation in terms of its effect. Unlike either phosphorylation or S-nitrosylation, this particular modification is thought to be less dynamic and more durable, and is frequently associated with oxidative stress [59]. In chronic and systemic pathologies, such as that which occurs in diabetes, this durability is frequently capitalized upon as a biomarker for stress as well as systemic pathological conditions. Because of this feature, 3-nitrotyrosine has been an attractive biomarker for detecting oxidative and nitrosative stress in the islet beta cell [60, 61]. However, few if any time points proximal to onset of either normophysiological and/or pathologic signaling have been extensively studied in the beta cell.

PART 2. NITROSATIVE POST-TRANSLATIONAL MODIFICATIONS IDENTIFIED IN BETA CELLS

As the stimulus-secretion coupling pathway leading to insulin secretion involves glycolytic and other oxidative events that stimulate an increase in the ratio of ATP relative to ADP within the cell, this increased metabolic function, in turn, leads to increased levels of reactive species, both oxygen- and nitrogen-centered [42, 62-64]. For nitrogen-centered reactive species, both •NO and ONOO⁻ have been shown to serve as signaling molecules, and an increasing body of literature demonstrates that these molecules serve as specific protein posttranslational modifiers. Early studies in neuronal secretion mechanisms hinted at the possibility [65, 66], yet only recently have nitrosative-based modifications of specific beta-cell insulin secretion-associated proteins been reported [8]. In terms of beta cell-specific physiology/pathophysiology, in fact, much of what is known regarding nitrosative post-translational protein modifications is by proxy, in that proteins known to be expressed in pancreatic beta cells and shown to play a role in insulin secretion have been identified as targets of modification in non-pancreatic model systems, as exemplified below.

S-Nitrosylation of Proteins in Beta Cells

Numerous reports insinuate the role of protein S-nitrosylation in regulation or modulation of insulin secretion, or in proteins known to be expressed in beta cells, and our own data indicate that numerous protein targets are S-nitrosylated, under both basal and glucose-stimulated conditions (Fig. 3). At present, just two proteins have been shown to be specifically S-nitrosylated in pancreatic beta cells or intact islets in response to secretagogues: Syntaxin 4 [32] and Glucokinase [40].

Syntaxin 4

We recently reported a pro-secretory effect corresponding with S-nitrosylation of Syntaxin 4, both in MIN6 beta cells as well as intact human islets [32]. We proposed that S-nitrosylation of Syntaxin 4 specifically at cysteine-141, which is centrally positioned within the Hc domain, promotes the dissociation of the N-terminal H_{abc} helical bundle from the H₃ SNARE domain, thus activating Syntaxin 4 to facilitate its participation in active SNARE core complexes with SNAP-25 and VAMP2 [67, 68]. Alternatively, S-nitrosylation may prevent premature deactivation of Syntaxin 4 [69, 70]. Interestingly, while Syntaxin 1 has also been shown to be a target of S-nitrosylation in neuronal cells and tissues [66, 71] it was not modified in response to glucose stimulation in beta cells. This finding may represent a harbinger of how a similar complement of exocytotic proteins can result in differing functional rate kinetics across different tissues. Importantly, S-nitrosylation of Syntaxin 4 occurred under diabetogenic pathophysiological conditions, simulated by acute cytokine stress, and in the absence of glucose stimulation [32]. As such, too much of the otherwise normal modification fosters unregulated and elevated basal insulin release, and elevated basal insulin release is a hallmark early event in the progression toward type 2 diabetes.

Glucokinase (GCK)

The work of Rizzo and others show that S-nitrosylation of GCK fosters its disassociation of from insulin secretory granules, thus promoting insulin secretion [40]. Furthermore, mutation of cysteine-371 to serine blocks S-nitrosylation of GCK and causes it to remain tightly bound to secretory granules. Moreover, subsequent reports indicated the existence of specific point mutations of GCK, rendering it S-nitrosylation-resistant in human patients presenting with maturity-onset diabetes of the young, termed MODY [72]. Interestingly, GCK also appears to be negatively regulated through a protein nitration mechanism, where chronic intake of 5% ethanol diet in mice, or in clonal beta cells exposed to exogenous ONOO⁻-donor compound SIN-1, caused reduced protein levels of GCK; GCK nitration—but specifically not its S-nitrosylation—triggered ubiquitin-mediated GCK degradation [73]. These findings indicate a potential scenario where low levels of RNS in response to physiological stimuli are beneficial and stimulatory, while pathological stimuli lead to elevated levels, which are ultimately detrimental.

Nitration of Proteins in Beta Cells

With regard to beta cell-specific tyrosine nitration events, in addition to the study by Kim *et al.* [73] described above where GCK undergoes chronic ethanol-induced nitration, another significant broad-based study using mass spectroscopy-based proteomic methods to identify nitration targets has been reported in pancreatic endocrine tissues [8], and these combined results are summarized in Table 3A. The focus of this latter study centered on the role of protein tyrosine nitration in the diminishment of mitochondrial function in beta cells and islets in response to sustained hyperglycemic conditions. Using a proteomic screening approach, 15 different tyrosine-nitrated proteins were identified in RIN-5F rat beta cells and intact isolated islets from male Sprague-Dawley rats, both exposed for extended periods (12-24 hr) to concentrations of glucose ranging from 6.5-11 mM, which was established from post-prandial blood glucose studies in human patients with impaired glucose tolerance (Table 3A). One protein of particular interest was mitochondrial heat-shock protein 60 (HSP60), which they showed that following *in vitro* nitration could suppress insulin secretion function in otherwise normal beta cells through a novel mechanism of association with proinsulin. Furthermore, these same authors published a different study showing a similar pattern of tyrosine nitration events using *in vitro*-differentiated NIH-3T3 adipocytes. Peripheral tissues, including adipocytes, utilize a suite of exocytotic proteins similar to beta cells in order to facilitate insulin-stimulated GLUT4 glucose transporter-bound vesicle exocytosis at the plasma membrane [74]. In the case of rat beta cells/islets and the mouse NIH-3T3 adipocyte models employed, there is a striking overlap of targets. Such parallel findings strongly implies a mechanistic pathological connection [75], and reaffirms speculation that two disparate events in Type 2 diabetes, namely peripheral tissue insulin resistance and pancreatic beta cell dysfunction, could be connected by similar biochemical mechanisms, including nitrosative modifications. In addition, a compelling set of molecular candidates for modification that exist in beta cells have been shown to be modified in a number of other cell types; the beta cell protein candidates await both confirmation and specific functional characterization (Table 3B). These beta cell candidate targets span a wide variety of functions, and demonstrate the significant potential for identification of new therapeutic targets and known nitration targets with previously unappreciated beta cell mechanistic relevance.

Part 3. THE TRANSITION FROM POST-TRANS-LATIONAL MODIFICATION TO PRO-APOPTOTIC SIGNAL: TOO MUCH OF A GOOD THING?

Since publication of the seminal work by Corbett and colleagues implicating a role for elevated •NO in the destruction of beta cells [28], the majority of attention in this field has

dealt with the role of NOS2 induction in this process. Works in particular from Lundquist and Salehi [76-81], Gross [39, 82, 83] and others [8, 84, 85] offer compelling evidence that •NO exerts a negative regulatory signal upon beta cell and islet insulin secretion, and lend strong credence that endogenous •NO production, and from NOS2 in particular, can negatively modulate insulin release from beta cells. Lundquist specifically argues that ROS lacking nitrogen, such as carbon monoxide, are in fact the positive signaling factors in insulin exocytosis, antagonizing the inhibitory functions of •NO. One explanation for this negative regulation is activation of AMP-activated protein kinase (AMPK), with the resultant signaling leading to downregulation of pro-secretion pathways and initiation of cytoprotective responses, including upregulation of the unfolded protein response [85]. Yet other investigators provide data suggesting that •NO can initiate cell death processes through multiple mechanisms, such as initiation of apoptosis [86, 87] and interference with zinc homeostasis [88-90]. Given that so many forms of cellular pathology arise when oxidative molecules surpass a given buffering antioxidant threshold, the hypothesis that RNS are uniformly negative signals, serving as protein inhibition and destabilization effectors is quite reasonable as it presents a significant weakness in the beta cell and an opportunity to go awry. As pro-inflammatory pressure increases through stimulation with cytokines or other forms of cell stress, beta cells increasingly suffer negative consequences beyond insulin secretion, leading ultimately to cell death [61, 73].

Nevertheless, findings continue to be communicated in which nitric oxide-mediated protein modification enhances or promotes activity in the direction of insulin secretion [32, 40, 66], or enhance beta cell survival and overall function [64, 91, 92]. Given that most of the work regarding RNS in beta cells utilized cell death or biomarkers consistent with full commitment to cell death pathways, a significant window of time *prior* to transcriptional responses (i.e. induction of NOS2 and inflammatory response factors) in beta cells remains an incompletely characterized area of investigation, and in many ways could be construed as mechanistically analogous to the immediate-early response in cells stimulated with growth factors. This omission is particularly emphasized by the fact that a key clinical observation at early stages of diabetic-associated pathology, chronic fasting hyperinsulinemia, cannot solely be attributed to peripheral tissue resistance. Work by Corkey and others have attempted to emphasize and reconcile this seemingly contradictory aspect of pre-diabetic physiology [93-95]. And so, while it could be proposed that either too much •NO or *any* ONOO⁻ above basal levels drives adverse consequences in pancreatic beta cells, it could also be argued that the physiologic window in beta cells is narrow and that the line between beneficial and detrimental outcomes is very fine [28].

Irrespective of which is the more accurate model, there is a definite need to refine the specific range of both ROS and RNS levels acceptable/advantageous from those considered toxic/pro-apoptotic in beta cells as well as intact islets. It has been noted that increases in either •NO or O₂•⁻ can result in equivalent fold-increases, or, in the case of elevation of both, exponential increases in ONOO⁻ (i.e. 10-fold increases in •NO and O₂•⁻ would result in a 100-fold increase in ONOO⁻) [34]. Indeed, the majority of nitric oxide-mediated adverse consequences reported in the literature are associated with induction of NOS2 activity, the protein expression of which can rise many-fold in response to stress and/or inflammatory stimuli. More to the point, it has been specifically demonstrated by multiple methods that mitigation or abrogation of NOS2 upregulation confers protection to beta cells [96], while the specific contribution of NOS1 and NOS3 isoforms remains largely unexplored.

In terms of future studies, in order to establish mechanistic consensus, we will need to take into account the physiological premise that redox processes function properly only in narrow range, and that oxidative processes may be as important as reductive (anti-oxidant)

processes. One clear example is the synthesis of insulin, which requires an appropriate oxidative ribosomal environment for the correct formation of disulfide bonds [97], and that reductive dissolution of proper bonding causes retention of pro-insulin complexes and may induce intracellular stress. Furthermore, this point is reinforced in the current literature, where only incremental benefit has been observed in patients in response to antioxidant therapies for various conditions [98].

Pharmacologic Pitfalls

One key technical hurdle is a current lack of consensus of what constitutes physiologically, pharmacologically, or clinically relevant concentrations of and durations of exposure to, the molecules in question. Studies in various tissue types typically report •NO production levels typically in a nanomolar range or perhaps as high as low micromolar range during acute pathophysiology [28, 99], and within the context of a steep diffusion gradient in the direction of the blood stream, resulting in an average lifetime of ~1 second. Given that levels which are several orders of magnitude higher than these are routinely used, it must be noted that use of bolus doses or even moderate doses for extended periods is fraught with risk of artefact, and care must be taken when considering experimental design. A number of different post-translational modifications utilize cysteine protein residues as the modification site, including prenylation and disulfide bond formation, such that inappropriate oxidation by nitric oxide or its derivatives can potentially hinder these endogenous processes. This could also be relevant with regard to appropriate intracellular sequestration and regulation of divalent transition metals, notably zinc, copper, and iron, where loss can drive cells into cell death pathways. Yet another example of a potential pitfall comes with use of ONOO⁻ to directly nitrate proteins. Given that ONOO⁻ is typically stabilized in basic buffer (i.e. 0.1M NaOH) one may adversely affect proteins through introduction of an inappropriate pH environment to a protein or molecule of interest. An additional consideration is potential obfuscating pharmacological effects from the resulting products of an •NO-donor compound upon release of •NO. One example is spermine NONOate, which upon release of •NO, the other product of this release is the polyamine compound spermine, which has documented effect upon cellular physiology [100]. Lastly, different modification effects have been observed using different methods, which raises the potential that *in vitro* methods may not reflect physiologically relevant conditions [101, 102].

Perspectives and Future Directions

Though discrepancy and controversy remain in this area, it is clear that with carefully-planned studies that much remains to be elucidated. There is significant evidence demonstrating that this area of beta cell research will uncover novel and events unique to beta cell function. As ever more specific and sensitive techniques are developed, they will yield exciting and clinically useful information in the battle to prevent and alleviate the worldwide epidemic that is Type 2 Diabetes. We propose that in order to overcome the apparent discrepancy with regard to the exact role of RNS in beta cells, it will require further appreciation of the idea that “too much of a good thing” is fundamentally the road to destruction. More to the point, specific attention must be given to the idea that the very molecules which may be driving the pathology are, in fact, required for appropriate signaling, and cannot be simply ablated, or conversely inundated with the molecules in question at concentrations and exposures far beyond even pathological levels, to arrive at a physiological understanding of their nature. In conclusion, we believe it is entirely possible that the manner in which studies conducted with regard to insulin secretion and studied undertaken to examine beta cell death has left a highly significant window of opportunity for experiments which have simultaneous relevance to both processes.

Acknowledgments

Supported by grants from the NIH (DK076614 and DK067912 to D.C.T., HL090198 and DK087811 to D.A.W.).

ABBREVIATIONS

•NO	nitric oxide
ONOO ⁻	peroxynitrite
RNS	reactive nitrogen species
ROS	reactive oxygen species
O ₂ • ⁻	superoxide
SNARE	Soluble NSF Attachment protein Receptor
NSF	N-ethylmaleimide sensitive fusion protein
NOS	nitric oxide synthase
GCK	glucokinase
SIN-1	3-morpholiniosydnonimine
TNM	tetranitromethane
GSNO	S-nitrosoglutathione
nNOS	“neuronal” nitric oxide synthase (NOS1)
iNOS	“inducible” nitric oxide synthase (NOS2)
eNOS	“endothelial” nitric oxide synthase (NOS3)

REFERENCES

- [1]. Lang DA, Matthews DR, Burnett M, Turner RC. Brief, irregular oscillations of basal plasma insulin and glucose concentrations in diabetic man. *Diabetes*. 1981; 30:435–9. [PubMed: 7014311]
- [2]. O’Rahilly S, Turner RC, Matthews DR. Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. *N Engl J Med*. 1988; 318:1225–30. [PubMed: 3283553]
- [3]. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest*. 1999; 104:787–94. [PubMed: 10491414]
- [4]. Asayama K, Kooy NW, Burr IM. Effect of vitamin E deficiency and selenium deficiency on insulin secretory reserve and free radical scavenging systems in islets: decrease of islet manganosuperoxide dismutase. *J Lab Clin Med*. 1986; 107:459–64. [PubMed: 3517203]
- [5]. Lenzen S. Oxidative stress: the vulnerable beta-cell. *Biochem Soc Trans*. 2008; 36:343–7. [PubMed: 18481954]
- [6]. Lenzen S, Drinkgern J, Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic Biol Med*. 1996; 20:463–6. [PubMed: 8720919]
- [7]. Malaisse WJ, Malaisse-Lagae F, Sener A, Pipeleers DG. Determinants of the selective toxicity of alloxan to the pancreatic B cell. *Proc Natl Acad Sci U S A*. 1982; 79:927–30. [PubMed: 7038690]
- [8]. Koeck T, Corbett JA, Crabb JW, et al. Glucose-modulated tyrosine nitration in beta cells: targets and consequences. *Arch Biochem Biophys*. 2009; 484:221–31. [PubMed: 19402213]

- [9]. Weber T, Zemelman BV, McNew JA, et al. SNAREpins: minimal machinery for membrane fusion. *Cell*. 1998; 92:759–772. [PubMed: 9529252]
- [10]. Spurlin BA, Thurmond DC. Syntaxin 4 facilitates biphasic glucosestimulated insulin secretion from pancreatic beta-cells. *Mol Endocrinol*. 2006; 20:183–93. [PubMed: 16099818]
- [11]. Ohara-Imaizumi M, Fujiwara T, Nakamichi Y, et al. Imaging analysis reveals mechanistic differences between first- and second-phase insulin exocytosis. *J Cell Biol*. 2007; 177:695–705. [PubMed: 17502420]
- [12]. Wang Z, Thurmond DC. Mechanisms of biphasic insulin-granule exocytosis - roles of the cytoskeleton, small GTPases and SNARE proteins. *J Cell Sci*. 2009; 122:893–903. [PubMed: 19295123]
- [13]. Jewell JL, Luo W, Oh E, et al. Filamentous actin regulates insulin exocytosis through direct interaction with Syntaxin 4. *J Biol Chem*. 2008; 283:10716–26. [PubMed: 18285343]
- [14]. Kalwat MA, Wiseman DA, Luo W, et al. Gelsolin associates with the N terminus of syntaxin 4 to regulate insulin granule exocytosis. *Mol Endocrinol*. 2011; 26:128–41. [PubMed: 22108804]
- [15]. Thurmond DC, Gonelle-Gispert C, Furukawa M, et al. Glucosestimulated insulin secretion is coupled to the interaction of actin with the t-SNARE (target membrane soluble N-ethylmaleimidesensitive factor attachment protein receptor protein) complex. *Mol Endocrinol*. 2003; 17:732–42. [PubMed: 12554769]
- [16]. Tomas A, Yermen B, Min L, et al. Regulation of pancreatic beta-cell insulin secretion by actin cytoskeleton remodelling: role of gelsolin and cooperation with the MAPK signalling pathway. *J Cell Sci*. 2006; 119:2156–67. [PubMed: 16638805]
- [17]. Bolaffi JL, Heldt A, Lewis LD, Grodsky GM. The third phase of *in vitro* insulin secretion. Evidence for glucose insensitivity. *Diabetes*. 1986; 35:370–3. [PubMed: 3512347]
- [18]. Buckley K, Kelly RB. Identification of a transmembrane glycoprotein specific for secretory vesicles of neural and endocrine cells. *J Cell Biol*. 1985; 100:1284–94. [PubMed: 2579958]
- [19]. Thomas-Reetz AC, De Camilli P. A role for synaptic vesicles in non-neuronal cells: clues from pancreatic beta cells and from chromaffin cells. *FASEB J*. 1994; 8:209–16. [PubMed: 7907072]
- [20]. Arnold WP, Mittal CK, Katsuki S, et al. Nitric oxide activates guanylate cyclase and increases guanosine 3':5'-cyclic monophosphate levels in various tissue preparations. *Proc Natl Acad Sci U S A*. 1977; 74:3203–7. [PubMed: 20623]
- [21]. Gruetter CA, Barry BK, McNamara DB, et al. Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosoamine. *J Cyclic Nucleotide Res*. 1979; 5:211–24. [PubMed: 39089]
- [22]. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 1987; 327:524–6. [PubMed: 3495737]
- [23]. Scher RL. Role of nitric oxide in the development of distant metastasis from squamous cell carcinoma. *Laryngoscope*. 2007; 117:199–209. [PubMed: 17277613]
- [24]. Beckman JS, Chen J, Crow JP, et al. Reactions of nitric oxide, superoxide and peroxynitrite with superoxide dismutase in neurodegeneration. *Prog Brain Res*. 1994; 103:371–80. [PubMed: 7886219]
- [25]. Petrie JR, Ueda S, Webb DJ, et al. Endothelial nitric oxide production and insulin sensitivity. A physiological link with implications for pathogenesis of cardiovascular disease. *Circulation*. 1996; 93:1331–3. [PubMed: 8641020]
- [26]. Corbett JA, Lancaster JR Jr. Sweetland MA, et al. Interleukin-1 beta-induced formation of EPR-detectable iron-nitrosyl complexes in islets of Langerhans. Role of nitric oxide in interleukin-1 beta-induced inhibition of insulin secretion. *J Biol Chem*. 1991; 266:21351–4. [PubMed: 1657959]
- [27]. Lukic ML, Stosic-Grujicic S, Ostojic N, et al. Inhibition of nitric oxide generation affects the induction of diabetes by streptozocin in mice. *Biochem Biophys Res Commun*. 1991; 178:913–20. [PubMed: 1831356]
- [28]. Corbett JA, Sweetland MA, Wang JL, et al. Nitric oxide mediates cytokine-induced inhibition of insulin secretion by human islets of Langerhans. *Proc Natl Acad Sci U S A*. 1993; 90:1731–5. [PubMed: 8383325]

- [29]. Knowles RG, Moncada S. Nitric oxide synthases in mammals. *Biochem J.* 1994; 298(Pt 2):249–58. [PubMed: 7510950]
- [30]. Beltran B, Mathur A, Duchen MR, et al. The effect of nitric oxide on cell respiration: A key to understanding its role in cell survival or death. *Proc Natl Acad Sci U S A.* 2000; 97:14602–7. [PubMed: 11121062]
- [31]. Moncada S, Erusalimsky JD. Does nitric oxide modulate mitochondrial energy generation and apoptosis? *Nat Rev Mol Cell Biol.* 2002; 3:214–20. [PubMed: 11994742]
- [32]. Wiseman DA, Kalwat MA, Thurmond DC. Stimulus-induced S-nitrosylation of Syntaxin 4 impacts insulin granule exocytosis. *J Biol Chem.* 2011; 286:16344–54. [PubMed: 21393240]
- [33]. Dulubova I, Sugita S, Hill S, et al. A conformational switch in syntaxin during exocytosis: role of munc18. *EMBO J.* 1999; 18:4372–82. [PubMed: 10449403]
- [34]. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev.* 2007; 87:315–424. [PubMed: 17237348]
- [35]. Seth D, Stamler JS. The SNO-proteome: causation and classifications. *Curr Opin Chem Biol.* 2010; 15:129–36. [PubMed: 21087893]
- [36]. Butler AR, Megson IL, Wright PG. Diffusion of nitric oxide and scavenging by blood in the vasculature. *Biochim Biophys Acta.* 1998; 1425:168–76. [PubMed: 9813307]
- [37]. Tsoukias NM, Popel AS. Erythrocyte consumption of nitric oxide in presence and absence of plasma-based hemoglobin. *Am J Physiol Heart Circ Physiol.* 2002; 282:H2265–77. [PubMed: 12003837]
- [38]. Lancaster JR Jr. Simulation of the diffusion and reaction of endogenously produced nitric oxide. *Proc Natl Acad Sci U S A.* 1994; 91:8137–41. [PubMed: 8058769]
- [39]. Lajoix AD, Reggio H, Chardes T, et al. A neuronal isoform of nitric oxide synthase expressed in pancreatic beta-cells controls insulin secretion. *Diabetes.* 2001; 50:1311–23. [PubMed: 11375331]
- [40]. Rizzo MA, Piston DW. Regulation of beta cell glucokinase by S-nitrosylation and association with nitric oxide synthase. *J Cell Biol.* 2003; 161:243–8. [PubMed: 12707306]
- [41]. Nunemaker CS, Buerk DG, Zhang M, et al. Glucose-induced release of nitric oxide from mouse pancreatic islets as detected with nitric oxide-selective glass microelectrodes. *Am J Physiol Endocrinol Metab.* 2007; 292:E907–12. [PubMed: 17122087]
- [42]. Henningsson R, Salehi A, Lundquist I. Role of nitric oxide synthase isoforms in glucose-stimulated insulin release. *Am J Physiol Cell Physiol.* 2002; 283:C296–304. [PubMed: 12055099]
- [43]. Spratt DE, Taiakina V, Palmer M, et al. Differential binding of calmodulin domains to constitutive and inducible nitric oxide synthase enzymes. *Biochemistry.* 2007; 46:8288–300. [PubMed: 17580957]
- [44]. Soum E, Drapier JC. Nitric oxide and peroxynitrite promote complete disruption of the [4Fe-4S] cluster of recombinant human iron regulatory protein 1. *J Biol Inorg Chem.* 2003; 8:226–32. [PubMed: 12459918]
- [45]. Radons J, Heller B, Burkle A, et al. Nitric oxide toxicity in islet cells involves poly(ADP-ribose) polymerase activation and concomitant NAD⁺ depletion. *Biochem Biophys Res Commun.* 1994; 199:1270–7. [PubMed: 8147870]
- [46]. Pacher P, Szabo C. Role of the peroxynitrite-poly(ADP-ribose) polymerase pathway in human disease. *Am J Pathol.* 2008; 173:2–13. [PubMed: 18535182]
- [47]. Jia M, Mateoiu C, Souchelnytskyi S. Protein tyrosine nitration in the cell cycle. *Biochem Biophys Res Commun.* 2011; 413:270–6. [PubMed: 21893039]
- [48]. Lopez CJ, Qayyum I, Mishra OP, et al. Effect of nitration on protein tyrosine phosphatase and protein phosphatase activity in neuronal cell membranes of newborn piglets. *Neurosci Lett.* 2005; 386:78–81. [PubMed: 16039061]
- [49]. Takakura K, Beckman JS, MacMillan-Crow LA, et al. Rapid and irreversible inactivation of protein tyrosine phosphatases PTP1B, CD45, and LAR by peroxynitrite. *Arch Biochem Biophys.* 1999; 369:197–207. [PubMed: 10486138]

- [50]. Mallozzi C, Ceccarini M, Camerini S, et al. Peroxynitrite induces tyrosine residue modifications in synaptophysin C-terminal domain, affecting its interaction with src. *J Neurochem*. 2009; 111:859–69. [PubMed: 19737347]
- [51]. Mallozzi C, Di Stasi AM, Minetti M. Activation of src tyrosine kinases by peroxynitrite. *FEBS Lett*. 1999; 456:201–6. [PubMed: 10452558]
- [52]. Buteau J, Foisy S, Joly E, et al. Glucagon-like peptide 1 induces pancreatic beta-cell proliferation *via* transactivation of the epidermal growth factor receptor. *Diabetes*. 2003; 52:124–32. [PubMed: 12502502]
- [53]. Dalle S, Ravier MA, Bertrand G. Emerging roles for beta-arrestin-1 in the control of the pancreatic beta-cell function and mass: new therapeutic strategies and consequences for drug screening. *Cell Signal*. 2011; 23:522–8. [PubMed: 20849951]
- [54]. Kepner EM, Yoder SM, Oh E, et al. Cool-1/betaPIX functions as a guanine nucleotide exchange factor in the cycling of Cdc42 to regulate insulin secretion. *Am J Physiol Endocrinol Metab*. 2011; 301:E1072–80. [PubMed: 21828338]
- [55]. Squadrito GL, Pryor WA. Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radic Biol Med*. 1998; 25:392–403. [PubMed: 9741578]
- [56]. Huie RE, Padmaja S. The reaction of no with superoxide. *Free Radic Res Commun*. 1993; 18:195–9. [PubMed: 8396550]
- [57]. Denicola A, Souza JM, Radi R. Diffusion of peroxynitrite across erythrocyte membranes. *Proc Natl Acad Sci U S A*. 1998; 95:3566–71. [PubMed: 9520406]
- [58]. Haddad IY, Pataki G, Hu P, et al. Quantitation of nitrotyrosine levels in lung sections of patients and animals with acute lung injury. *J Clin Invest*. 1994; 94:2407–13. [PubMed: 7989597]
- [59]. Ischiropoulos H. Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species. *Arch Biochem Biophys*. 1998; 356:1–11. [PubMed: 9681984]
- [60]. Reddy S, Bradley J. Immunohistochemical demonstration of nitrotyrosine, a biomarker of oxidative stress, in islet cells of the NOD mouse. *Ann N Y Acad Sci*. 2004; 1037:199–202. [PubMed: 15699517]
- [61]. Suarez-Pinzon WL, Szabo C, Rabinovitch A. Development of autoimmune diabetes in NOD mice is associated with the formation of peroxynitrite in pancreatic islet beta-cells. *Diabetes*. 1997; 46:907–11. [PubMed: 9133563]
- [62]. Lajoix AD, Pugniere M, Roquet F, et al. Changes in the dimeric state of neuronal nitric oxide synthase affect the kinetics of secretagogue-induced insulin response. *Diabetes*. 2004; 53:1467–74. [PubMed: 15161750]
- [63]. Leloup C, Tourrel-Cuzin C, Magnan C, et al. Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. *Diabetes*. 2009; 58:673–81. [PubMed: 19073765]
- [64]. Schmidt HH, Warner TD, Ishii K, et al. Insulin secretion from pancreatic B cells caused by L-arginine-derived nitrogen oxides. *Science*. 1992; 255:721–3. [PubMed: 1371193]
- [65]. Matsushita K, Morrell CN, Cambien B, et al. Nitric oxide regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. *Cell*. 2003; 115:139–50. [PubMed: 14567912]
- [66]. Palmer ZJ, Duncan RR, Johnson JR, et al. S-nitrosylation of syntaxin 1 at Cys(145) is a regulatory switch controlling Munc18-1 binding. *Biochem J*. 2008; 413:479–91. [PubMed: 18452404]
- [67]. Foster LJ, Yeung B, Mohtashami M, et al. Binary interactions of the SNARE proteins syntaxin-4, SNAP23, and VAMP-2 and their regulation by phosphorylation. *Biochemistry*. 1998; 37:11089–96. [PubMed: 9693005]
- [68]. Sollner T, Whiteheart SW, Brunner M, et al. SNAP receptors implicated in vesicle targeting and fusion. *Nature*. 1993; 362:318–24. [PubMed: 8455717]
- [69]. Schneider SW. Kiss and run mechanism in exocytosis. *J Membr Biol*. 2001; 181:67–76. [PubMed: 11420594]
- [70]. Tsuboi T, Rutter GA. Insulin secretion by 'kiss-and-run' exocytosis in clonal pancreatic islet beta-cells. *Biochem Soc Trans*. 2003; 31:833–6. [PubMed: 12887316]

- [71]. Pongrac JL, Slack PJ, Innis SM. Dietary polyunsaturated fat that is low in (n-3) and high in (n-6) fatty acids alters the SNARE protein complex and nitrosylation in rat hippocampus. *J Nutr.* 2007; 137:1852–6. [PubMed: 17634254]
- [72]. Ding SY, Tribble ND, Kraft CA, et al. Naturally occurring glucokinase mutations are associated with defects in posttranslational S-nitrosylation. *Mol Endocrinol.* 2010; 24:171–7. [PubMed: 19934346]
- [73]. Kim JY, Song EH, Lee HJ, et al. Chronic ethanol consumption induced pancreatic {beta}-cell dysfunction and apoptosis through glucokinase nitration and its down-regulation. *J Biol Chem.* 2010; 285:37251–62. [PubMed: 20855893]
- [74]. Foley K, Boguslavsky S, Klip A. Endocytosis, recycling, and regulated exocytosis of glucose transporter 4. *Biochemistry.* 2011; 50:3048–61. [PubMed: 21405107]
- [75]. Koeck T, Willard B, Crabb JW, et al. Glucose-mediated tyrosine nitration in adipocytes: targets and consequences. *Free Radic Biol Med.* 2009; 46:884–92. [PubMed: 19135148]
- [76]. Akesson B, Henningson R, Salehi A, et al. Islet constitutive nitric oxide synthase and glucose regulation of insulin release in mice. *J Endocrinol.* 1999; 163:39–48. [PubMed: 10495405]
- [77]. Akesson B, Lundquist I. Evidence for nitric oxide mediated effects on islet hormone secretory phospholipase C signal transduction mechanisms. *Biosci Rep.* 1998; 18:199–213. [PubMed: 9877233]
- [78]. Akesson B, Mosen H, Panagiotidis G, et al. Interaction of the islet nitric oxide system with L-arginine-induced secretion of insulin and glucagon in mice. *Br J Pharmacol.* 1996; 119:758–64. [PubMed: 8904652]
- [79]. Mosen H, Ostenson CG, Lundquist I, et al. Impaired glucose-stimulated insulin secretion in the GK rat is associated with abnormalities in islet nitric oxide production. *Regul Pept.* 2008; 151:139–46. [PubMed: 18662725]
- [80]. Panagiotidis G, Akesson B, Rydell EL, et al. Influence of nitric oxide synthase inhibition, nitric oxide and hydroperoxide on insulin release induced by various secretagogues. *Br J Pharmacol.* 1995; 114:289–96. [PubMed: 7533613]
- [81]. Panagiotidis G, Alm P, Lundquist I. Inhibition of islet nitric oxide synthase increases arginine-induced insulin release. *Eur J Pharmacol.* 1992; 229:277–8. [PubMed: 1283375]
- [82]. Beffy P, Lajoix AD, Masiello P, et al. A constitutive nitric oxide synthase modulates insulin secretion in the INS-1 cell line. *Mol Cell Endocrinol.* 2001; 183:41–8. [PubMed: 11604223]
- [83]. Gross R, Roye M, Manteghetti M, et al. Mechanisms involved in the effect of nitric oxide synthase inhibition on L-arginine-induced insulin secretion. *Br J Pharmacol.* 1997; 120:495–501. [PubMed: 9031755]
- [84]. Hughes KJ, Meares GP, Hansen PA, et al. FoxO1 and SIRT1 regulate beta-cell responses to nitric oxide. *J Biol Chem.* 2011; 286:8338–48. [PubMed: 21196578]
- [85]. Meares GP, Hughes KJ, Naatz A, et al. IRE1-dependent activation of AMPK in response to nitric oxide. *Mol Cell Biol.* 2011; 31:4286–97. [PubMed: 21896783]
- [86]. Ansari MM, Ansari M. Nitric oxide involvement in pancreatic beta cell apoptosis by glibenclamide. *Nitric Oxide.* 2006; 14:39–44. [PubMed: 16256381]
- [87]. Nakata M, Uto N, Maruyama I, et al. Nitric oxide induces apoptosis via Ca²⁺-dependent processes in the pancreatic beta-cell line MIN6. *Cell Struct Funct.* 1999; 24:451–5. [PubMed: 10698259]
- [88]. Tartler U, Kroncke KD, Meyer KL, et al. Nitric oxide interferes with islet cell zinc homeostasis. *Nitric Oxide.* 2000; 4:609–14. [PubMed: 11139369]
- [89]. Wiseman DA, Sharma S, Black SM. Elevated zinc induces endothelial apoptosis via disruption of glutathione metabolism: role of the ADP translocator. *Biometals.* 2009; 23:19–30. [PubMed: 19768661]
- [90]. Wiseman DA, Wells SM, Wilham J, et al. Endothelial response to stress from exogenous Zn²⁺ resembles that of NO-mediated nitrosative stress, and is protected by MT-1 overexpression. *Am J Physiol Cell Physiol.* 2006; 291:C555–68. [PubMed: 16723513]
- [91]. Bachar E, Ariav Y, Cerasi E, et al. Neuronal nitric oxide synthase protects the pancreatic beta cell from glucolipotoxicity-induced endoplasmic reticulum stress and apoptosis. *Diabetologia.* 2010; 53:2177–87. [PubMed: 20596694]

- [92]. Smukler SR, Tang L, Wheeler MB, et al. Exogenous nitric oxide and endogenous glucose-stimulated beta-cell nitric oxide augment insulin release. *Diabetes*. 2002; 51:3450–60. [PubMed: 12453899]
- [93]. Corkey BE. Banting lecture 2011: hyperinsulinemia: cause or consequence? *Diabetes*. 2011; 61:4–13. [PubMed: 22187369]
- [94]. Pi J, Zhang Q, Fu J, et al. ROS signaling, oxidative stress and Nrf2 in pancreatic beta-cell function. *Toxicol Appl Pharmacol*. 2010; 244:77–83. [PubMed: 19501608]
- [95]. Nichols CG, Koster JC, Remedi MS. beta-cell hyperexcitability: from hyperinsulinism to diabetes. *Diabetes Obes Metab*. 2007; 9(Suppl 2):81–8. [PubMed: 17919182]
- [96]. Bai-Feng L, Yong-Feng L, Ying C. Silencing inducible nitric oxide synthase protects rat pancreatic islet. *Diabetes Res Clin Pract*. 2010; 89:268–75. [PubMed: 20541824]
- [97]. Gorr SU, Huang XF, Cowley DJ, et al. Disruption of disulfide bonds exhibits differential effects on trafficking of regulated secretory proteins. *Am J Physiol*. 1999; 277:C121–31. [PubMed: 10409115]
- [98]. Iannitti T, Palmieri B. Antioxidant therapy effectiveness: an up to date. *Eur Rev Med Pharmacol Sci*. 2009; 13:245–78. [PubMed: 19694341]
- [99]. Tominaga T, Sato S, Ohnishi T, et al. Electron paramagnetic resonance (EPR) detection of nitric oxide produced during forebrain ischemia of the rat. *J Cereb Blood Flow Metab*. 1994; 14:715–22. [PubMed: 8063867]
- [100]. Kroncke KD, Brenner HH, Rodriguez ML, et al. Pancreatic islet cells are highly susceptible towards the cytotoxic effects of chemically generated nitric oxide. *Biochim Biophys Acta*. 1993; 1182:221–9. [PubMed: 8395219]
- [101]. Zhang Y, Lu N, Gao Z. Hemin-H₂O₂-NO₂(-) induced protein oxidation and tyrosine nitration are different from those of SIN-1: a study on glutamate dehydrogenase nitrative/oxidative modification. *Int J Biochem Cell Biol*. 2009; 41:907–15. [PubMed: 18835362]
- [102]. Kanski J, Alterman MA, Schoneich C. Proteomic identification of age-dependent protein nitration in rat skeletal muscle. *Free Radic Biol Med*. 2003; 35:1229–39. [PubMed: 14607522]
- [103]. Aronstam RS, Martin DC, Dennison RL, et al. S-nitrosylation of m2 muscarinic receptor thiols disrupts receptor-G-protein coupling. *Ann N Y Acad Sci*. 1995; 757:215–7. [PubMed: 7611675]
- [104]. Azad N, Vallyathan V, Wang L, et al. S-nitrosylation of Bcl-2 inhibits its ubiquitin-proteasomal degradation. A novel antiapoptotic mechanism that suppresses apoptosis. *J Biol Chem*. 2006; 281:34124–34. [PubMed: 16980304]
- [105]. Carvalho-Filho MA, Ropelle ER, Pauli RJ, et al. Aspirin attenuates insulin resistance in muscle of diet-induced obese rats by inhibiting inducible nitric oxide synthase production and S-nitrosylation of IRbeta/IRS-1 and Akt. *Diabetologia*. 2009; 52:2425–34. [PubMed: 19730809]
- [106]. Chanvorachote P, Nimmannit U, Stehlik C, et al. Nitric oxide regulates cell sensitivity to cisplatin-induced apoptosis through S-nitrosylation and inhibition of Bcl-2 ubiquitination. *Cancer Res*. 2006; 66:6353–60. [PubMed: 16778213]
- [107]. Choi YB, Tenneti L, Le DA, et al. Molecular basis of NMDA receptor-coupled ion channel modulation by S-nitrosylation. *Nat Neurosci*. 2000; 3:15–21. [PubMed: 10607390]
- [108]. Chung KK, Thomas B, Li X, et al. S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. *Science*. 2004; 304:1328–31. [PubMed: 15105460]
- [109]. Ckless K, Lampert A, Reiss J, et al. Inhibition of arginase activity enhances inflammation in mice with allergic airway disease, in association with increases in protein S-nitrosylation and tyrosine nitration. *J Immunol*. 2008; 181:4255–64. [PubMed: 18768883]
- [110]. Erwin PA, Lin AJ, Golan DE, Michel T. Receptor-regulated dynamic S-nitrosylation of endothelial nitric-oxide synthase in vascular endothelial cells. *J Biol Chem*. 2005; 280:19888–94. [PubMed: 15774480]
- [111]. Greco TM, Hodara R, Parastatidis I, et al. Identification of S-nitrosylation motifs by site-specific mapping of the S-nitrosocysteine proteome in human vascular smooth muscle cells. *Proc Natl Acad Sci U S A*. 2006; 103:7420–5. [PubMed: 16648260]
- [112]. Gu Z, Kaul M, Yan B, et al. S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. *Science*. 2002; 297:1186–90. [PubMed: 12183632]

- [113]. Hao G, Xie L, Gross SS. Argininosuccinate synthetase is reversibly inactivated by S-nitrosylation *in vitro* and *in vivo*. *J Biol Chem*. 2004; 279:36192–200. [PubMed: 15192091]
- [114]. Hashemy SI, Holmgren A. Regulation of the catalytic activity and structure of human thioredoxin 1 *via* oxidation and S-nitrosylation of cysteine residues. *J Biol Chem*. 2008; 283:21890–8. [PubMed: 18544525]
- [115]. Hashemy SI, Johansson C, Berndt C, et al. Oxidation and S-nitrosylation of cysteines in human cytosolic and mitochondrial glutaredoxins: effects on structure and activity. *J Biol Chem*. 2007; 282:14428–36. [PubMed: 17355958]
- [116]. Jian K, Chen M, Cao X, et al. Nitric oxide modulation of voltage-gated calcium current by S-nitrosylation and cGMP pathway in cultured rat hippocampal neurons. *Biochem Biophys Res Commun*. 2007; 359:481–5. [PubMed: 17544367]
- [117]. Kawano T, Zoga V, Kimura M, et al. Nitric oxide activates ATP-sensitive potassium channels in mammalian sensory neurons: action by direct S-nitrosylation. *Mol Pain*. 2009; 5:12. [PubMed: 19284878]
- [118]. Kim S, Wing SS, Ponka P. S-nitrosylation of IRP2 regulates its stability *via* the ubiquitin-proteasome pathway. *Mol Cell Biol*. 2004; 24:330–7. [PubMed: 14673166]
- [119]. Leclerc PC, Lancot PM, Auger-Messier M, Escher E, Leduc R, Guillemette G. S-nitrosylation of cysteine 289 of the AT1 receptor decreases its binding affinity for angiotensin II. *Br J Pharmacol*. 2006; 148:306–13. [PubMed: 16565729]
- [120]. Li F, Sonveaux P, Rabbani ZN, et al. Regulation of HIF-1 α stability through S-nitrosylation. *Mol Cell*. 2007; 26:63–74. [PubMed: 17434127]
- [121]. Li J, Billiar TR, Talanian RV, Kim YM. Nitric oxide reversibly inhibits seven members of the caspase family *via* S-nitrosylation. *Biochem Biophys Res Commun*. 1997; 240:419–24. [PubMed: 9388494]
- [122]. Marshall HE, Stamler JS. Inhibition of NF- κ B by S-nitrosylation. *Biochemistry*. 2001; 40:1688–93. [PubMed: 11327828]
- [123]. Martinez-Ruiz A, Villanueva L, Gonzalez de Orduna C, et al. S-nitrosylation of Hsp90 promotes the inhibition of its ATPase and endothelial nitric oxide synthase regulatory activities. *Proc Natl Acad Sci U S A*. 2005; 102:8525–30. [PubMed: 15937123]
- [124]. Nott A, Watson PM, Robinson JD, Crepaldi L, Riccio A. S-Nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons. *Nature*. 2008; 455:411–5. [PubMed: 18754010]
- [125]. Ozawa K, Whalen EJ, Nelson CD, et al. S-nitrosylation of beta-arrestin regulates beta-adrenergic receptor trafficking. *Mol Cell*. 2008; 31:395–405. [PubMed: 18691971]
- [126]. Park HS, Huh SH, Kim MS, et al. Nitric oxide negatively regulates c-Jun N-terminal kinase/stress-activated protein kinase by means of S-nitrosylation. *Proc Natl Acad Sci U S A*. 2000; 97:14382–7. [PubMed: 11121042]
- [127]. Rahman MA, Senga T, Ito S, et al. S-nitrosylation at cysteine 498 of c-Src tyrosine kinase regulates nitric oxide-mediated cell invasion. *J Biol Chem*. 2009; 285:3806–14. [PubMed: 19948721]
- [128]. Reynaert NL, Ckless K, Korn SH, et al. Nitric oxide represses inhibitory κ B kinase through S-nitrosylation. *Proc Natl Acad Sci U S A*. 2004; 101:8945–50. [PubMed: 15184672]
- [129]. Riederer IM, Schiffrin M, Kovari E, et al. Ubiquitination and cysteine nitrosylation during aging and Alzheimer's disease. *Brain Res Bull*. 2009; 80:233–41. [PubMed: 19427371]
- [130]. Schonhoff CM, Gaston B, Mannick JB. Nitrosylation of cytochrome c during apoptosis. *J Biol Chem*. 2003; 278:18265–70. [PubMed: 12646553]
- [131]. Shi Q, Feng J, Qu H, et al. A proteomic study of S-nitrosylation in the rat cardiac proteins *in vitro*. *Biol Pharm Bull*. 2008; 31:1536–40. [PubMed: 18670085]
- [132]. So HS, Park RK, Kim MS, et al. Nitric oxide inhibits c-Jun N-terminal kinase 2 (JNK2) *via* S-nitrosylation. *Biochem Biophys Res Commun*. 1998; 247:809–13. [PubMed: 9647775]
- [133]. Sun J, Xin C, Eu JP, et al. Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO. *Proc Natl Acad Sci U S A*. 2001; 98:11158–62. [PubMed: 11562475]
- [134]. Thang SH, Yasuda Y, Umezawa M, et al. Inhibition of phospholipase A(2) activity by S-nitroso-cysteine in a cyclic GMP-independent manner in PC12 cells. *Eur J Pharmacol*. 2000; 395:183–91. [PubMed: 10812048]

- [135]. Tsang AH, Lee YI, Ko HS, et al. S-nitrosylation of XIAP compromises neuronal survival in Parkinson's disease. *Proc Natl Acad Sci U S A*. 2009; 106:4900–5. [PubMed: 19273858]
- [136]. Wang G, Moniri NH, Ozawa K, et al. Nitric oxide regulates endocytosis by S-nitrosylation of dynamin. *Proc Natl Acad Sci U S A*. 2006; 103:1295–300. [PubMed: 16432212]
- [137]. Wang X, Michael D, de Murcia G, Oren M. p53 Activation by nitric oxide involves down-regulation of Mdm2. *J Biol Chem*. 2002; 277:15697–702. [PubMed: 11867628]
- [138]. Whalen EJ, Foster MW, Matsumoto A, et al. Regulation of beta-adrenergic receptor signaling by S-nitrosylation of G-proteincoupled receptor kinase 2. *Cell*. 2007; 129:511–22. [PubMed: 17482545]
- [139]. Xu L, Eu JP, Meissner G, et al. Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science*. 1998; 279:234–7. [PubMed: 9422697]
- [140]. Yoshida T, Inoue R, Morii T, et al. Nitric oxide activates TRP channels by cysteine S-nitrosylation. *Nat Chem Biol*. 2006; 2:596–607. [PubMed: 16998480]
- [141]. Kumar S, Barthwal MK, Dikshit M. Cdk2 nitrosylation and loss of mitochondrial potential mediate NO-dependent biphasic effect on HL-60 cell cycle. *Free Radic Biol Med*. 2010; 48:851–61. [PubMed: 20079829]
- [142]. Charbonneau A, Marette A. Inducible nitric oxide synthase induction underlies lipid-induced hepatic insulin resistance in mice: potential role of tyrosine nitration of insulin signaling proteins. *Diabetes*. 2010; 59:861–71. [PubMed: 20103705]
- [143]. Csibi A, Communi D, Muller N, Bottari SP. Angiotensin II inhibits insulin-stimulated GLUT4 translocation and Akt activation through tyrosine nitration-dependent mechanisms. *PLoS One*. 2010; 5:e10070. [PubMed: 20383279]
- [144]. Fujisawa Y, Kato K, Giulivi C. Nitration of tyrosine residues 368 and 345 in the beta-subunit elicits FoF1-ATPase activity loss. *Biochem J*. 2009; 423:219–31. [PubMed: 19650768]
- [145]. Guingab-Cagmat JD, Stevens SM Jr, Ratliff MV, et al. Identification of tyrosine nitration in UCH-L1 and GAPDH. *Electrophoresis*. 2011; 32:1692–705. [PubMed: 21706495]
- [146]. Kanski J, Hong SJ, Schoneich C. Proteomic analysis of protein nitration in aging skeletal muscle and identification of nitrotyrosine-containing sequences *in vivo* by nanoelectrospray ionization tandem mass spectrometry. *J Biol Chem*. 2005; 280:24261–6. [PubMed: 15851474]
- [147]. Nomiya T, Igarashi Y, Taka H, et al. Reduction of insulin-stimulated glucose uptake by peroxynitrite is concurrent with tyrosine nitration of insulin receptor substrate-1. *Biochem Biophys Res Commun*. 2004; 320:639–47. [PubMed: 15240096]
- [148]. Osoata GO, Yamamura S, Ito M, et al. Nitration of distinct tyrosine residues causes inactivation of histone deacetylase 2. *Biochem Biophys Res Commun*. 2009; 384:366–71. [PubMed: 19410558]
- [149]. Viner RI, Ferrington DA, Williams TD, et al. Protein modification during biological aging: selective tyrosine nitration of the SERCA2a isoform of the sarcoplasmic reticulum Ca²⁺-ATPase in skeletal muscle. *Biochem J*. 1999; 340:657–69. [PubMed: 10359649]
- [150]. Yakovlev VA, Bayden AS, Graves PR, et al. Nitration of the tumor suppressor protein p53 at tyrosine 327 promotes p53 oligomerization and activation. *Biochemistry*. 2010; 49:5331–9. [PubMed: 20499882]
- [151]. Hellberg CB, Boggs SE, Lapetina EG. Phosphatidylinositol 3-kinase is a target for protein tyrosine nitration. *Biochem Biophys Res Commun*. 1998; 252:313–7. [PubMed: 9826526]
- [152]. Wagner AH, Hildebrandt A, Baumgarten S, et al. Tyrosine nitration limits stretch-induced CD40 expression and disconnects CD40 signaling in human endothelial cells. *Blood*. 2011; 118:3734–42. [PubMed: 21832282]
- [153]. Garcia-Heredia JM, Diaz-Moreno I, Nieto PM, et al. Nitration of tyrosine 74 prevents human cytochrome c to play a key role in apoptosis signaling by blocking caspase-9 activation. *Biochim Biophys Acta*. 2010; 1797:981–93. [PubMed: 20227384]

Fig. (1). Glucose-stimulated insulin secretion in pancreatic beta cells

[1] Glucose enters the beta cell from the extracellular space *via* constitutively-expressed GLUT2 transporters positioned in the plasma membrane; [2] Glucose is immediately metabolized by glycolysis; [3] Mitochondrial ATP synthesis ensues, and the intracellular ATP-to-ADP ratio increases; [4] The elevation in ATP/ADP ratio causes ATP-sensitive potassium channels to close, inducing depolarization of the plasma membrane; [5] Voltage-sensitive calcium channels then open in response to depolarization, thus intracellular calcium increases, triggering SNARE core complex formation [6 and 7], and ultimately; [8] SNARE-mediated fusion of the insulin granule with the plasma membrane and release of insulin into the extracellular space. S-nitrosylation of Syntaxin 4 occurs within minutes of glucose stimulation, to facilitate the opening and activation of the t-SNARE for subsequent participation in granule fusion (solid arrow); the potential remains for modification(s) to other factors involved in the secretion mechanism (dotted arrow).

Fig. (2). Multiple effects of •NO-mediated signaling within cells; Syntaxin S-nitrosylation in beta cells exemplifies •NO signaling

A). NO can react directly with macromolecules, such as cysteine residues in proteins (in the case of S-nitrosylation), which akin to phosphorylation, can activate or inhibit protein function. Depending on the oxidative state of the cell, •NO can potentially serve as either oxidant *or* antioxidant, and it is likely that •NO contributes to the “decision” of a cell as to whether or not to undergo apoptosis. **B)** S-nitrosylation of Syntaxin 4 in beta cells. Threaded crystal structure of S-nitrosylated Syntaxin 4, based on sequence homology with the original crystal structure derived from Syntaxin 1A. S-nitrosylation is both longitudinally and radially centered within the coiled-coil motif for the syntaxin 4 H_{abc} SNARE regulatory domain.

Fig. (3). Numerous proteins are S-nitrosylated in pancreatic beta cells

MIN6 mouse pancreatic beta cells were incubated for 2 hours under glucose-free conditions, then left unstimulated or were stimulated with 16.7 mM glucose for 5 min. Cells were harvested in 1% NP-40 lysis buffer and lysates were clarified by centrifugation. Identical 2 mg quantities of MIN6 solubilized and clarified cell lysate protein (as determined by Coomassie Brilliant Blue G-250 protein assay) were subjected to a biotin-switch assay with detection of all S-nitrosylated proteins using avidin overlay. Biotinylated proteins (indicative of S-nitrosylated proteins) were resolved by 12% SDS-PAGE for subsequent transfer to PVDF for detection using streptavidin-HRP. Of significance are the multitudes of bands detected under both basal and acute glucose-stimulated conditions.

Table 1

Protein Targets of S-Nitrosylation and their Known Functions

S-Nitrosylation Target	Function	Ref.
Glucokinase	Exocytosis	[40]
Syntaxin 1A		[66]
Syntaxin 4		[32]
NSF		[65]
SNAP-25		[71]
Type 1 Angiotensin II Receptor	Receptor Signaling	[119]
Insulin Receptor		[105]
NMDA Receptor		[107]
Muscarinic M2 Receptor		[103]
Ryanodine Receptor		[133]
B-Arrestin 2		[125]
MAP Kinase 8	Signal Transduction	[126]
c-Jun		[132]
c-Src		[127]
IRS-1		[105]
Akt		[105]
β Androgen Receptor Kinase 2		[138]
Bcl-2	Apoptosis	[104,106]
Caspase Family		[121]
E3 Ubiquitin Ligase (XIAP)		[135]
Arginase-1	•NO Synthesis	[109]
Argininosuccinate synthetase		[113]
eNOS		[110]
Dynamin-1/2	Endocytosis	[136]
Glutathione reductase	Redox Homeostasis	[115]
Thioredoxin-1		[114]
HSP70-1	Heat Shock	[129]
HSP90- α		[123]
Elongation factor 1- α (eEF1a)	Gene Transcription	[111]
HDAC 2		[124]
HIF-1 α		[120]
I κ B kinase (IKKB)		[128]
Mdm2		[137]
NF κ B p105 subunit		[122]
ATP-sensitive K ⁺ channel	Cation Channel	[117]
L-type Ca ²⁺ channel		[116]
TRP channels		[140]

S-Nitrosylation Target	Function	Ref.
Cytochrome c oxidase subunit 2	Electron Transport	[130]
Protein disulfide isomerase	Protein Homeostasis	[131]
Parkin		[108]
Cytosolic phospholipase A2	Other	[134]
IREBP2		[118]
MMP-9		[112]
Cdk2		[141]

Table 2

Acute Intracellular Events Following Onset of Glucose Stimulation

Intracellular Event	Time of Onset
Increased NADPH Levels	1-3 s
Increased FAD ⁺	1-3 s
Onset of •NO Synthesis	<20 s
ATP-to-ADP Ratio Increase	15-25 s
Increased Oxygen Consumption	17-41 s
Rise in intracellular pH (pH _i)	43-53 s
Increased intracellular Ca ²⁺	104-124s

Table 3AProtein Tyrosine Nitration Targets in Pancreatic β -Cells

Tyrosine Nitration Target	Location
Glucokinase	Cytosol
RACK 1(activated protein kinase C receptor)	Cytosol
G3PDH (glyceraldehyde-3-phosphate dehydrogenase)	Cytosol
Glutamate dehydrogenase	Cytosol/Mitochondria
Malate dehydrogenase	Cytosol/Mitochondria
Aldolase A	Cytosol/Nucleus
Protein disulfide isomerase (ER60)	Mitochondria
Aconitase 2 (mitochondrial)	Mitochondria
Glutamate oxaloacetate transaminase 2 (mitochondrial)	Mitochondria
L-3-hydroxyacyl-coA dehydrogenase	Mitochondria
MnSOD (manganese superoxide dismutase, SOD2)	Mitochondria
Phosphoglycerate kinase	Mitochondria
VDAC-1, -2, -3 (voltage-dependent anion-selective channel)	Mitochondria
Heterogenous nuclear ribonucleoprotein A2/B1 (hnRNP-A2/B1)	Nucleus
HSP60 (heat shock protein 60)	Perimembrane
eEF1a1 (eukaryotic translation elongation factor 1 α 1)	Ribosome

Table 3B

Potentially Relevant Protein Tyrosine Nitration Targets in Other Cell Types

Protein Target	Nitrating Agent	Dose	Ref.
CD40	SIN-1	0.1-2 mM	[152]
PI3-Kinase	ONOO ⁻	1 mM	[151]
Cytochrome c	ONOO ⁻	0-3 mM	[153]
SERCA-2a	ONOO ⁻	0-200 μ M	[149]
GADPH	ONOO ⁻	0.7-3.5 mM	[145]
Myosin light chain	ONOO ⁻	0-1 mM	[146]
IRS-1	ONOO ⁻ , SIN-1	50 μ M, 0-8 mM	[147]
P53	DETA NONOate	0-200 μ M	[150]
HDAC2	ONOO ⁻ , SIN-1	1 mM, 500 μ M	[148]
Akt	SIN-1	0-100 μ M	[143]
ERK1/2	SIN-1	0-100 μ M	[143]
IR-beta	ONOO ⁻	50 μ M	[142]
F1-ATPase	TNM	0-1.5 mM	[144]