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Editorial Focus: *Unraveling the molecular machinery that promotes pancreatic β-cell dysfunction during oxidative stress*: focus on "Phagocyte-like NADPH oxidase promotes cytokine-induced mitochondrial dysfunction in pancreatic β-cells: evidence for regulation by Rac1"

Martin Jastroch

Buck Institute for Age Research, Novato, California

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INSULIN SECRETION BY PANCREATIC β-cells regulates glucose uptake from the blood into peripheral tissues, such as liver, muscle, and adipose tissue. Therefore, pancreatic β-cells possess a central role in controlling systemic glucose homeostasis and energy balance. The relative proportion of this organ to body mass, however, is exceptionally small, comprising about 0.002% of body mass in rats (8). Furthermore, there is a low capacity of β-cell regeneration and if the cell number declines below 10^6 cells/kg body mass, animals become glucose intolerant (15). These facts imply that disturbances in β-cell function may easily impair glucose homeostasis.

In humans, this is reflected in the pandemic disease diabetes mellitus. In the United States alone, about 8% of the population has diabetes, and 1.6 million new cases are reported each year (http://www.diabetes.org/diabetes-basics/diabetes-statistics/). The sequelae of diabetes are numerous and diverse (4), but a direct dysfunction of β-cells is one of the most frequent causes (3). Abnormalities in β-cell physiology, which promote dysfunction and eventually cell death, are usually attributed to "stress." In particular, oxidative stress has been identified by a lot of studies as a major player, as β-cells, in particular, are sensitive toward excessive or chronically elevated amounts of reactive oxygen species (ROS). The vulnerability of the β-cell is caused by relatively low amounts of antioxidant defense systems (7). Although the low concentration of antioxidants may allow using ROS as an adjustable cellular signal, excessive amounts of ROS may not be buffered. In particular, imbalances of nutrient metabolism or inflammatory responses enhance ROS production and oxidative damage.

The mitochondrial respiratory chain complexes have been conventionally considered as major producers of cellular ROS, but there is increasing evidence that the phagocyte-like NADPH oxidase (PHOX) may also contribute significantly to ROS upon activation. This enzyme was initially identified in phagocytes and the nonmitochondrial ROS generation by PHOX was solely thought to be microbicidal (6). Over the years, PHOX and its isoforms have been identified in other cell types, but their physiological role is still poorly understood. The enzyme catalyzes a single electron transfer to molecular oxygen and thereby produces superoxide. Superoxide usually dismutates spontaneously to hydrogen peroxide. Superoxide and hydrogen peroxide can be further processed to other reactive molecules that are collectively termed as ROS. ROS may assist to fight viral or bacterial invaders or act as a signaling molecule. Under pathological conditions, the activation of NADPH-derived ROS has been implicated in numerous metabolic diseases. Upon an activating stimulus, the membrane-bound catalytic core of the enzyme is dissociated from regulatory subunits. Multiple steps are involved to translocate the cytosolic subunits to the catalytic core for the assembly of the holoenzyme.

Pancreatic β-cells express NADPH oxidases (12), and although there is no physiological function addressed so far, the contribution to cellular dysfunction during oxidative stress has been clearly shown. Elevated glucose, saturated fatty acids, or proinflammatory cytokines mediate PHOX activation in β-cells and may promote cellular damage (9).

Given these observations, a major step toward the understanding of β-cell dysfunction during oxidative stress is the identification of the molecular events leading to PHOX activation. Eventually, knowledge on the multiple regulatory steps of PHOX activity may allow pharmacological intervention and therapeutically targeting either the activating signals, the regulatory units of PHOX, or further downstream products of the enzyme.

Subasinghe et al. (14) study the regulatory elements of PHOX in a pancreatic β-cell culture model. In a pathologically relevant context, the study uses proinflammatory cytokines to activate PHOX, thereby mimicking immune reactions, as they may be found during the progress of β-cell dysfunction and type 1 diabetes.

The authors show that PHOX is activated and ROS production is increased upon cytokine stimulation. Specifically, the response of subunit $p47^{phox}$ and its crucial role in ROS production is shown by two approaches: *1*) pharmacological inhibition of ROS production with the PHOX-inhibitor apocynin and *2*) silencing of $p47^{phox}$ with siRNA.

Apocynin is an antioxidant and may interfere with ROS production per se, thereby, ambiguously reporting specific inhibition of $p47^{phox}$ (5). Silencing of $p47^{phox}$, however, also attenuates ROS production and, therefore, unequivocally demonstrates the involvement of $p47^{phox}$ in cytokine-promoted PHOX-derived ROS production. These findings support previous studies on PHOX in pancreatic β-cells (9, 10), and in the following experiments, Subasinghe et al. (14) comprehensively focus on the PHOX regulator Rac1, which belongs to the Rho subfamily of Ras-related GTPases. Rac1 in its active, GTP-bound form is essential for PHOX holoenzyme assembly. The

Address for reprint requests and other correspondence: M. Jastroch, Buck Institute for Age Research, 8001 Redwood Blvd., Novato, CA 94945 (e-mail: mjastroch@buckinstitute.org).

authors use pharmacological intervention to show that Rac1 is transiently activated upon inflammatory stimulus and controlled by Tiam1, a guanine nucleotide exchange factor, and prenylation. Strikingly, "mitochondrial defects" that occur upon cytokine treatment, measured as a loss of mitochondrial membrane potential, are prevented by inhibitors of Rac1. Taken together, the study highlights Rac1 as a key regulatory compound of PHOX activity in the β-cell. The authors (14) suggest that PHOX-derived ROS promote "mitochondrial defects," leading to the release of cytochrome *c*, activation of caspase 3, and cell death.

The work by Subasinghe et al. (14) improves our understanding of how oxidative stress in pancreatic β-cells occurs, dissects the molecular, regulatory subunits of the major ROS-generating enzyme of this stress response, and demonstrates a central role of Rac1 in the regulation of cellular ROS and mitochondrial dysfunction. Although the authors admit that the pharmacological interventions used in this study may not be applicable, as other essential cellular processes may be affected, targeting the activation of PHOX may open new therapeutic avenues for the treatment of diabetes. The fundamental molecular regulatory pathways of PHOX activation during inflammatory response may also help understand oxidative stress in other pathological conditions and cell types.

Important findings are shown in this study that raise new questions but also emphasize further experimental refinements. Considering the significance of PHOX for intracellular stress, the topology of superoxide production requires further attention: to date, there is no convincing consensus in the field as to whether superoxide is produced toward the extracellular space, as originally suggested for phagocytes (6), or whether it may be generated on the cytosolic side in β-cells (2, 11). These molecular mechanisms may have profound implications in identifying the physiological role of PHOX-derived ROS in pancreatic β-cells and downstream mechanisms.

In future experiments activating PHOX, it might be useful to systematically test single compounds of the proinflammatory cocktail cytomix, which consists of interleukin-1β (IL-1β), interferon-γ (IF-γ), and tumor necrosis factor-α (TNF-α), and is broadly used to mimic inflammation. Unfortunately, pathways of caspase 3 activation not involving PHOX are also induced by cytomix. Some preliminary results suggest, however, that Rac1 activation may be solely due to IL-1 β (14).

Interestingly, nitric oxide (NO) is also increased during inflammatory response, independent of PHOX activity and regulators, and apparently without effects on mitochondrial membrane potential. The authors suggest that NO may be involved in apoptosis but future approaches need to inhibit NO and reevaluate NO-dependent apoptosis, possibly including other crucial apoptotic parameters, such as cytochrome *c* release. To partially understand how ROS and NO may promote apoptosis together, we may consult analogies occurring during inflammatory neurodegeneration (1). In neurons, only the combination of PHOX and iNOS forming peroxynitrite ($ONOO^-$) causes cell death. Therefore, the combined action of PHOX and iNOS may be required to fully activate apoptotic pathways in β-cells.

The questions remain whether in response to PHOX-mediated β-cell dysfunction, mitochondria are used as vehicles to signal apoptosis, or whether the loss in mitochondrial membrane potential pivotally affects energy transduction leading to β-cell dysfunction prior to cell death. The importance of these questions derives from the special bioenergetic design of β-cell function: proton motive force (which can be estimated as the mitochondrial membrane potential) has to be increased during glucose stimulation to secrete insulin (13). Loss of mitochondrial membrane potential by PHOX activation would compromise insulin secretion, adding further dysfunction at an earlier stage than apoptosis.

The important finding of mitochondrial membrane potential loss by PHOX in the pancreatic β-cell provides an exciting

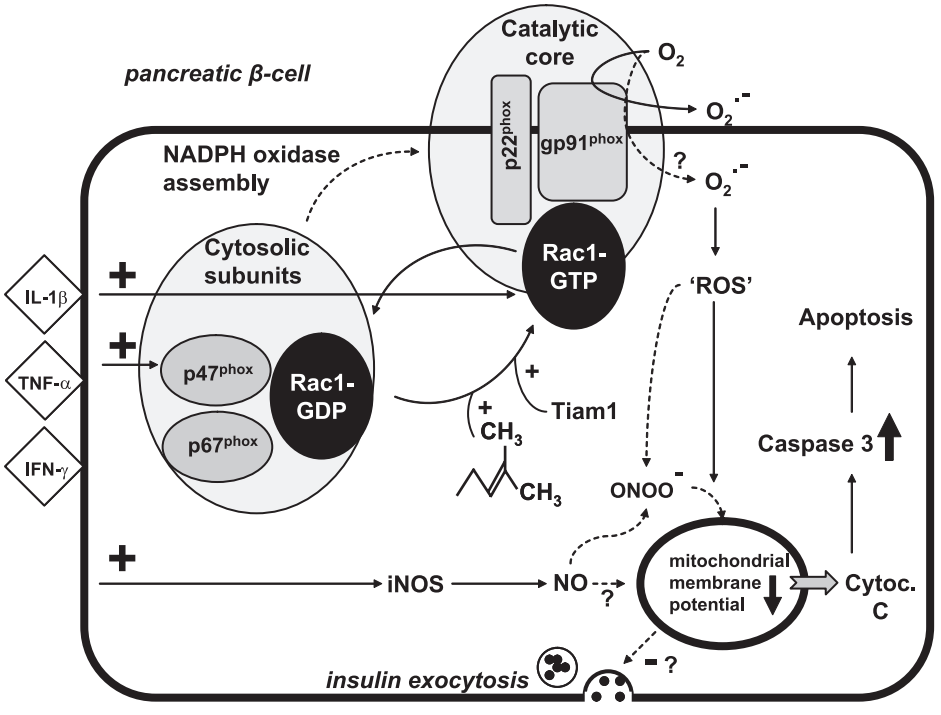

Fig. 1. Simplified scheme on the regulation of the phagocyte-like NADPH oxidase and oxidative stress responses in the pancreatic β-cell. Cytokines (IL-1 β, IF-γ, TNF-α) promote the assembly of the phagocyte-like NADPH oxidase (PHOX) by upregulating $p47^{phox}$ and increasing activated Rac1-GTP. The transformation of inactive Rac1-GDP to active Rac1-GTP during cytokine exposure is regulated by Tiam1, a guanine nucleotide exchange protein, and by prenylation. The translocation of the cytosolic subunits allows the assembly of the holoenzyme and activates the generation of superoxide. Superoxide, either released extracellularly or intracellularly, will generate further reactive oxygen species (ROS) that compromise mitochondrial membrane potential. Simultaneously to processes involving PHOX, cytokines also activate the inducible nitric oxide synthase (iNOS). Nitric oxide (NO) may be involved in activation of caspase 3. In this scheme, it is also suggested that *1*) the topology of superoxide by PHOX is still controversial, *2*), the simultaneous release of reactive oxygen and nitrogen species will generate peroxynitrite ($ONOO^-$) that may potentiate deleterious effects on cellular death, and *3*) the loss in mitochondrial membrane potential may directly impair glucose-stimulated insulin secretion.

new field to link "mitochondrial defects" with detailed functional studies on mitochondria, glucose-stimulated insulin secretion, and apoptosis (Figure 1).

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DISCLOSURES

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