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FORUM REVIEW ARTICLE

Nox, Nox, Are You There? The Role of NADPH Oxidases in the Peripheral Nervous System

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Abstract

Significance: Reactive oxygen species (ROS) contribute to multiple aspects of peripheral nervous system (PNS) biology ranging from physiological processes (*e.g.*, axonal outgrowth and regeneration) to pathophysiology (*e.g.*, nerve degeneration). Although ROS are derived from multiple sources, NADPH oxidase (Nox) family members are dedicated to ROS generation. Noxs are expressed in the PNS, and their overexpression is associated with detrimental effects on nerve function and contributes, at least in part, to peripheral neuropathies.
Recent Advances: Of the seven members, studies mostly focused on Nox1, Nox2, and Nox4, which are expressed in the PNS in a cell-specific manner. We have also recently identified human Nox5 in sural nerve biopsies. When maintained at homeostatic levels, Noxs regulate several aspects of peripheral nerve health, most notably neurite outgrowth and axonal regeneration following nerve lesion. While Nox2 and Nox4 dysregulation is a major source of oxidative stress in PNS disorders, including neuropathic pain and diabetic peripheral neuropathy, recent evidence also implicates Nox1 and Nox5.
Critical Issues: Although there is compelling evidence for a direct role of Noxs on nerve function, little is known about their subcellular localization, intercellular regulation, and interaction. These, together with redox signaling, are considered crucial components of nerve redox status. In addition, the lack of isoform-specific inhibitors limits conclusions about the physiological role of Noxs in the PNS and their therapeutic potential in peripheral neuropathies.
Future Directions: Future research using isoform-specific genetic and pharmacological approaches are therefore needed to better understand the significance of Nox enzymes in PNS (patho) physiology. *Antioxid. Redox Signal.* 37, 613–630.

Keywords: NADPH oxidases (Nox), neuron, neuropathy, peripheral nervous system (PNS), reactive oxygen species, Schwann cells

Introduction

THE PERIPHERAL NERVOUS system (PNS) refers to the portion of the nervous system that lies outside the central nervous system (CNS) and serves as a connecting point between the CNS and peripheral tissues. It consists of a complex network of cranial and spinal nerves, composed of neurons, their axons, and supporting Schwann cells. Afferent sensory neurons and their associated axons transmit information from sensory receptors in the PNS back to the CNS, whereas motor efferent neurons and their axonal extensions transmit information from the CNS to the muscles and glands (38). This back-and-forth communication between the PNS and the CNS is pivotal for the physiological regulation of the internal system as well as the interactions with the external environment.

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The need to rapidly carry nerve impulses over long distances of up to 2 m or more in length poses a unique challenge to the PNS and makes it highly susceptible to metabolic, mechanical, toxic, and immune insults (141). When injured, the PNS exhibits abnormalities in nerve structure and function that disrupt the ability of the CNS to communicate *via* the PNS with effector organs and muscles.

Peripheral neuropathies are a heterogeneous group of disorders that occur secondary to peripheral nerve damage, leading to reduced quality of life (43). They affect more than 8% of the general population, and this number rises to 15% in patients 40 years or older (46). Peripheral neuropathies are often associated with axon degeneration, impaired regeneration, as well as disruptions in calcium (Ca^{2+}) signaling, electrophysiological function, mitochondrial function, and substrate utilization (38, 135, 141). Redox signaling modulates many of these processes, and accumulating clinical and preclinical evidence has shown increased nerve reactive oxygen species (ROS) following peripheral nerve dysfunction. However, untargeted antioxidant therapies to treat PNS disorders have only exhibited limited therapeutic potential in the clinical setting (83, 110).

In addition to the incorrect selection of antioxidant dosages and treatment durations, these failures are mainly attributed to the lack of antioxidant specificity against the ROS source(s) altered in a disease- and tissue-specific manner. Untargeted antioxidant treatment can result in off-target effects or lead to global suppression of ROS-producing enzymes, including those required for normal physiology (133). Therefore, it is critical to abandon the previous conventional approach that ''blindly'' targets all antioxidant activity. The new and needed way forward is to identify specific ROS sources that are altered during disease course, so that targeted antioxidant therapies can be developed to treat PNS diseases.

The major sources of ROS in peripheral nerves include mitochondria, xanthine oxidase, and NADPH oxidases (Noxs) (Fig. 1) (38, 124, 143). Among these sources, the Nox family of enzymes consists of seven members (Nox1–5 and dual oxidases [Duox] 1 and 2) specialized for ROS production (103). Although the role of Noxs in the PNS is not completely understood, evidence implicates Nox family members in cellular functions ranging from the immune response and neuronal development to pathophysiological involvement and neurodegeneration (35, 73, 96, 143). In this review, we first provide a general overview of ROS and antioxidant generation in the PNS. We then focus on Nox expression in the PNS, their physiological roles, how Nox-derived ROS contribute to PNS disorders, and the novel concepts of Nox signaling, which may be relevant to nerve redox homeostasis and PNS function.

ROS and Antioxidants: General Overview

ROS are a family of oxygen containing molecules resulting from cellular metabolism, which can avidly react with biomolecules, including nitric oxide, proteins, lipids, carbohydrates, and DNA (119). Because of their ability to modify biomolecules, ROS generation was initially considered

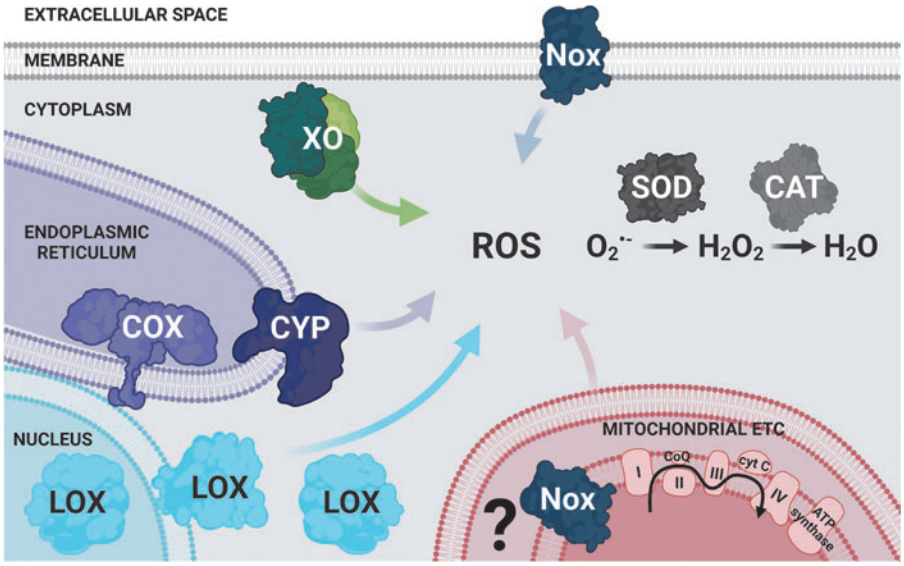

FIG. 1. ROS sources and metabolism in the PNS. The mitochondrial ETC is considered a major source of ROS in the PNS and can inadvertently lead to $O_2^{-\bullet}$ generation under physiological conditions (38). Other sources that generate ROS as a by-product of metabolism include XO located in the cytoplasm (116, 127), CYP and COX (128) that reside in the ER, as well as LOX (147) found in the nuclear and cytosolic regions and membranes (not shown in the figure for simplicity) (124). Noxs are a specialized source of ROS (120). They localize to the plasma membrane and intracellular compartments, including the mitochondria (shown in the figure with a question mark that highlights a possible localization to the inner mitochondrial space), the ER, and the nuclear envelope (not shown in the figure for simplicity). Under physiological conditions, $O_2^{-\bullet}$ generated by these ROS-producing enzymes is rapidly converted to the more stable and easily diffusible H_2O_2 by SOD. H_2O_2 is then detoxified by CAT to form H_2O (124). CAT, catalase; COX, cyclooxygenases; CYP, cytochrome P450 monooxygenases; ER, endoplasmic reticulum; ETC, electron transport chain; H_2O, water; H_2O_2, hydrogen peroxide; LOX, lipoxygenases; Nox, NADPH oxidase; $O_2^{-\bullet}$, superoxide anion; PNS, peripheral nervous system; ROS, reactive oxygen species; SOD, superoxide dismutase; XO, xanthine oxidase. Created with BioRender.com.

cytotoxic and believed to occur only under pathological conditions. However, it is now well established that homeostatic ROS levels are crucial for cellular physiology and regulate processes such as growth, apoptosis, signal transduction, cellular respiration, and host defense.

In the nervous system, ROS production is involved in blood pressure regulation, cognitive function, tissue repair, and immune response (103). However, when overproduced, these highly reactive molecules become deleterious and can promote cell damage in disease states. The increased understanding of both the beneficial and harmful effects of ROS is critical to the design of rationale, mechanism-based therapies for PNS disorders.

ROS are classified into two groups: radical and nonradical species. Radical ROS include superoxide anion ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$), and nitrogen-based species such as the nitric oxide radical ($NO^{\bullet}$), whereas nonradical ROS include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$) (119). Many ROS types are involved in redox signaling, defined as the reversible redox modifications exerted by ROS to a specific biomolecule (14). As an example, $O_2^{-\bullet}$ exerts its effects at the site of generation and is rapidly converted to the more stable and easily diffusible H_2O_2; both $O_2^{-\bullet}$ and H_2O_2 are considered major ROS involved in redox signaling through iron/sulfur cluster modification and cysteine oxidation.

More potent oxidants such as $^{\bullet}OH$, exert irreversible redox reactions (14). In peripheral nerves, redox signaling is implicated in physiological processes, including axonal outgrowth and regeneration, as well as pathophysiology, including pain processing and nerve degeneration (35, 72, 143).

Intracellular ROS levels in the PNS are maintained in check *via* antioxidant defense mechanisms, which consist of enzymes and nonenzymatic scavengers. Enzymes are mainly under the control of the transcription factor NF-E2-related factor 2 (Nrf2) and include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (71, 88, 137). On the contrary, nonenzymatic scavengers are primarily of dietary origins and include α-tocopherol (vitamin E), β-carotene, and ascorbate (vitamin C).

Neurons and Schwann cells can initially increase Nrf2-dependent antioxidant signaling in the face of cellular stressors at early disease stages before progression to irreversible damage (53, 137). Interestingly, we have shown that Schwann cells have a high basal antioxidant potential under physiological conditions, which is further increased during metabolic stress. This feature is thought to confer resistance to oxidative damage relative to the more vulnerable neurons (137).

An imbalance between ROS generation and the ability of the antioxidant defense mechanisms to clear excess ROS, or effectively repair the resulting damage, is deleterious and is associated with irreversible oxidative modifications to macromolecules as well as impaired redox signaling. These processes eventually lead to uncontrolled ROS generation commonly referred to as oxidative stress (119), an effect that has been implicated in nerve degeneration and PNS disorders.

Sources That Generate ROS As a By-Product of Metabolism in the PNS

Multiple cellular sources generate ROS in the PNS as a by-product of oxidative phosphorylation and metabolism. The mitochondrial electron transport chain is perhaps the most studied ROS source in the PNS, which can generate $O_2^{-\bullet}$ as a result of an electron leak leading to a 1-electron reduction from oxygen to $O_2^{-\bullet}$, instead of water (2).

Compared with other cell types, mitochondria make up half of the cytoplasmic volume of high energy consuming peripheral neurons, which require up to 4.7 billion adenosine triphosphate (ATP) molecules per second under normal physiologic conditions (153) to maintain their membrane potential across a large surface area (115). Because of this high mitochondrial content, the electron transport chain was long thought to be the major ROS source that sustains the oxidative potential in the PNS under physiological conditions besides being a substantial ROS source in PNS disorders (3, 16). Interestingly, Nox enzymes, mainly Nox4 and Nox5, might localize to mitochondria, turning the mitochondrial electron transport chain into both an oxidation target and an ROS source (10, 81, 93).

In addition to the mitochondrial electron transport chain, multiple enzymes in the PNS can produce ROS as by-products of their catalytic activities (Fig. 1). These include xanthine oxidase, located in the cytoplasm (116, 127), cytochrome P450 monooxygenases (unpublished data), and cyclooxygenases (128), residing in the endoplasmic reticulum (ER), as well as lipoxygenases (147), found in the nuclear and cytosolic regions and membranes (124). Studies have mostly evaluated the function of these ROS-generating enzymes in PNS pathophysiology and their role in normal PNS physiology is an avenue warranting further investigation.

NADPH Oxidases of the Nox Family As a Dedicated Source of ROS

In contrast to the other ROS-generating systems, NADPH oxidases of the Nox family (Nox) are transmembrane proteins with no known metabolic function, except catalyzing ROS generation across biological membranes (120). This occurs *via* electron transfer from NADPH as an electron donor. Two NADPH molecules transfer two electrons to flavin adenine dinucleotide, which passes the electrons to two iron-containing heme groups. The electrons are then transferred to oxygen as an electron acceptor, thereby yielding $O_2^{-\bullet}$ largely thought to be the major product of the electron transfer (Fig. 2). In most mammals, the Nox enzyme family consists of seven members: Nox1 through 5 and Duox 1 and 2. Interestingly, the Nox5 isoform is only expressed in higher mammals and is absent in rats and mice, which limits our understanding of its physiological and pathophysiological significance in humans (39).

The prototype NADPH oxidase, Nox2, originally discovered in neutrophils, is one of the best-characterized members of the Nox family and is critical for innate immunity (25). It consists of two transmembrane catalytic subunits ($gp91^{phox}$ [commonly referred to as Nox2], and the regulatory subunit $p22^{phox}$), three cytosolic subunits ($p47^{phox}$, $p67^{phox}$, and $p40^{phox}$), and a small Rho GTP-binding protein (Rac1 or Rac2). These subunits are disassociated in the inactive state, but assemble upon enzyme stimulation to produce $O_2^{-\bullet}$ (112).

While other Nox members share a certain extent of structural homology with Nox2, the mechanisms by which they are activated may vary; for example, similar to Nox2,

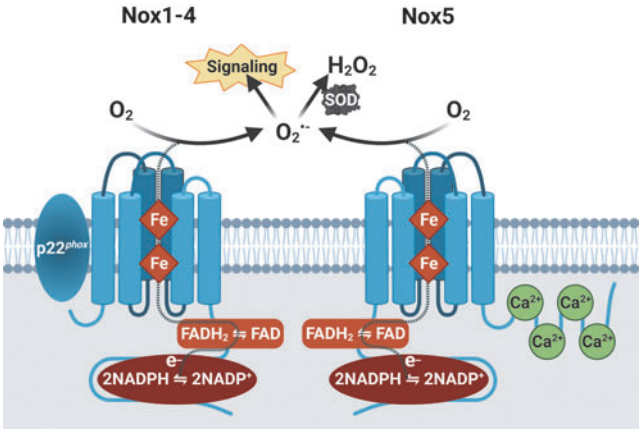

FIG. 2. NADPH oxidase family of enzymes as a dedicated source of ROS. Nox enzymes are a family of transmembrane proteins. They bind within their C-terminus, NADPH, which acts as the electron (e^-) donor. Two NADPH molecules transfer two e^- to FAD, also bound within the C-terminus, which passes the e^- to two Fe-containing heme groups. Finally, the e^- are transferred to O_2, the terminal acceptor, catalyzing the transformation to $O_2^{-\bullet}$, although Nox4 and Nox5 release H_2O_2. Nox1–4 share a certain extent of structural homology and all require interaction with the regulatory subunit $p22^{phox}$ to produce ROS. Nox5 does not require $p22^{phox}$, but is Ca^{2+}-dependent and thus contains N-terminal EF-hand domains with four Ca^{2+}-binding sites (103). Ca^{2+}, calcium; FAD, flavin adenine dinucleotide; Fe, iron; O_2, molecular oxygen. Created with BioRender.com.

Nox1–4 full activation also requires interaction with $p22^{phox}$ to produce ROS (112). Nox1 through 3 enzymes require cytosolic subunits ($p47^{phox}$ and $p67^{phox}$, or homologues) to form a fully functional enzyme complex (112). However, unlike the other isoforms, Nox4 is constitutively active and does not require any cytosolic subunits for full activation (92). Nox5 and Duox enzymes contain N-terminal EF-hand domains with four binding sites for Ca^{2+}, which is required for enzyme activation (103).

Under physiological conditions, Noxs are maintained at a relatively low level of constitutive activity to regulate redox signaling in the vicinity of target molecules. While Nox-dependent redox signaling mainly regulates cell differentiation, proliferation, and apoptosis, some Nox isoforms exert cell-specific functions. For example, as mentioned above, phagocyte Nox2 is heavily involved in the innate immune response, while Nox3 is highly expressed in the inner ear and plays a role in otoconia biogenesis (84).

With respect to the ROS type, Nox isoforms generally produce $O_2^{-\bullet}$ as their primary product. However, data have demonstrated that Nox4, Duox1, and Duox2 generate H_2O_2 (26, 105). Evidence also shows that H_2O_2 can be detected following Nox5 activation (20, 118). While the biochemical mechanism underlying H_2O_2 generation is unknown, a rapid conversion of $O_2^{-\bullet}$ into H_2O_2 before release from the enzyme has been suggested as a contributing factor. It has been further demonstrated that Nox4-dependent H_2O_2 generation relies on a histidine (His-222) residue, localized in the extracellular loop of the enzyme (105, 126), a mechanism that requires validation in the other isoforms.

In the following sections, we review Nox cellular distribution, as well as their physiological and pathophysiological involvement in the PNS. Because the roles of Nox3 and Duox1-2 in the PNS are unclear, we focus this review on Nox1, Nox2, Nox4, and Nox5.

Nox Expression in the PNS

Nox subcellular localization

Nox enzymes reside both at the plasma membrane and in intracellular compartments, including the ER, mitochondria, and the nuclear envelope (103). Under resting conditions, Nox2 is closely associated with $p22^{phox}$ and together primarily localize to intracellular and plasma membranes, while cytosolic subunits are typically located in the cytoplasm (102). Upon cell activation, the cytosolic subunits translocate to membrane-bound Nox2 to form a fully functional enzyme complex (102). Nox4 on the contrary is expressed intracellularly and can be found in the ER (146), the nuclear envelope (22), and the mitochondria (10). While both Nox1 and Nox5 have been identified in the plasma membrane (49, 130), reports have also detected Nox5 at several intracellular sites, including the perinuclear area, the mitochondria, and the ER (130).

Because of their high reactivity, this diverse subcellular localization of Noxs has several consequences, including how Nox isoforms influence redox signaling as well as how Nox isoforms function under normal and disease states. For example, Nox2 assembly and activation on the plasma membrane of neutrophils are essential for ROS generation and pathogen elimination at the injury site (36). A recent report identified mitochondrial Nox4 as an energetic sensor, whose activity is directly regulated by ATP in renal cells (117). These results suggest a close connection between ATP turnover and Nox4 redox signaling and could be of relevance to the PNS.

With respect to the PNS, axons are intimately associated with Schwann cells, and growing evidence highlights the importance of the Schwann cell/axon cross talk in peripheral nerve metabolic support (11). This in turn raises the possibility that ROS release from axons into the extracellular space may directly influence the surrounding Schwann cells and *vice versa*. Specifically, Nox4-derived oxidative stress in sensory dorsal root ganglion neurons is accompanied by Schwann cell injury and dysmyelination in a mouse model of neuropathic pain (72). While the underlying mechanism is unknown, one can speculate that Nox4-derived ROS in axons could damage Schwann cells in a paracrine manner, similar to what has been observed in the vasculature (12, 13, 98). This novel aspect of Nox signaling is discussed in detail below.

Nox cellular distribution in the PNS

Relative to the better characterized CNS (103), the cellular distribution of Nox enzymes in the PNS has not been comprehensively analyzed (Table 1). Data, however, demonstrate that Nox isoforms can be expressed simultaneously at multiple PNS sites (28, 73). In addition, it is generally thought that Nox expression in the PNS is maintained at low basal levels under physiological conditions, and upregulated in disease states (103).

Table 1. Nox Cellular Distribution in the Peripheral Nervous System

PNS cellular localization	*Nox isoforms*	*Accessory proteins*	*References*
Dorsal root ganglion neurons	Nox1, Nox2, and Nox4	$p22^{phox}$, $p47^{phox}$, and Rac1	(17, 59, 72, 111, 136)
Schwann cells	Nox1 and Nox4	Not identified	(28, 35)
Macrophages	Nox2 and Nox4	$p22^{phox}$ and $p47^{phox}$	(9, 28, 70)

Nox, NADPH oxidase; PNS, peripheral nervous system.

Dorsal root ganglion neurons express Nox1, Nox2, and Nox4 (59, 72, 136). Studies have also reported the expression of Nox accessory proteins $p22^{phox}$, $p47^{phox}$, and Rac1 in rodent primary cultures of dorsal root ganglion neurons (17, 111, 136).

As we mentioned above, Nox subcellular localization in dorsal root ganglion neurons is a crucial determinant of the downstream signaling effects of intracellular *versus* extracellular Nox-derived ROS. While this is still an area requiring further research, multiple studies have used dihydroethidium (DHE) for detecting $O_2^{-\bullet}$ in neuronal cultures or whole peripheral nerve samples (35, 57), which suggests a role for Nox-derived ROS in intracellular redox signaling. Increasing studies are, however, assessing ROS release into the extracellular space using cytochrome *c* assay and Amplex Red (23, 72), which may reflect a role in cell-to-cell communication and in the propagation of ROS signals into neighboring cells.

Schwann cells mainly express Nox1 and Nox4 (28, 35), with no available reports on Nox2 protein expression.

As a whole, Nox subcellular localization in Schwann cells remains largely unexplored. Recent data, however, have shown that Nox1 can generate both intra- and extracellular oxidants in Schwann cells in a neuropathic pain model. The authors suggest that intracellular ROS maintain mechanical allodynia, while extracellular H_2O_2 promotes Nox2-expressing macrophage recruitment to the perineurial space (28). Simultaneous expression of Schwann cell Nox1 and macrophage Nox2 in the PNS leads to a sustained feed-forward loop of oxidative injury. These results suggest a potential interaction between different Nox isoforms to regulate redox signaling, a novel concept that is further discussed below.

Macrophages mostly express Nox2 (28, 70), which is low in the absence of a stimulus (77). Once activated, reports demonstrate the Nox2-mediated oxidative burst at the injury site in rodent models of PNS disorders (28, 70). Besides Nox2, Nox4 is upregulated in spinal cord macrophages at late stages of neuropathic pain and, together with Nox2, modulates macrophage polarization (9). Whether Nox2 and Nox4 are simultaneously coexpressed in PNS macrophages and the significance of this coexpression on peripheral nerve health are areas requiring additional studies.

Nox Signaling in the PNS Under Physiological Conditions

Under physiological conditions, Nox enzymes regulate redox signaling by generating low ROS levels, in a spatially confined manner, which induces conformational changes in the target molecule, in turn impacting its interactions and downstream function (14). Signaling proteins containing active-site and structural cysteine residues are perhaps the most susceptible targets for redox modifications and include kinases, phosphatases, ion channels, and transcription factors (108). Oxidation targets that may be especially important for peripheral nerve function include redox switches Nrf2 and NFκB (69, 82), as well as sensory neuron ion transient receptor potential (TRP) channels (40).

The direct contributions of Nox enzymes to these processes in the PNS remain unclear and are not discussed in this review. Instead, we consider examples of cellular functions of Nox enzymes in neurite outgrowth and axon regeneration (Fig. 3).

Neurite outgrowth

Redox signaling is implicated in the regulation of neurite outgrowth, a highly coordinated process that allows exact pathfinding for developing and regenerating neurons in response to environmental cues (150). In this context, an early study showed that exposing cultured neurons to nerve growth factor (NGF) induces neurite outgrowth in an ROS-dependent manner (125). More recent data revealed that Rac1, the Nox cytosolic subunit, and increased Nox activity are both required for neurite outgrowth *in vitro* (78).

When evaluating the contribution of specific Nox isoforms to this process, Ibi *et al.* found that Nox1-dependent ROS suppress neurite outgrowth (58). However, studies in *Aplysia* bag cell neurons showed that Nox2 and its cytosolic subunit $p40^{phox}$ localize in growth cones, dynamic structures composed of an actin and microtubule cytoskeleton located at the tip of elongating neurites, which allows outgrowth and guidance to the proper target.

The authors suggest that Nox2/$p40^{phox}$ may modulate neurite outgrowth by oxidizing the actin cytoskeleton and changing its polymerization state (101). Findings in cultured hippocampal neurons further demonstrated that Nox2-derived H_2O_2 can also oxidize ryanodine receptors (RyR) localized in the ER, releasing Ca^{2+}. This Ca^{2+}-dependent process regulates the different aspects of neurite outgrowth, including axonal development and polarization (144). Together, these data suggest that physiological Nox levels are required for neurite outgrowth during development (Fig. 3A). Moreover, Nox isoforms (*e.g.*, Nox1 *vs.* Nox2) may have differential effects on neurite outgrowth, an idea that requires further research.

Tissue repair and axonal regeneration

As we mentioned above, neurons and their long axonal processes have high energy demands with considerable ATP consumption. Neuron maintenance and repair require efficient signaling over long distances, which can be achieved through bidirectional protein and organelle transport between cell body and axons (114).

Indeed, lesions to peripheral nerves trigger well-orchestrated cellular and molecular events, including an inflammatory response at the injury site to induce axonal

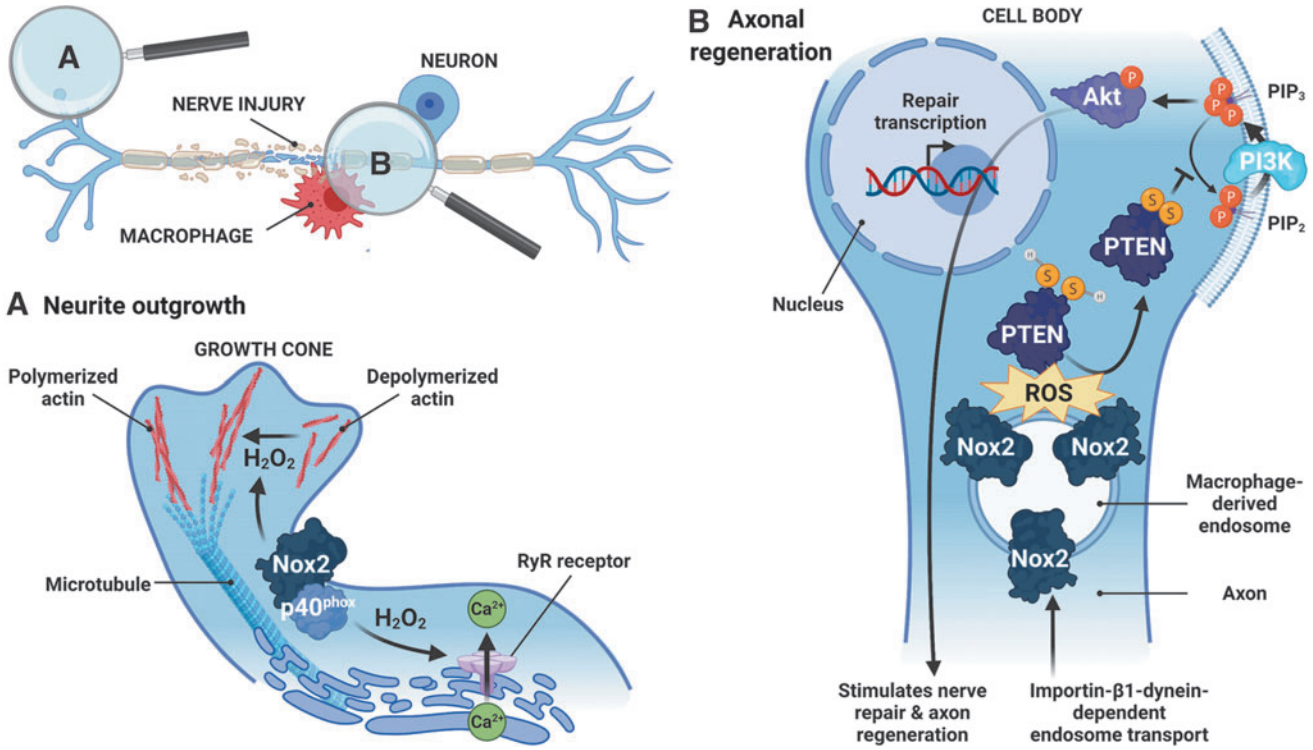

FIG. 3. NADPH oxidase signaling in the PNS under physiological conditions. Nox-derived ROS promote neurite outgrowth **(A)** and axon regeneration **(B)** under physiological conditions. **(A)** Nox2 and its cytosolic subunit $p40^{phox}$ localize in growth cones, dynamic structures composed of an actin and microtubule cytoskeleton located at the tip of elongating neurites, which allows outgrowth and guidance to the proper target. Nox2-derived H_2O_2 oxidizes the actin cytoskeleton, changing its polymerization state and favoring neurite outgrowth (101). Nox2-derived H_2O_2 can also oxidize RyR localized in the ER, releasing Ca^{2+}. This Ca^{2+}-dependent process regulates different aspects of neurite outgrowth, including axonal development and polarization (144). **(B)** Following peripheral nerve lesion, macrophages release exosomal Nox2, which is taken up by injured axons *via* endocytosis and retrogradely transported to the cell body in an importin-β1–dynein-dependent mechanism. Endosomal Nox2 oxidizes PTEN, triggering a disulfide bond (SS) formation, which inactivates PTEN. PTEN inactivation favors PIP_2 phosphorylation (P) to PIP_3 and PI3K-Akt pathway activation, promoting nerve repair and axon regeneration (50). PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homologue; RyR, ryanodine receptors. Created with BioRender.com.

regeneration (7). This inflammatory response favors a highly oxidizing environment, and accumulating evidence suggests that ROS are in turn essential for axonal regeneration and functional recovery after peripheral nerve injury (29, 50, 113). Earlier studies mainly examined axonal regeneration in *Drosophila* and zebrafish [reviewed in Terzi and Suter (129)], which consistently pointed to a key role of Duox-mediated ROS in axon reinnervation and wound healing (76, 104, 113).

More recently, studies in genetically modified mouse models revealed that macrophage-derived Nox2 promotes axon growth and regeneration in mouse dorsal root ganglion neurons (50). Hervera *et al.* found that exosomal Nox2 is taken up by injured axons *via* endocytosis and retrogradely transported to the cell body in endosomes in an importin-β1–dynein-dependent mechanism. Endosomal Nox2 oxidizes phosphatase and tensin homologue (PTEN), triggering a disulfide bond formation, which inactivates PTEN. PTEN inactivation favors phosphatidylinositol 4,5-bisphosphate (PIP_2) phosphorylation to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) and PI3K-Akt pathway activation, promoting nerve repair and axon regeneration (50).

The same team went on to show that *in vivo* activation of neuronal Nox2 promotes axonal regeneration and partial restoration of sensory nerve function after spinal cord injury (29).

Overall, these studies strongly support a role for Nox-dependent ROS as signaling molecules required for tissue regeneration after PNS injury (Fig. 3B). The idea of Nox shuttling from macrophages to damaged axons is a novel concept, which advocates a new way forward in our understanding of physiological redox signaling in the PNS (51). In addition, these results are particularly exciting in light of the growing interest in axo-glial metabolic communication (6) and require further investigation in *in vitro* and *in vivo* models of PNS disorders.

Nox Pathophysiological Involvement in PNS

Table 2 outlines the involvement of major Nox isoforms and their potential roles in PNS disorders detailed below.

Neuropathic pain

Neuropathic pain is a common and debilitating complication associated with a range of PNS disorders, including

Table 2. Nox Isoform Involvement in Peripheral Nervous System Disorders

PNS disorder	*Nox isoform(s) involved*	*Potential role(s)*	*References*
Neuropathic pain	(a) Nox1 (b) Nox2 (c) Nox4	(a) Macrophage infiltration; thermal and mechanical hyperalgesia (b) Central and peripheral immune regulation in pain sensitization after peripheral nerve injury (c) Peripheral pain processing, neuroinflammation, and dysmyelination	(28, 42, 59, 70, 72)
CIDP	Nox2	Unclear	(91)
CIPN	Nox4	Pain hypersensitivity; increased proinflammatory mediators	(96, 97)
DPN	(a) Nox2 (b) Nox4	(a) Pain processing and allodynia (b) Schwann cell injury; neurophysiological defects	(21, 35, 136)

CIDP, chronic inflammatory demyelinating polyneuropathy; CIPN, chemotherapy-induced peripheral neuropathy; DPN, diabetic peripheral neuropathy.

diabetic neuropathy, amputation, acute peripheral nerve injury, and chemotherapy-induced neuropathy (64). It presents as hypersensitivity with allodynia and hyperalgesia or continuous pain sensations (38). Current therapies only marginally provide relief due to the lack of understanding of pain processing (19).

Preclinical studies in animal models of neuropathic pain implicate oxidative stress as a prominent pathogenic factor in pain sensitization (73). Specifically, increasing evidence suggests that Nox enzymes contribute to pain processing (42, 70, 140), and the most studied isoforms are Nox1, Nox2, and Nox4 (Fig. 4). Nox2-dependent ROS are a key mediator of oxidative stress in pain sensitization after peripheral nerve injury (28, 70, 77, 86). Kallenborn-Gerhardt *et al.* also demonstrated that increased Nox2 signaling in infiltrating macrophages promotes dorsal root ganglion damage and neuropathic pain after peripheral nerve injury, an observation not present in Nox2-deficient mice (70). Besides its action on sensory neurons, Nox2 induction in infiltrating macrophages can also target Schwann cells, which in turn activate Nox1, as we discussed above (28).

In addition to its effect on peripheral immune cells, Nox2 can modulate neuropathic pain after peripheral nerve injury by its actions on microglia located in the dorsal horn of the spinal cord, a mechanism that requires Toll-like receptor 2 (TLR2)-dependent activation of the inflammatory response (77, 86). These findings suggest that Nox2-derived ROS in central and peripheral immune cells mediate, at least in part, neuropathic pain. This raises the possibility of inhibiting excess Nox2 activity as a meaningful therapeutic strategy. The caveat with this approach lies in the critical roles of Nox2-derived ROS in the innate immune response (133). In addition, we have discussed in a previous section the emerging role of Nox2 in axonal regeneration following PNS injury (50).

These results conflict with the findings that Nox2 mediates pain processing and suggest that the role of Nox2 may differ at different stages of peripheral nerve injury, which calls for caution in the use of Nox2 inhibitors as these may limit regeneration. Furthermore, genetic deletion of Nox2 functional subunits $p47^{phox-/-}$ and $gp91^{phox-/-}$ leads to arthritis, joint inflammation, and increased bone destruction (134). Thus, inhibiting Nox2 could be of limited therapeutic use for treating neuropathic pain due to its physiological roles in the immune response and axonal outgrowth (50).

Nox4 is also implicated in pain processing after peripheral nerve injury (42, 72, 140). Geis *et al.* found that Nox4 upregulation contributes to early neuropathic pain and increases proinflammatory cytokine release at the lesion site (42), worsening the pain sensation (121). While Nox4 genetic deletion prevented this acute neuropathic state, the authors found that pharmacological inhibition using the dual Nox1/4 inhibitor GKT136901 did not prevent pain in the later stages of neuropathy (42), suggesting that targeting Nox4 should occur early in the disease course.

Yet, another study showed that Nox4 upregulation in nociceptive primary afferent neurons maintains neuropathic

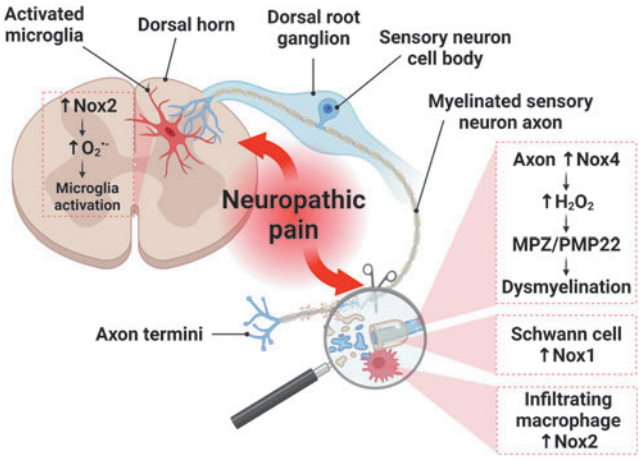

FIG. 4. Role of NADPH oxidases in pain processing. Nox2 induction in both central and peripheral immune cells contributes to neuropathic pain. In the dorsal horn of the spinal cord, Nox2 activation following peripheral nerve injury leads to $O_2^{-\bullet}$ generation, microglial activation, and pain hypersensitivity (77, 86). In addition, Nox2 upregulation in infiltrating macrophages promotes sensory neuron damage and neuropathic pain after peripheral nerve injury (70). Besides its action on sensory neurons, Nox2 induction in infiltrating macrophages can also target Schwann cells, which in turn activate Nox1 (28). This initiates a pro-oxidative feed-forward loop, leading to sustained macrophage infiltration to the damaged area, further exacerbating neuroinflammation and neuropathic pain (not shown in the figure). Nox4 is also involved in peripheral pain processing through H_2O_2 release in nociceptive primary afferent neurons, which is associated with neuropathic pain and dysmyelination, as evidenced by peripheral myelin protein MPZ and PMP22 degradation (72). Created with BioRender.com.

pain in both the subacute and late phases after peripheral nerve injury by mechanisms involving Schwann cell injury and dysmyelination, as evidenced by peripheral myelin protein MPZ and PMP22 degradation (72). This group went on to demonstrate, using mice with sensory neuron-specific Nox4 deletion, that the Ca^{2+}-binding protein S100A4 is an oxidation target of Nox4, which mediates hypersensitivity downstream of Nox4 (140).

These studies suggest that Nox4 signaling may vary during different stages of neuropathic pain. Future studies are needed to identify Nox4 signaling kinetics in neuropathic pain by direct comparisons across different clinically relevant animal models, on different genetic backgrounds, using pharmacological inhibitors and global and tissue-specific genetic manipulations (60) to identify potential therapeutic windows for pharmacological intervention.

In addition to the role of Nox1 in Schwann cells (28), Ibi *et al.* have demonstrated that Nox1-derived ROS mediate thermal and mechanical hyperalgesia in dorsal root ganglia using Nox1 knockout mice (59). Nox1-dependent pain processing is thought to involve the enhanced activity of the inflammatory sensor transient receptor potential vanilloid 1 (TRPV1) (59).

Chronic inflammatory demyelinating polyneuropathy

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an autoimmune disease of the PNS (85), which typically presents as a slowly progressive and symmetric neuropathy, with impaired sensorimotor function (85). While immune therapies are generally effective, at least half of CIDP patients require prolonged treatment to prevent disease relapse (45). Identifying targetable pathways in CIDP is therefore needed to develop more effective therapies and to improve disease outcomes.

Numerous pathogenic mechanisms are implicated in human and mouse CIDP, including neuroinflammation, Schwann cell dysfunction, and oxidative stress (74). Because of its known role in neuroinflammation (122), it is perhaps not surprising that Nox2 activity is increased in granulocytes and monocytes of CIDP patients (91). Interestingly, treatment with intravenous immunoglobulin increases this activity compared with pretreatment values. The significance of this increased Nox2-mediated ROS following treatment remains unclear. However, another study found that Nox2 may play a role in combating neuroinflammation in multiple sclerosis (100). While a similar mechanism may occur in CIDP, future studies are required to validate this hypothesis in clinically relevant mouse models and determine the precise mechanisms downstream of Nox2.

Chemotherapy-induced peripheral neuropathy

Chemotherapy-induced peripheral neuropathy (CIPN), a disabling consequence of cancer therapies (109), is predominantly a sensory and painful neuropathy (89). Currently, there are no effective CIPN treatments beyond symptomatic relief, and a better understanding of disease pathogenesis is essential for developing much needed mechanism-based neuroprotective therapies.

One of the established mechanisms by which chemotherapeutic agents induce cancer cell apoptosis is *via* ROS generation (148). Yet, ROS production is not only restricted to the tumor environment, but can diffuse to the neighboring healthy cells and induce damage as well (79). Importantly, sensory dorsal root ganglion neurons and their axons, which are the first to be affected during CIPN, have low antioxidant potential and high mitochondrial content, and, unlike the CNS, lack a protective vascular barrier, rendering them more susceptible to oxidative damage (38, 137). While increased ROS levels and lipid peroxidation are present in the peripheral nerves of multiple experimental CIPN models (31, 33, 149), much less is known about the exact source of ROS in CIPN.

While some studies report ROS overproduction secondary to mitochondrial dysfunction (75, 94), there remains little direct evidence on the primary ROS source in the context of disease. With respect to Noxs, increased NADPH oxidase activity in the spinal cord of a rat model of CIPN results in neurotoxic peroxynitrite accumulation and CIPN development (32, 62). Miao *et al.* reported a role for Nox4 signaling in the dorsal root ganglion and the dorsal horn of the spinal cord during painful CIPN (96, 97). These findings suggest that Nox4 may be a primary source of ROS in CIPN. However, the lack of studies assessing changes in other Nox subunits, both in the CNS and PNS in the presence or absence of specific inhibitors, limits conclusions about the therapeutic potential of Noxs in CIPN.

Diabetic peripheral neuropathy

Diabetic neuropathy is a common and debilitating complication affecting more than 50% of all diabetic patients. While diabetes-induced nerve damage can present in multiple forms, the most common form is diabetic peripheral neuropathy (DPN), a length-dependent and symmetric peripheral nerve degeneration (37). There are no disease-modifying therapies for DPN beyond glycemic control, which often fails to slow or reverse progression, especially in prediabetes and type 2 diabetes (T2D) (15). It is therefore critical to identify specific pathogenic factors contributing to DPN development to develop targeted mechanism-based therapies.

Experimental and clinical data, including our own, have shown that oxidative stress is a major component of cellular and molecular injury in DPN (4, 35, 56, 136, 137, 154). Mechanisms of injury downstream of hyperglycemia converge and lead to oxidative stress. Moreover, high-fat diet (HFD)-fed mice, which develop robust peripheral neuropathy closely resembling human disease, have increased nerve oxidative stress (136). These findings indicate that other metabolic stressors, such as dyslipidemia, an independent DPN risk factor, may also induce ROS and contribute to DPN at early disease stages before progressing to overt T2D. However, untargeted antioxidant therapy, including our own clinical trial of allopurinol, α-lipoic acid, and nicotinamide (110), has only exhibited limited therapeutic potential (154).

Among the sources of ROS in the PNS, we have previously shown that Nox activity is increased in dorsal root ganglion neurons following multiple metabolic stressors, including hyperglycemia and hyperlipidemia (136, 138), implicating Nox enzymes in DPN pathogenesis. What is more important, however, is the specific Nox isoform altered in DPN-relevant cell types, which may underlie oxidative damage in DPN (summarized in Fig. 5).

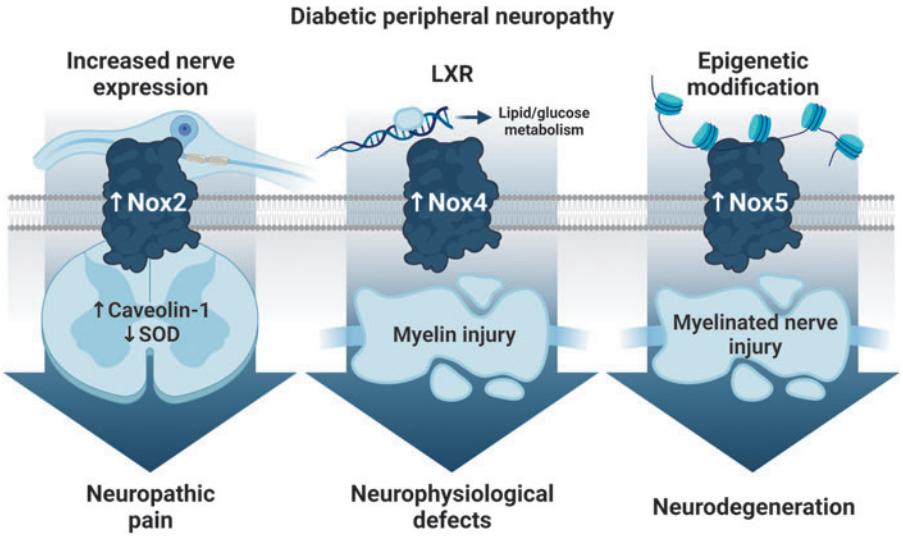

FIG. 5. Schematic summary of Nox-dependent mechanisms involved in diabetic peripheral neuropathy. Nox2, 4, and 5 are induced in peripheral nerves under diabetic conditions. Nox2 is activated in both dorsal root ganglion neurons and spinal cord. Increased spinal cord Nox2 is associated with reduced SOD activity and neuropathic pain (152). Nox2 is thought to mediate pain hypersensitivity by interacting with caveolin-1, a regulatory protein involved in lipid homeostasis (21). The LXR, a master regulator of lipid and glucose homeostasis, is inhibited in diabetes. This inhibition is accompanied by Nox4 induction in the sciatic nerves of type 1 diabetic mice and in cultured Schwann cells exposed to high glucose conditions. Nox4-derived ROS results in myelin injury and neurophysiological defects in hyperglycemia-induced peripheral neuropathy (35). The human Nox5 isoform is hypomethylated at the promoter region (47), which leads to increased Nox5 gene and protein expression in sural nerve biopsies of type 2 diabetic subjects with peripheral neuropathy (34). LXR, liver X receptor. Created with BioRender.com.

Studies show that Nox2 functional subunits $p47^{phox}$ and $gp91^{phox}$ are upregulated in the spinal cord of streptozotocin (STZ)-induced type 1 diabetic (T1D) rats, which develop neuropathic pain (152). These changes are associated with increased ROS generation and lipid peroxidation, and reduced SOD activity, ultimately resulting in tactile allodynia (152). Nox2-derived ROS also contributed to neuropathic pain in a T2D rat model rendered diabetic through an HFD and a single low STZ dose (21). Mechanistic analyses further showed that Nox2 induction in spinal cord microglia leads to chronic pain through a direct interaction with caveolin-1 (Cav-1), a regulatory protein involved in lipid homeostasis (21).

In line with these findings, we have previously shown that $p47^{phox}$ is increased in dorsal root ganglion neurons treated with oxidized low-density lipoproteins to mimic the dyslipidemic milieu in DPN (136). Taken together, these results provide supporting evidence for a role for Nox2 in the development of DPN and neuropathic pain, which may be conserved across diabetes type.

In addition to Nox2, we were the first to show that Nox4 is a major ROS source in peripheral nerves of STZ-induced T1D mice and that its pharmacological inhibition using GKT137831 prevents hyperglycemia-induced nerve dysfunction (35). Interestingly, we also showed that Nox4 mRNA levels were significantly increased in skin biopsies of T2D patients without clinical signs of DPN, an effect that was further enhanced in T2D patients with DPN (35). These clinical data are complemented by preclinical findings, which show increased Nox4-derived ROS in sciatic nerves from prediabetic HFD-fed mice even in the absence of hyperglycemia (unpublished data) and hyperglycemic T2D *db/db* mice (151).

Overall, these results support the hypothesis that Nox4-derived ROS are instrumental for human and murine DPN progression in prediabetes, type 1 diabetes, and T2D. Thus, in addition to hyperglycemia, additional studies exploring the link between Nox4 and different metabolic drivers of prediabetes, T2D, and DPN, such as dyslipidemia and insulin resistance, will be critical to validate Nox4 therapeutic efficacy in DPN.

Beyond Nox4, the human Nox5 isoform has emerged as a pathogenic factor in diabetic complications (30, 66). Particularly, data from humanized transgenic mice expressing Nox5 in different kidney cell populations have identified a role for Nox5 in promoting diabetic kidney disease, even in the absence of the Nox4 effect (66, 67). Consistent with these findings, our preliminary observations in sural nerve biopsies from T2D participants with DPN indicate that the human Nox5 promoter is hypomethylated (47), which promotes increased Nox5 gene and protein expression (34).

These data suggest that in addition to Nox2 and Nox4, Nox5-derived ROS may play a key role in human DPN development. We are currently examining the selective expression of Nox5 in Schwann cells and dorsal root ganglion neurons in transgenic mice to evaluate cell-specific Nox5 effects in the presence or absence of diabetes. These data will also allow us to evaluate the relative effects and the oxidation targets of Nox4 *versus* Nox5 in DPN.

Nox Inhibition As a Therapeutic Target for PNS Diseases

Given the emerging evidence implicating specific Nox isoforms as critical mediators of oxidative stress and nerve injury, Nox inhibition could be a promising therapeutic strategy to treat PNS diseases. Below we discuss these advantages.

Antioxidants

In the past two decades, untargeted antioxidant therapy aimed at neutralizing ROS overproduction was considered the only approach to reduce oxidative stress in the PNS. Although promising results were obtained using antioxidant therapy to treat PNS diseases in the experimental setting (18, 38), clinical trials, especially for DPN, were either of limited efficacy or were inconclusive (83, 110). This failure was mainly attributed to the lack of antioxidant specificity against the ROS source altered in a disease- and tissue-specific manner. In fact, it is thought that antioxidant supplementation could result in off-target effects or lead to global suppression of other ROS-generating enzymes or Nox isoforms, including those required for normal physiology (133).

Another limitation is related to the rapid oxidization by ROS, which leads to cellular damage even before initiation of antioxidant beneficial effects (103).

Nonspecific Nox inhibition

Diphenyleneiodonium (DPI) and apocynin are the two most commonly used nonspecific Nox inhibitors. Besides Nox inhibition, DPI inhibits other flavoproteins, such as xanthine oxidase and nitric oxide synthase (145). DPI as a therapy has been primarily studied in diabetic complications where it has cytoprotective effects in complication-prone tissues (61), including peripheral nerves (61, 68). However, its use *in vivo* is associated with insolubility and toxicity issues (133), rendering DPI a poor therapeutic option.

Apocynin may interfere with ROS detection by chemiluminescence and displays variable efficacy and potency (145). Unlike DPI, apocynin inhibition efficiency *in vitro* is low and very high concentrations are required for the antioxidant effect (52), which explains why it is more suitable and more commonly used *in vivo* (133, 145). Experimental evidence shows that apocynin alleviates neuropathic pain in the presence or absence of diabetes (48, 107). Apocynin treatment restored nerve conduction velocity and corrected blood flow and vascular conductance deficits in STZ-induced T1D rats (24). Although available safety data show low toxicity (123), to our knowledge, there are no studies addressing apocynin efficacy in patients with PNS disorders.

Specific Nox inhibition

A specific Nox inhibitor is essential to determine the therapeutic potential of targeting Noxs in PNS diseases. As opposed to global genetic deletion, administering a specific Nox inhibitor at a particular dose *in vivo* will be crucial for restoring Nox activity back to the homeostatic levels, rather than completely abolishing enzyme activity (134). Accordingly, recent high-throughput screening campaigns have effectively identified compound classes with enhanced selectivity against Nox enzymes, including the orally available, small-molecule Nox1/Nox4 allosteric inhibitors of the pyrazolopyridine chemical series: GKT136901 and its close analogue GKT137831. GKT compounds preferentially inhibit Nox1 and Nox4, and to a lesser extent Nox5 (1).

These dual Nox1/Nox4 inhibitors have gained considerable attention mainly because of their ability to prevent the development of diabetic complications, including DPN in the preclinical setting (30, 44). Specifically, we have shown that GKT137831 treatment improves nerve conduction velocity, sensorimotor deficits, and thermal sensitivity in neuropathic STZ-induced T1D mice, effects attributed to Nox4 inhibition (35). Experimental advances using GKT137831, particularly in the area of diabetic kidney disease, led to a randomized phase II trial in T2D participants with advanced diabetic kidney disease treated with a renin/angiotensin/aldosterone system inhibitor for 12 weeks (Genkyotex Innovation SAS; NCT02010242, GSN000200, completed).

The dual Nox1/Nox4 inhibitor had a favorable safety profile and improved several secondary outcome measures. Unfortunately, it did not effectively improve albuminuria, the primary outcome measure. Many reasons for drug failure were cited, such as inclusion of participants with very advanced kidney disease, short trial duration, low drug dose, as well as the heterogeneous nature of T2D-induced kidney disease relative to type 1 diabetes (133).

Accordingly, a new ongoing clinical trial addressing many of these concerns will shed light on the efficacy of GKT137831 in T1D patients with persistent albuminuria, using a longer treatment duration and a higher drug dose (27) (Genkyotex Innovation SAS; ACTRN12617001187336, UTN U1111-1187-2609). While DPN pathogenesis differs significantly between type 1 diabetes and T2D (15), experimental evidence suggests that Nox4 may be a viable therapeutic target for DPN conserved across diabetes type. It would therefore be interesting to evaluate the therapeutic efficacy of Nox4 inhibition using GKT137831 on DPN in type 1 diabetes, T2D, or preferably both.

In addition to DPN, the neuroprotective effects of the GKT compounds were tested in a rodent model of CIPN (97). GKT137831 improved mechanical and thermal sensitivity in CIPN rats, which was accompanied by reduced neuronal oxidative stress, proinflammatory cytokines, and increased Nrf2 signaling (97).

VAS2870 is a less specific pan-Nox inhibitor with a slight preference for Nox2 inhibition (5). While the therapeutic potential of VAS2870 is unknown for PNS dysfunction, reports show that VAS2870 treatment reduces neurodegeneration and improves neural function in a mouse model of acute ischemic stroke, an effect possibly mediated by Nox2 and/or Nox4 (80, 132). The therapeutic potential of VAS2870 is an area worthy of further investigation in PNS diseases, particularly in the context of neuropathic pain and CIDP, where Nox2 has emerged as a prominent pathogenic factor.

Novel Aspects of Nox Signaling: Potential Relevance to PNS Diseases?

Nox-derived ROS production in microparticles

Over the past decade, there has been a paradigm shift in PNS research, from focusing solely on neurons and their axonal extensions as an isolated system, to studying the interactions between axons and other nerve cell populations, namely Schwann cells and macrophages. Indeed, growing evidence points to the importance of Schwann cells and the Schwann cell/axon cross talk in axon viability and function (38, 135), such as through energy substrate transfer from Schwann cells to axons during periods of high energy demand and ROS scavenging by Schwann cells (6, 137).

Under conditions of metabolic dysfunction, however, studies show that Schwann cells can transfer lipotoxic species into the axon, promoting neurodegeneration (55, 135).

Interestingly, this shuttling activity can be mediated by Schwann cell-derived extracellular vesicles (EVs) under both normal and stress conditions (87, 142). EVs are a heterogenous group of membranous vesicles, released by all cells into body fluids or tissues (41). They mediate intercellular communication by transferring molecular cargo enriched with enzymes, nucleic acids, and metabolites to recipient cells (90). According to their biogenesis and size, EVs are commonly classified into three main types: exosomes (40–150 nm), originating from the endocytic pathway, microparticles (MPs; 100–1000 nm), derived from plasma membranes, and apoptotic bodies (50–5000 nm), released following apoptosis. EVs are implicated in neurodegeneration in disorders of the CNS [reviewed in Hill (54)].

In the PNS, most studies to date focus on the contribution of Schwann cell-derived exosomes to nerve regeneration after axonal injury and DPN through microRNA (miRNA), growth factor, and metabolite transfer (87, 142). Yet, the role of EVs in nerve redox signaling and the effect of oxidative stress on EV molecular cargo remain understudied.

Newly emerging ideas in the Nox field include the concept of intercellular ROS shuttling in MPs (98). The role of MPs has been generally studied in the context of vascular dysfunction, and increased MP levels positively correlate with adverse cardiovascular events and dyslipidemia (8, 106). Interestingly, increasing data implicate MPs in processes such as angiogenesis, vasorelaxation, inflammation, as well as oxidative stress (12, 95, 131), and further suggest that MPs may themselves be metabolically active and generate ROS (12).

Relevant to this review, multiple studies report that endothelium-derived MPs contain the Nox4 isoform as well as the regulatory subunit $p22^{phox}$ and produce Nox-dependent ROS (13, 63, 98). Secreted MPs can disrupt endothelial and vascular smooth muscle cell function in a paracrine/autocrine manner *via* a pro-oxidative feed-forward loop of injury, leading to apoptosis, inflammation, and impaired vascular tone (12, 13, 98).

Similar to the vasculature, it is tempting to speculate that disruption of the redox status between glial cells and axons, or the transfer of Nox-derived ROS from glia to axons *via* MPs, may contribute to nerve degeneration and PNS disorders (Fig. 6).

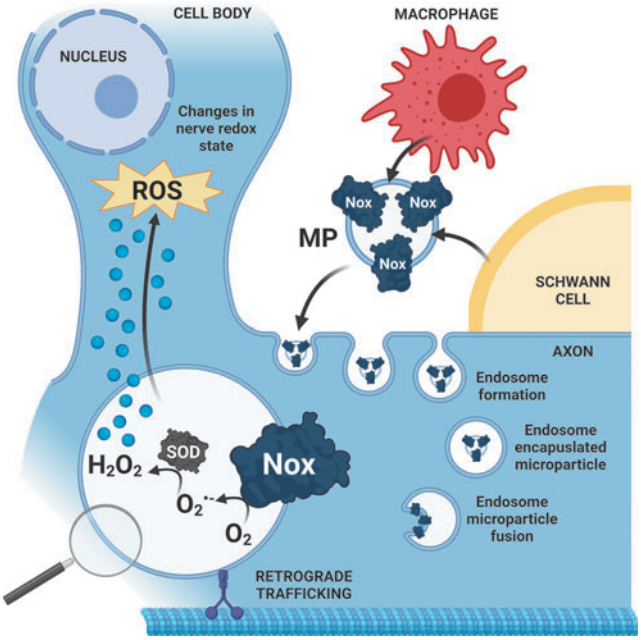

FIG. 6. Diagram summarizing our proposed mechanism of Nox-containing MPs in PNS diseases. We speculate that Nox-derived ROS are released from macrophages and/or Schwann cells *via* MPs, and engulfed by axons by endocytosis at the injury site. This ROS-producing MP transfer may disrupt the axo-glial redox state, contributing to nerve degeneration and PNS diseases. MP, microparticle. Created with BioRender.com.

Nox isoform interaction: is it biologically relevant?

As detailed above, PNS cell types simultaneously express several Nox isoforms, each exerting a distinct role (28). Emerging data suggest that these isoforms can interact and regulate ROS generation promoting sustained oxidative stress in disease states (98). Indeed, previous data reported the ability of Nox homologues such as Nox2 and Nox4 to dimerize (139). Furthermore, in cultured human endothelial cells with normal Nox2 and Nox4 expression, Nox5 knockdown is sufficient to abolish Nox-dependent ROS generation (99). In addition, this response is not accompanied by compensatory Nox2 or Nox4 increases, suggesting a potential regulatory effect of Nox5 on ROS generation.

More recently, Jha *et al.* reported similar results in transgenic models expressing human Nox5 in kidney mesangial cells, highlighting the ability of Nox5 to promote kidney disease progression, even in the absence of Nox4 upregulation (66). Perhaps more importantly, the same group showed that Nox5 may interact with Nox4 to regulate redox signaling in cultured renal cells exposed to metabolic stressors (65). While the precise mechanisms underlying these observations remain unclear, the findings do suggest, at least partly, an interdependent effect of Nox4 and Nox5 on the cellular oxidative response.

Thus, future studies are warranted to determine the relative Nox isoform expression and function in PNS cell types, their interactions, and their independent and interdependent effects on ROS levels. These findings will in turn be critical to understanding the cellular oxidative response and damage in PNS diseases.

Conclusion

In summary, oxidative stress research has evolved from the traditional view that ROS are exclusively harmful with focus on untargeted antioxidant therapies, to new advances centered on understanding the intricacies of redox signaling and the regulation of ROS sources in a cell- and disease-specific manner. The NADPH oxidase family, specialized for ROS generation, appears to be particularly important in the PNS for multiple cellular functions, ranging from neurite outgrowth and tissue repair to pathophysiological implications and neurodegeneration.

How specific Nox isoforms mediate peripheral nerve damage has fostered preclinical research examining the effect of Nox genetic and pharmacological manipulation on nerve function. While Nox inhibitors are currently being tested in the context of PNS diseases, these are not isoform

specific and may simultaneously inhibit several Nox isoforms. Thus, the development of isoform-specific Nox inhibitors with improved specificity is more promising for a better understanding of Nox biology in PNS health and disease.

The growing appreciation of the importance of axo-glial metabolic cross talk on nerve health is a recent area of interest among PNS researchers and may be of potential relevance to nerve redox status. Indeed, more research is needed in the area of Nox shuttling in MPs and how Nox isoforms interact to regulate nerve redox status and function in the axo-glial milieu. While much remains to be elucidated, increased experimental and clinical knowledge in the Nox field may facilitate the development of much needed mechanism-based therapies for the treatment of peripheral neuropathies.

Authors' Contributions

S.A.E. and E.L.F. contributed equally to the concept and design of the review. S.A.E. had final responsibility for the decision to submit for publication. S.A.E. completed the literature search, drafted, and, with E.L.F., finalized the review. M.G.S., A.A.E., and E.L.F. provided critical review of the article. M.G.S. created the figures, and, with S.A.E., conducted the literature search associated with the figures. All authors have reviewed and approved the article before submission. The review has been submitted solely to *Antioxidant & Redox Signaling* and is not published, in press, or submitted elsewhere.

Author Disclosure Statement

All authors declare no competing interests.

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Abbreviations Used

ATP = adenosine triphosphate
Ca^{2+} = calcium
CAT = catalase
CIDP = chronic inflammatory demyelinating polyneuropathy
CIPN = chemotherapy-induced peripheral neuropathy
CNS = central nervous system
COX = cyclooxygenases
CYP = cytochrome P450 monooxygenases
DPI = diphenyleneiodonium
DPN = diabetic peripheral neuropathy
Duox = dual oxidases
ER = endoplasmic reticulum
ETC = electron transport chain
EV = extracellular vesicle
FAD = flavin adenine dinucleotide
Fe = iron
H_2O = water
H_2O_2 = hydrogen peroxide
HFD = high-fat diet

Abbreviations Used (Cont.)

LOX = lipoxygenases
LXR = liver X receptor
MP = microparticle
Nox = NADPH oxidase
Nrf2 = NF-E2-related factor 2
O_2 = molecular oxygen
$O_2^{-\bullet}$ = superoxide anion
$^{\bullet}OH$ = hydroxyl radical
PIP_2 = phosphatidylinositol 4,5-bisphosphate
PIP_3 = phosphatidylinositol (3,4,5)-trisphosphate
PNS = peripheral nervous system
PTEN = phosphatase and tensin homologue
ROS = reactive oxygen species
RyR = ryanodine receptors
SOD = superoxide dismutase
STZ = streptozotocin
T1D = type 1 diabetic
T2D = type 2 diabetes
XO = xanthine oxidase