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Forum Review

NADPH Oxidases of the Brain: Distribution, Regulation, and Function

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ABSTRACT

The NADPH oxidase is a multi-subunit enzyme that catalyzes the reduction of molecular oxygen to form superoxide ($O_2^{\bullet-}$). While classically linked to the respiratory burst in neutrophils, recent evidence now shows that $O_2^{\bullet-}$ (and associated reactive oxygen species, ROS) generated by NADPH oxidase in nonphagocytic cells serves myriad functions in health and disease. An entire new family of NADPH Oxidase (Nox) homologues has emerged, which vary widely in cell and tissue distribution, as well as in function and regulation. A major concept in redox signaling is that while NADPH oxidase-derived ROS are necessary for normal cellular function, excessive oxidative stress can contribute to pathological disease. This certainly is true in the central nervous system (CNS), where normal NADPH oxidase function appears to be required for processes such as neuronal signaling, memory, and central cardiovascular homeostasis, but overproduction of ROS contributes to neurotoxicity, neurodegeneration, and cardiovascular diseases. Despite implications of NADPH oxidase in normal and pathological CNS processes, still relatively little is known about the mechanisms involved. This paper summarizes the evidence for NADPH oxidase distribution, regulation, and function in the CNS, emphasizing the diversity of Nox isoforms and their new and emerging role in neuro-cardiovascular function. In addition, perspectives for future research and novel therapeutic targets are offered. *Antioxid. Redox Signal.* 8, 1583–1596.

INTRODUCTION

THE NADPH OXIDASE is a multi-subunit enzyme that catalyzes the reduction of molecular oxygen and oxidation of NADPH to generate superoxide radicals ($O_2^{\bullet-}$) (5, 53). Originally discovered in polymorphonuclear neutrophils over 4 decades ago, this protein was first shown to provide host defense against bacteria via a rapid respiratory burst of $O_2^{\bullet-}$. The subunits localize in both membrane-bound (cytochrome b_{558}, comprised of $p22^{phox}$ and $gp91^{phox}$) and cytoplasmic ($p40^{phox}$, $p47^{phox}$, and $p67^{phox}$) locations (5, 53). Upon stimulation, activation of a low-molecular weight G protein (Rac1 or Rac2) and phosphorylation of $p47^{phox}$ initiates migration of the cytosolic elements to the plasma membrane, whereby a functional complex forms that generates $O_2^{\bullet-}$ (5, 38, 53).

Over the last decade, however, the classical paradigm of NADPH oxidase solely as a means to an immunological end has become outdated. As discussed elsewhere in this review series, a vast number of studies have redefined the NADPH oxidase in a wide array of cells and tissues in the context of a diverse set of functions—from mediating normal intracellular signaling to regulating cell growth and death, from modulating inflammatory responses to regulating endothelial function (9, 36, 81). This began with the seminal discovery by Griendling, Lambeth, and colleagues of a $gp91^{phox}$ homologue in a nonphagocytic cell type named NADPH oxidase 1 (Nox1). Their discovery of Nox1 in vascular smooth muscle cells, along with its critical role in cell growth regulation (93), rapidly spurred an enormous amount of research on this enzyme complex. We now know there is an entire family of

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Nox proteins: Nox1, gp91^{phox} (Nox2), Nox3, Nox4, Nox5, and Dual Oxidase 1 and 2 (Duox1 and Duox2) (9, 36, 53). In conjunction with p22^{phox}, the Nox homologues are considered to be the catalytic component from which NADPH oxidase derives its functional capacity, since this subunit facilitates electron transfer by interacting with heme, NADPH, and flavin adenine dinucleotide elements (53). Whereas close structural and functional similarities exist between the different homologues, each is diversely expressed and regulated across distinct tissue and cell types (6, 9, 36, 53, 54, 62). Importantly, multiple Nox isoforms can coexist within an individual cell and can serve distinct cellular functions (36, 54), thus increasing the complexity of regulation of the oxidative environment. Additional complexity arises due to the fact that $O_2^{\bullet -}$ can be further reduced to produce additional reactive oxygen species (ROS, such as hydrogen peroxide [H_2O_2] and hydroxyl radicals [OH^-]). While oxidative levels are normally kept in check by endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, these enzymes (*e.g.,* SOD) can generate new ROS which can also participate in intracellular redox signaling.

The focus of this review will be on the expression, regulation, and function of NADPH oxidase and redox mechanisms in the brain. Given the enormous diversity of central nervous system (CNS) function—from coordinating body movement to interpreting senses, controlling behavior, establishing memory, and regulating cardiovascular homeostasis—understanding a complex enzyme such as NADPH oxidase in the CNS poses a considerable challenge. However, exciting new evidence demonstrating the importance of Nox enzymes in normal neuronal function and in CNS diseases has recently begun to emerge. For example, it is becoming increasingly clear that many of the neurodegenerative diseases of the CNS arise from a common defect, namely excessive free radical levels in particular brain regions. So-called oxidative stress—due to augmented production of ROS and/or decreased antioxidant capacity of cells to detoxify these molecules—is involved in Parkinson's and Alzheimer's diseases (11, 43, 85). This has prompted investigations into the basis of ROS production, and the NADPH oxidase complex has emerged as an important potential source of ROS generation in the neuronal cell death that characterizes these diseases (98, 113). Another important CNS function where oxidant mechanisms and NADPH oxidase are now known to be critical is the central neural regulation of the cardiovascular system. For example, angiotensin II (Ang-II), a well-known peptide that regulates central sympathetic outflow, vasopressin release, and fluid homeostasis, ultimately impacting blood pressure, cardiac function, and renal physiology, has been shown to do so in part via ROS/NADPH oxidase mechanisms (59, 119, 120). Furthermore, dysregulation of these central redox pathways, including the NADPH subunits, is now strongly implicated in hypertension (121) and heart failure (30, 56).

The tremendous potential importance of NADPH oxidase in the CNS, coupled with a keen appreciation of the complexity of this enzyme family, provides the impetus for this review. Here we will summarize our current understanding of this complex in the brain, highlighting the expression patterns and differential functional roles of the homologues and subunits. We will be relatively brief in discussing NADPH oxidase in the context of memory, neurodegenerative pathologies, and ischemic cerebral vessel injury since these topics have been reviewed elsewhere (67, 74, 99, 113). Instead, we will focus more on the recent and growing body of evidence suggesting a pivotal role for the NADPH oxidase as a mediator of central neuro-cardiovascular function and disease.

NADPH OXIDASE DISTRIBUTION AND FUNCTION IN NONCARDIOVASCULAR REGIONS OF THE CNS

Despite increasing evidence for redox signaling and NADPH oxidase in normal CNS processes, as well as a number of brain diseases (85, 113), still relatively little is known about the expression, regulation, and function of this enzyme complex in cerebral cells or tissues. As in the periphery, the dearth of easily available specific antibodies, especially for the Nox homologues, and a lack of tissue and/or cell type-specific knockout animal models or other gene silencing strategies have slowed progress. Nonetheless, new advances have been made and considerable evidence has accumulated that definitively establish an important role for NADPH oxidase in the CNS. We begin here with a summary of what is known about its distribution in the brain.

Spatial localization of NADPH oxidase subunits

One of the first demonstrations of NADPH oxidase in neural cells was a study by Tammariello *et al*. (98). In this study they showed that all the classical subunits of the Nox2 enzyme were expressed in rat isolated sympathetic neuron cultures, which were necessary to generate $O_2^{\bullet -}$ and stimulate programmed cell death in the absence of nerve growth factor. Since then, immunohistochemical approaches in rat (46) and mouse (88) brain samples have been instrumental in determining localization of NADPH oxidase subunits in different regions of the CNS. Serrano *et al*., using polyclonal antibodies to p40^{phox}, p47^{phox}, and p67^{phox}, p22^{phox}, and Nox2, demonstrated widespread expression of each of these five proteins separately in serial microsections of mouse fore-, mid-, and hindbrain (88). The staining was most prominent in neurons of the hippocampus, cortex, amygdala, striatum, and thalamus. Although immunostaining for the cytoplasmic subunits was diffuse throughout the cell, membrane-associated p22^{phox} and Nox2 exhibited punctate staining, suggestive of a traditional subcellular localization of these subunits. In rat, there was also strong neuronal immunoreactivity for p47^{phox} and Nox2 in regions of hippocampus and cerebral cortex; however, these authors observed little or no staining for these subunits in thalamus, brainstem, or amygdala (46). Likewise, rat cerebellar and hypothalamic neurons also showed NADPH oxidase immunoreactivity, whereas corresponding mouse tissues did not (46, 88). Some of the differences seen between these two immunohistochemical studies are likely due to different antibodies and staining methods used.

Interestingly, neither of these studies detected NADPH oxidase subunits in microglia, which function as resident immune cells of the CNS (24). This finding is surprising, since microglia have been shown to generate NADPH oxidase-derived

$O_2^{\bullet-}$ in response to phorbol ester stimulation (60, 84). In addition, mRNA transcripts of all the classical phagocytic subunits have been detected in isolated fetal human microglia, and $p22^{phox}$, $p47^{phox}$, and Nox2 proteins have been detected by immunostaining in perivascular cells in rats (34). Furthermore, microglia from $p47^{phox}$-mutant mice are unable to initiate an oxidative response to phorbol ester; however, this response can be restored with retroviral transduction of the p47phox gene (55). Taken together, these results strongly suggest NADPH oxidase-dependent $O_2^{\bullet-}$ production in microglia. As described in more detail below, microglial Nox2 also appears to play a prominent role in Parkinson's disease pathology.

In addition to Nox2, recent studies have detected other Nox isoforms in cerebral tissue as well. Cheng *et al*. (16) have detected low levels of Nox4 and Nox5 expression in adult human brain tissue. Interestingly, fetal human brain tissue shows robust Nox4 and Nox5 expression as well as low levels of Nox1, suggesting a role for these homologues in early development. In support of this, Nox4 expression has also been detected in cortical neurons and newly-formed cerebral capillaries after ischemia (103). In addition, a recent study using degenerate PCR primers detected Nox1, Nox3, and Duox1 expression at low levels in rat brain tissue (63). Given the current lack of cellular resolution, it is difficult to speculate on the function of these Nox isoforms in specific neuronal circuits or locations.

NADPH oxidase in hippocampal-dependent memory

The pronounced immunostaining of NADPH oxidase subunits in regions of mouse and rat hippocampus described above suggests that the intracellular redox environment may be important for normal cellular processes of these neurons, such as long-term potentiation (LTP) and hippocampus-dependent memory. Indeed, $O_2^{\bullet-}$ has been strongly implicated in LTP in mice, since transgenic models overexpressing extracellular SOD (EC-SOD) show impaired memory function which can be reversed through pharmacological inhibitors to the enzyme (100). Furthermore, neuron–glia signaling in the hippocampus has been shown to generate ROS, which can impact post-translational modifications in neighboring cells and enhance LTP (3). A role for NADPH oxidase in LTP and memory is also supported by the recent detection of enzyme subunits p22phox, Nox2, p47phox, and p67phox at synaptic sites in hippocampal neurons, which were further shown to assemble and generate $O_2^{\bullet-}$ when stimulated with phorbol ester (99).

It is important to note that while intracellular redox signaling—in part due to NADPH oxidase function—appears necessary for normal LTP and memory functions of hippocampal neurons, excessive oxidative stress can also impair cellular processes and even lead to neuronal death (Fig. 1). For example, hydrogen peroxide, a product of $O_2^{\bullet-}$ dismutation, has been shown to inhibit LTP in rat hippocampal slices (4). Furthermore, excessive $O_2^{\bullet-}$ generation imparted by NADPH oxidase activation contributes to hippocampal neuronal death in chemically-induced seizures in rats (75). Interestingly, this chemically-induced neuronal death was exacerbated in EC-SOD knockout mice, while transgenic animals overexpressing EC-SOD were protected (75). Taken together, these findings suggest that normal neuronal functions of the hippocampus—LTP and memory—rely on a carefully modulated redox environment and can be impaired by both lack of as well as overproduction of ROS (Fig. 1).

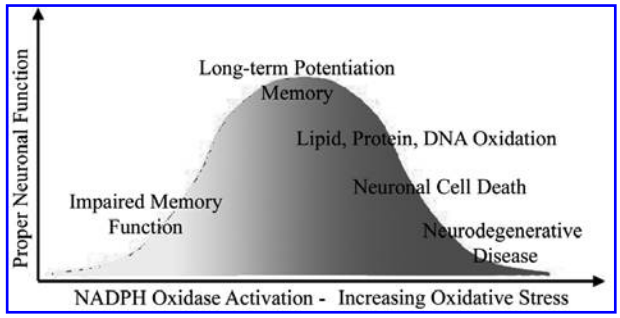

FIG. 1. Normal CNS function depends on a balanced oxidant environment. Hippocampal processes, such as long-term potentiation and memory, are inhibited in mice in which basal ROS levels are reduced by scavengers. On the other hand, excessive production of oxidant radicals is linked to improper neuronal signaling in this and other brain regions, and in some instances can lead to neuronal cell death. Neurodegenerative diseases such as Alzheimer's and Parkinson's are characterized by increased oxidative stress, lipid, protein, and DNA oxidation, and neuronal cell death in brain regions associated with these diseases. More recently, NADPH oxidase upregulation and activation in both neurons and microglia has emerged as an important mechanism of oxidative stress in CNS.

NADPH oxidase in neurodegenerative diseases

The importance of uncontrolled ROS production in mediating cellular pathology, described in Figure 1, also serves as a good paradigm for neurodegenerative diseases. For example, chronic oxidative stress has been implicated in neuronal cell death, protein aggregations, and the pathogenesis of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (33, 50, 72). Despite the established link between chronic ROS overproduction and pathological neurodegeneration, the source of ROS and the cell type involved remains unknown. While mitochondrial dysfunction can lead to increased oxidative stress in neurons and glia (85), a growing body of evidence supports a role for abnormal NADPH oxidase activation in these pro-oxidative conditions. For example, post-mortem brain tissue from Alzheimer's disease patients exhibits an increased accumulation of cytosolic NADPH oxidase subunits $p47^{phox}$ and $p67^{phox}$ at the cell surface of diseased brain regions, suggesting chronic activation of this complex (89). Moreover, addition of β-amyloid fragments derived from Alzheimer's patients to cultured primary rat microglial cells has been shown to elicit $O_2^{\bullet-}$ generation via an NADPH oxidase-dependent mechanism (7). Increased $O_2^{\bullet-}$ generation and subsequent H_2O_2 formation resulting from β-amyloid-stimulated NADPH oxidase activates neutral sphingomyelinase in neuronal cells, which may promote neuronal apoptosis in the diseased areas (42). Although the mechanisms of persistent NADPH oxidase activation are not completely understood, it has been suggested that blood-derived factors often observed in Alzheimer's brains (8), presumably from a compromised blood–brain barrier, may be involved in NADPH oxidase activation and neuronal apoptosis. In support of this, Choi *et al*. (17) have

shown that cerebral injection of thrombin, one such blood-derived factor, elicits Nox2, $p47^{phox}$, and $p67^{phox}$ upregulation in rat microglia, as well as NADPH oxidase assembly at the cell surface. Importantly, they showed that thrombin-induced neuronal apoptosis could be prevented by NADPH oxidase inhibitors, further implicating this complex in the neurodegenerative process. As summarized in Fig. 1, these studies clearly link NADPH oxidase-derived ROS with protein oxidation, aggregation, and neuronal death in Alzheimer's disease; however the specific molecules, cell types, and mechanisms remain to be elucidated.

Similarly, new evidence suggests abnormal NADPH oxidase function in the pathogenesis of Parkinson's disease, although the underlying mechanisms are incompletely understood. This syndrome, characterized by degeneration of dopaminergic neurons of the zona compacta of substantia nigra (73), exhibits high levels of oxidative damage to the neuronal milieu of this region. Using dopaminergic-specific neurotoxins to model the disease process, a critical role for oxidative stress imparted by microglial NADPH oxidase has been elucidated. Gao *et al*. (28) isolated primary mesencephalic neurons and associated microglia from Nox2 knock-out mice to test the hypothesis that this Nox homologue is an important source of $O_2^{\bullet-}$ generation during rotenone-induced dopaminergic neuron degeneration. In this study, normal cultured dopaminergic neurons co-cultured with microglia from the knock-out mice were more resistant to rotenone-induced neurotoxicity, as compared to wild-type cultures. On the other hand, purified dopaminergic neuronal cultures from the knock-out mice were more susceptible to rotenone-induced cell death when mixed with Nox2-containing microglia, further underscoring the potential importance of Nox2 activation in these cells in mediating the effects of the neurotoxin. Nox2 knock-out models have also been used to establish NADPH oxidase dysfunction in other models of Parkinson's disease, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced degeneration of dopamine-specific neurons (29). More recently, the neuroprotective effects of dextromethorphan in an *in vivo* MPTP model of Parkinson's disease have been linked to microglial NADPH oxidase inhibition (116). Collectively, these findings provide convincing evidence for microglial NADPH oxidase-derived oxidative stress in Parkinson's disease (Fig. 1).

NADPH oxidase in cerebral ischemic injury

Another CNS disorder in which oxidative stress is well established as a key player is cerebral ischemia (51, 74). Walder *et al*. (104) were the first to show a pivotal role for NADPH oxidase-derived $O_2^{\bullet-}$ generation in ischemic stroke injury. Using Nox2 knockout mice, they demonstrated that brain injury in response to middle cerebral artery occlusion (MCAO) was markedly attenuated in mice lacking functional NADPH oxidase. Similarly, several recent reports have shown protection against hypoxia/reperfusion-induced brain infarctions in Nox2 knockout mice (41, 114). In further support of this, Kusaka *et al*. (52) reported that focal cerebral ischemia induced by MCAO/reperfusion was accompanied by an increase in the Nox2 and $p22^{phox}$ mRNA levels in the ipsilateral hemisphere, and this increase was further augmented in diabetic rats. Using the Ang-II AT-1 receptor inhibitor candesartan, their results further demonstrated that ischemia/reperfusion-induced cerebral infarction and NADPH oxidase induction was mediated by Ang-II-AT-1 receptor-dependent pathways.

In addition to the crucial role of Nox2, recent efforts also suggest involvement of Nox4-containing NADPH oxidase in experimental brain ischemia. For example, Vallet *et al*. (103) have shown Nox4 expression in selective populations of neurons located in brain areas susceptible to ischemic damage. Further, these investigators demonstrated that Nox4 expression is increased in infarcted brain tissue five-fold 1–2 weeks after ischemia/reperfusion injury. In addition, Nox4 expression is observed in rat basilar and middle cerebral arteries (68, 74), as well as endothelial cells isolated from rat basilar artery (1). Interestingly, expression of Nox4 and ROS production is 10–100 times more in cerebral arteries as compared to peripheral vessels (68), and it has been suggested that Nox4-derived ROS may play a critical role in the regulation of cerebral vascular tone under physiological conditions. In addition, using immunohistochemical localization of Nox4, Vallet *et al*. (103) have shown that its expression is markedly increased in newly-formed capillaries in the peri-infarct zone of ischemia-injured brain tissue, and have suggested that Nox4 oxidase-derived ROS may promote vasculogenesis in the ischemic zone.

These findings are particularly interesting in light of a recent study by Martyn *et al.* (62). These authors have shown that transfection of Nox4 into HEK293 cells results in constitutive production of H_2O_2 rather than $O_2^{\bullet-}$, and that Nox4, in conjunction with p22phox, does not require cytosolic proteins p47phox or p67phox for activation. One possibility is that constitutive activation of Nox4-containing NADPH oxidase could account for the markedly higher concentrations of ROS observed in cerebral arteries compared to peripheral vessels (68). Interestingly, H_2O_2 has been shown to function as a vasodilator in rat cerebral arteries via opening of calcium channels in vascular smooth muscle cells (74), and pretreatment with catalase has been shown to abolish the vasodilating effect of NADPH oxidase activation (74). It is intriguing to speculate if NADPH oxidase-mediated vasodilatation in cerebral vessels may in part occur via constitutive production of H_2O_2 from a Nox4-containing complex.

Taken together, these studies implicate both Nox2 and Nox4-containing NADPH oxidases in the pathophysiology of ischemia/reperfusion-induced neuronal injury and subsequent vessel replacement. However, it remains to be seen whether specific neuronal and vessel populations differentially express Nox2 and Nox4, and whether these enzymes are expressed and activated in microglia during ischemic stroke. Similarly, defining the relative contribution of neuronal versus vascular Nox2 and Nox4 in the pathophysiology of stroke will require further studies, since these enzymes are implicated in both normal cerebral vascular physiology (68), and pathology of neurovascular diseases (44, 110).

THE CNS AS A POTENT REGULATOR OF CARDIOVASCULAR FUNCTION

The CNS exerts a profound influence on the cardiovascular system to maintain control of blood pressure, volume balance, and cardiac function. It receives, processes, and inte-

grates a host of neural and humoral signals that reflect the cardiovascular status of the organism, and orchestrates appropriate effector systems (*e.g.,* autonomic nerves and neurohormones) to maintain homeostasis. In fact, most cardiovascular diseases involve some type of dysfunction in CNS mechanisms. For example, many humans with chronic hypertension exhibit exaggerated sympathetic nervous system activity (26). Likewise, chronic heart failure is characterized by augmented sympathetic nerve function, baroreflex function, and abnormal hormone mechanisms, and the most effective treatments to improve survival in patients target pathological neurohumoral excitation (27). Emerging evidence also suggests overactivity of the CNS in metabolic disorders known to be major risk factors for cardiovascular disease, including diabetes and obesity (79). In spite of the critical role of the CNS in controlling cardiovascular balance and mediating cardiovascular pathology, surprisingly little is known about the molecular substrates of the central neural pathways involved.

A host of central and peripheral factors contribute to the pathogenic function of CNS mechanisms in cardiovascular disease. Of these, Ang-II has emerged as a key player (20). It has long been known that injection of Ang-II directly in specific brain regions inside the blood–brain barrier (BBB) elicits profound blood pressure, heart rate, and dipsogenic responses, suggesting the evidence of Ang-II processing systems in the CNS (61). Since then, all of the substrates and enzymes necessary for Ang-II production and responses (*e.g.,* Ang-II receptors, AT-1 and AT-2) have been identified in the brain (83), and it is well established that Ang-II functions as a neurotransmitter/neuromodulator in cardiovascular regulatory circuits (20). Evidence that overactivation of brain Ang-II signaling leads to hypertension and related diseases further underscores the importance of this peptidergic system in neurocardiovascular regulation (20).

In addition to its role inside the CNS, Ang-II is also a major factor in the systemic circulation that signals blood pressure and fluid status to the CNS. However, since the CNS is largely isolated from blood-borne peptides such as Ang-II because it is too large to cross the BBB, communication between the circulation and the CNS takes place at specialized brain regions lacking such a barrier, termed circumventricular organs (CVOs) (20). These CVOs, including the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) in the forebrain, contain high concentrations of AT-1 receptors that bind circulating Ang-II (20). This stimulates downstream circuits in the brain, ultimately leading to CNS effector pathway activation and neurocardiovascular responses. For example, blood-borne Ang-II acting at the SFO subsequently leads to stimulation of the paraventricluar nucleus (PVN) (66). The PVN, in turn, sends excitatory projections to the sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM), ultimately causing increased sympathetic outflow (45). Activation of the PVN is also involved in the synthesis and release of vasopressin. Additionally, high concentrations of AT-1 receptors have been detected in the hindbrain CVO region area postrema (AP) (22). Ang-II binds these neurons, which send projections to neurons of the nucleus tractus solitarius (NTS) and RVLM, also rich in AT-1 receptors (95), which in turn synapse upon preganglionic neurons of the spinal cord (39). Collectively, these and other neuronal circuits relay signals derived from the circulation and other peripheral sites and couple them with networks involved in maintaining cardiovascular homeostasis.

NADPH OXIDASE AND Ang-II SIGNALING IN THE CNS

Abundant research now suggests that a key mechanism through which Ang-II influences blood pressure and other cardiovascular endpoints is via its ability to activate ROS signaling pathways. It has been over a decade now since Harrison, Griendling, and colleagues first discovered that Ang-II activates an NADPH oxidase in vascular smooth muscle cells (37, 80). This launched a new field from which a critical concept in cardiovascular physiology emerged: that ROS are pivotal primary effectors in a variety of Ang-II-mediated processes in cardiovascular cells, and that dysregulation of redox mechanisms are strongly implicated in cardiovascular diseases such as hypertension, atherosclerosis, and heart failure (9, 36, 81). More recently, accumulating evidence from our laboratory and others suggests that, like peripheral cardiovascular cells, neurocardiovascular cells of the CNS also require ROS to carry out crucial functions related to central control of blood pressure and other cardiovascular parameters (118).

In 2002, we made the observation that $O_2^{\bullet -}$ was necessary to elicit the vasopressor, bradycardiac, and dipsogenic responses produced by intracerebroventricular (ICV) administration of Ang-II in conscious mice (120). Using recombinant viral vectors to target human SOD transgenes selectively to forebrain CVOs in a highly robust and stable manner (91), our studies showed that scavenging $O_2^{\bullet -}$ in this brain region completely abolished the cardiovascular and dipsogenic actions of Ang-II. Importantly, these studies also demonstrated that the requirement for $O_2^{\bullet -}$ was specific to Ang-II since the pressor response to another central pressor agent, the muscarinic agonist carbachol, was completely intact in SOD-treated mice (120). Finally, we also provided direct evidence in these studies that Ang-II causes robust increases in $O_2^{\bullet -}$ production in cultured CNS neurons from this forebrain CVO area.

While it is known that the increase in blood pressure caused by central Ang-II involves sympathoexcitation, our studies did not address this directly. Recent work by several groups has provided important evidence linking central Ang-II, ROS, and sympathoexcitation. CNS administration of the SOD mimetic Tempol or PEG-encapsulated SOD attenuated ICV Ang-II-induced increases in renal sympathetic nerve activity (RSNA), norepinephrine secretion, and blood pressure (12, 13, 59). Similarly, bilateral microinjection of Tempol directly into RVLM has been shown to inhibit Ang-II-induced increases in blood pressure, tachycardia, and renal sympathoexcitation, suggesting a role for ROS in excitatory responses of Ang-II in RVLM (65). These recent findings on the involvement of ROS in sympathetic activity are interesting in light of studies from 5 years ago by Zanzinger that showed direct administration of SOD into RVLM inhibits RSNA in pigs chronically subjected to oxidative stress by long-term treatment with organic nitrates (112).

While ROS generation has proven necessary to mediate the *in vivo* effects of Ang-II signaling in CNS cardiovascular circuits, only recently has the source of these free radicals been

investigated. Using adenovirus-mediated gene transfer of a dominant-negative mutant of Rac1 (AdN17Rac1), a cofactor essential for NADPH oxidase assembly and activation (35), we have recently demonstrated a requirement for NADPH oxidase in Ang-II-stimulated ROS generation in primary neurons derived from the forebrain lamina terminalis, a region that encompasses the CVOs (119). More importantly, inhibition of NADPH oxidase assembly by AdN17Rac1 resulted in complete loss of pressor, bradycardiac, and dipsogenic responses to Ang-II, further underscoring the importance of ROS–and the NADPH oxidase complex–in this signaling cascade.

Although the studies described above implicate NADPH oxidase-derived $O_2^{\bullet-}$ in Ang-II-induced signaling in cardiovascular nuclei in the CNS, the downstream mechanisms of ROS-mediated neuronal activation are incompletely understood. Recent evidence suggests that ROS may enhance neuronal activation by increases in intracellular calcium. For example, Wang *et al*. (105) utilized a double-label immunoelectron microscopy approach to demonstrate co-localization of the Nox2 subunit of NADPH oxidase with AT-1 receptors in dorsomedial neurons of the NTS. Further, these investigators demonstrated that inhibition of this complex via the pharmacological inhibitor of NADPH oxidase apocynin or an inhibitory Nox2 peptide docking sequence (gp91-ds-tat) (82) decreased Ang-II-stimulated increases in cytosolic calcium and neuronal potentiation. Similarly, we have shown that treatment with Ang-II produces a rapid influx of calcium into neuronal cells, and that this response was abolished by overexpression of the dominant-negative AdN17Rac1 or AdCuZnSOD, thereby implicating an active NADPH oxidase-derived $O_2^{\bullet-}$ in this signaling pathway (122). These findings were later confirmed by Sun *et al*. through the use of pharmacological and peptide inhibitors of NADPH oxidase (94). Importantly, these investigators also provided evidence for a critical link between Nox2 and Ang-II in neuronal firing, and further implicated a role for the active complex in inhibiting a delayed rectifier potassium channel in these neurons (94).

A recent elegant study by Chan *et al*. (14) has further advanced our understanding of Ang-II/NADPH oxidase signaling mechanisms in central cardiovascular neurons. They showed that bilateral Ang-II injections into the RVLM increases NADPH oxidase activation, $O_2^{\bullet-}$ generation, and stimulates the mitogen-activated protein kinase (MAPK) signaling cascade. Ang-II caused phosphorylation of p38 MAPK as well as extracellular signal-related protein kinase 1/2 (ERK 1/2) in RVLM, and this could be inhibited by co-injection of pharmacological inhibitors of the AT-1 receptor or the NADPH oxidase, as well as Tempol or antisense oligonucleotides targeted to NADPH subunits. Furthermore, addition of a MAPK phosphorylation inhibitor significantly attenuated Ang-II-stimulated pressor responses from the RVLM, as well as abolished the frequency of glutamate-sensitive postsynaptic excitatory currents. These findings strongly suggest that redox activation of MAPK signaling is involved in the sympathoexcitatory effect of Ang-II, possibly through the stimulated release of glutamate from presynaptic neurons at the RVLM.

Together, these studies provide clear evidence that intracellular $O_2^{\bullet-}$ generation is required for activation of cardiovascular neurons, at least in some brain regions, in response to Ang-II. The mechanisms by which Ang-II activates NADPH oxidase in neurons, and how subsequent $O_2^{\bullet-}$ signals to cause changes in neurohumoral responses and regulation of cardiovascular function is an area of intense investigation in several laboratories, including ours. Elucidating these pathways will advance our understanding of neurocardiovascular regulation.

NADPH OXIDASE IN CENTRAL NEUROCARDIOVASCULAR DISEASES

Hypertension

The evidence that human hypertension is characterized by dysfunction of central neural pathways is now extremely compelling (23). Ang-II has emerged as a primary culprit, driving central neurohumoral abnormalities in hypertension, although the precise molecular mechanisms and physiological circuits remain largely unknown.

As discussed above, abundant evidence now points to oxidative stress as a key mechanism in Ang-II-dependent hypertension and other diseases in the periphery. Furthermore, it has become clear that ROS are important in the pressor effects of Ang-II administered directly in the CNS. Recently we tested the hypothesis that hypertension caused by elevated circulating Ang-II might also involve an increase in intracellular $O_2^{\bullet-}$ levels in CNS neurons (121). Using chronic systemic infusion of subpressor doses of Ang-II, the so-called "slow-pressor" model of hypertension in mice [which closely mimics human hypertension (25)], we observed marked increase in $O_2^{\bullet-}$ production selectively in the forebrain CVO, SFO. Importantly, this increase in $O_2^{\bullet-}$ radical production paralleled the increase in MAP and reached a maximum on day 16, the time of peak hypertension. Both the oxidant production in SFO and the hypertension were abolished in mice that had received ICV delivery of a recombinant adenovirus expressing CuZnSOD, clearly demonstrating the requirement of $O_2^{\bullet-}$ in this vasopressor response. Interestingly, viral gene transfer of EC-SOD neither reduced ROS in the SFO nor affected hypertension, demonstrating that Ang-II effects are mediated by intracellular but not extracellular $O_2^{\bullet-}$ generation in neurons. These results suggest that Ang-II-induced increases in $O_2^{\bullet-}$ production in SFO plays a key role in the development of hypertension. Interestingly, recent results from Raizada's group (87) have demonstrated that soluble epoxide-mediated blood pressure regulation in the spontaneously hypertensive rat (SHR) is mediated by NADPH oxidase-derived $O_2^{\bullet-}$ generation in forebrain CVOs, further supporting an important role for ROS signaling in these regions in hypertension.

The hypothesis that chronic increases in ROS production in the CNS is involved in neurogenic hypertension is further supported by recent studies demonstrating increased levels of ROS in RVLM of SHR rats, as compared to Wistar–Kyoto (WKY) rats (49). Importantly, bilateral microinjection of SOD mimetics into RVLM or overexpression of MnSOD decreased both blood pressure and sympathetic nerve activity in SHR but not WKY rats (49, 97). Furthermore, chronic increases in ROS levels in normotensive WKY rat RVLM by Ad-mediated overexpression of inducible-NOS (iNOS) enzyme produced hypertension which was abrogated by bilateral microinjection of Tempol (47). Together these studies underscore the importance of chronic increase in ROS levels in key cardiovascular regulatory nuclei in the brain in neurogenic hypertension.

Ang-II-stimulated increases in ROS may also act by compromising sympathoinhibitory mechanisms in the CNS as well, thereby contributing to hypertension. The NTS and RVLM have been shown to generate nitric oxide (NO), which decreases sympathetic activity and depresses blood pressure (111). Whereas transgenic mice lacking endothelial nitric oxide synthase (eNOS) develop hypertension (40), viral delivery of eNOS selectively into the NTS causes a significant decrease in blood pressure in spontaneously hypertensive rats (96). Since $O_2^{\bullet -}$ can react with NO, thereby producing peroxynitrite and reducing NO concentrations, it is postulated that reductions in NO-mediated sympathoinhibition by this mechanism may play an important role in hypertension.

These findings, along with studies discussed above demonstrating a requirement for a Rac1-dependent NADPH oxidase in forebrain Ang-II signaling (119), suggest the involvement of this enzyme complex in cardiovascular regulation mediated by this region. Interestingly, expression of NADPH oxidase complex proteins has also recently been observed in brainstem cardiovascular regulatory nuclei of the CNS. Zucker and colleagues showed expression of Nox2, $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$ in RVLM of rabbits, and levels of all these proteins were increased by ICV Ang-II injection (30, 32). Similarly, Chan *et al*. have demonstrated the presence of Nox2, $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$ mRNA in rat RVLM, and importantly, Ang-II increased the serine phosphorylation of $p47^{phox}$, suggesting an increase in NADPH oxidase assembly and activation (14). Furthermore, bilateral injection of antisense oligonucleotides directed against either $p22^{phox}$ or $p47^{phox}$ inhibited Ang-II-induced $O_2^{\bullet -}$ production, clearly demonstrating the involvement of Nox2 NADPH oxidase in Ang-II signaling in RVLM (14). Importantly, Nox2 immunoreactivity has been observed in other central sites of Ang-II-mediated autonomic regulation, including the NTS (105) and PVN (46).

In contrast to the above findings, a recent study by Touyz *et al*. using double transgenic mice in which Nox2 is deleted and the endogenous renin–angiotensin system is chronically upregulated [including the brain RAS (78)], has suggested that loss of functional Nox2 does not prevent development of hypertension and cardiac hypertrophy in this model (102). In this study, while the double-transgenic progeny exhibited significantly reduced ROS levels in cardiac, aortic, and renal tissues compared to control single transgenic mice overexpressing renin, these animals still developed chronic Ang-II-dependent hypertension. Importantly, compensatory increases in Nox1 and Nox4 expression were not observed in cardiac and renal tissues of the double Nox2 knockout/renin overexpressing mice, raising questions about the links between Ang-II, ROS, and hypertension. Although it is not clear at this time, an intriguing possibility is that increased circulating Ang-II may be producing hypertension in this model by neurogenic mechanisms as described above (121). As such, the hypertension and related sequelae observed in this model may still be the result of ROS, but working through compensatory increases in expression of other Nox homologues within the CNS in this model.

Sympathetic activation in heart failure

Dysregulation of autonomic function—including a persistent overactivation of the sympathetic nervous system—is well established in the etiology of chronic heart failure (HF) (27). Cardiac ventricular impairment, initiated by any of a number of pathological states, elicits the release of various neurohumoral substances including Ang-II into the circulation and from peripheral organs, which collectively contribute to the maintenance of tissue perfusion and arterial pressure via vasoconstriction, increased blood volume, enhanced heart rate, and augmented neurocardiovascular output (27, 123). While these compensatory mechanisms are beneficial in the short-term, chronic neurohumoral activation contributes to the deterioration of myocardial function over time (123).

During HF, blood-borne factors, such as Ang-II, are increased (107). Elevated Ang-II, for example, is in constant contact with forebrain and hindbrain CVOs via AT-1 receptors, which in turn can stimulate sympathetic nerve activity as described above (22). Enhanced sympathetic activity has widespread effects, including promotion of excess sodium retention and therefore fluid volume, leading to edema, lung congestion, and cardiac stress (108). Direct effects of excessive sympathetic effects on the heart are also a factor. Evidence for a critical role of the CNS in driving this neurohumoral state comes from experiments in which coronary artery ligation (myocardial infarction, MI) induced impairment of arterial baroreflex control of heart rate, and RSNA were ameliorated by chronic ICV infusion of AT-1 inhibitor losartan (115). Similarly, microinjection of antisense oligodeoxynucleotides to the AT-1 receptor in PVN has been shown to blunt resting sympathetic activity in CHF rats, once again implicating increased central Ang-II signaling in the neurohumoral response during HF (117).

Given the involvement of Ang-II-mediated activation of CVOs and downstream sympathetic areas of the brain in HF, we recently tested the hypothesis that redox signaling may be an underlying mechanism in this response. Normal mice subjected to MI to induce HF exhibited hallmarks of this disorder, including increased central neural activation, shown by increased immuno-Fos staining in some cardiovascular nuclei, along with a significant increase in sympathetic output as evidenced by elevated urinary norepinephrine and pronounced cardiovascular responses to the ganglionic blocker hexamethonium (56). Interestingly, each of these indicators of excessive neurohumoral activation in the HF mice was normalized by gene transfer of AdCuZnSOD to forebrain CVOs at the time of coronary ligation (56). Furthermore, this translated into improved cardiac performance and reduced mortality rates in the HF animals (57). These results suggest that increased redox signaling in forebrain CVOs is involved in the pathogenesis of MI-induced sympathoexcitation and HF.

Since then, additional evidence has accumulated implicating NADPH oxidase as a source of ROS generation in the sympathoexcitatory loop of HF. For example, studies by Zucker and colleagues (30, 32) showed that ICV injection of apocynin, a pharmacological inhibitor of the NADPH oxidase complex, was able to both normalize ROS levels observed in the RVLM and prevent Ang-II-mediated increases in RSNA in rabbits with pacing-induced HF. Interestingly, these authors confirmed that HF rabbits also exhibited significant increases in mRNA and protein for the AT-1 receptor, as well as for subunits of the NADPH oxidase complex, including $p40^{phox}$, $p47^{phox}$, and Nox2 in RVLM (30, 31). Taken together, these experiments provide convincing evidence for NADPH oxidase-

derived ROS in abnormal CNS/Ang-II signaling that causes enhanced sympathetic activity in HF.

The ability to normalize HF-induced sympathoexcitation and improve outcomes by reducing ROS levels in the CNS, as described above, are particularly intriguing in light of recent clinical trials where treatment of chronic HF patients with a sustained-release form of moxonidine (MOXCON), a centrally-acting sympathetic inhibitor, resulted in higher mortality rates compared to HF patients given placebo (18, 77). Certainly the beneficial effects of sympathetic activation in HF, at least initially, are well documented (27, 86, 90), and the results of this trial may further support this. Although the causes of the adverse effects of MOXCON are not known, the high doses and widespread effects on sympathetic outflow to multiple end-organs may explain the excess mortality (18). In the studies described above where the redox inhibition was targeted to highly localized CNS regions of sympathetic control, it is possible that this resulted in a more specific modulation of sympathetic traffic. Indeed, although CNS gene transfer of Ad-SOD caused reductions in general markers of sympathetic tone, resting heart rate was not affected (56). In addition, it is possible that inhibition of oxidant signaling in these particular forebrain (56) and brainstem sites (31, 32) may have provided additional therapeutic effects through modulation of other neurohumoral mechanisms underlying HF (27). Clearly, a greater understanding of NADPH oxidase and other signaling pathways involved in the sympathoexcitatory loop of HF is necessary in the development of treatments for this disease.

Diversity of Nox enzymes in CNS cardiovascular circuits

While the studies described above have demonstrated primarily the involvement of Nox2 in neurocardiovascular circuits, the roles of the other Nox enzymes have not been investigated in these sites. To fully understand how NADPH oxidase is involved in neurocardiovascular disease, and to be able to target it precisely, either experimentally or therapeutically, information about the expression patterns of the Nox homologues is needed. To this end, we have recently compared the expression levels of Nox1, Nox2, and Nox4 in different regions of mouse brain using real-time PCR. We have taken this approach because of the lack of available specific antibodies. As shown in Fig. 2, Nox2 as well as Nox4 are the predominant homologues expressed in fore-, mid-, and hindbrain of mice, while Nox1 is detectable but at very low levels. Interestingly, the relative distribution of Nox2 and Nox4 differs in these regions. For example, Nox2 levels are highest in forebrain tissue, whereas Nox4 mRNA is most abundant in midbrain. Both Nox 2 and Nox 4 are expressed at nearly comparable levels in hindbrain, although Nox4 is slightly more abundant. Already this provides a hint of the complexity of this Nox family of enzymes in different brain regions. Currently we are undertaking a more specific mapping strategy to delineate Nox expression profiles in specific neurocardiovascular nuclei such as the SFO, PVN, and RVLM under normal and pathological conditions. Indeed, emerging evidence in a variety of cell types suggests that Nox transcript levels at baseline do not necessarily predict stimulus-induced activation of the enzymes, and opposing functions of various enzymes have been detected under different physiological conditions

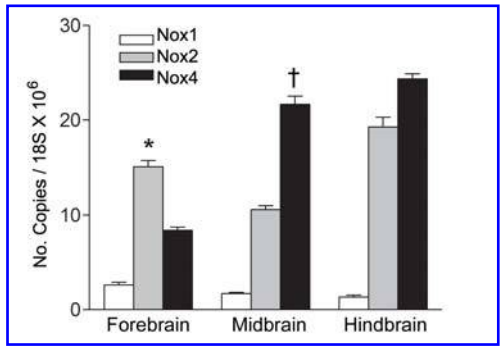

FIG. 2. Basal levels of Nox1, Nox2, and Nox4 expression in adult C57BL/6 mouse brain estimated by quantitative real-time PCR. Summary data are presented (mean ± SEM, $n = 3$) for Nox1, Nox2, and Nox4 transcript numbers in fore-, mid-, and hindbrain tissue. Copy numbers of Nox homologues were calculated from standard curves generated with purified plasmids. Data are expressed as copy number per 18S RNA and are for 100 ng input RNA in each sample. *$p < 0.01$ versus Nox1 and Nox4, † $p < 0.01$ versus Nox 1 and Nox2.

(36, 54, 68, 69, 74). Likewise, our preliminary studies suggest a distinct and complex distribution pattern of the homologues across these specific nuclei, with an even more elaborate profile under experimental conditions of cardiovascular disease (Infanger, Sharma, Davisson 2006 unpublished data).

These data, along with evidence presented above suggests that an increase in NADPH oxidase-derived ROS in CVOs, hypothalamic nuclei, and brainstem sites play a central role in the neurocardiovascular dysfunction observed in hypertension and HF. The expression of the Nox homologues, cytosolic regulatory subunits, and mechanisms of their activation in different brain circuits are the subject of intense ongoing investigations. Figure 3 summarizes our current knowledge of the expression profile of different NADPH oxidase components in these central pathways which utilize redox signaling mechanisms. Undoubtedly a better understanding of the expression patterns of this complex family of enzymes will spur the field forward to a greater appreciation of their function.

SUMMARY AND PERSPECTIVES

Over the past decade, an enormous amount of research has been devoted to the study of the NADPH oxidase complex in nonphagocytic cells and tissues, which has led to the identification of an entire family of Nox enzymes. While these isoforms are common in their ability to generate ROS, each is uniquely regulated and distributed, and play distinct roles in various cell types. Despite agreement about the tremendous potential importance of Nox enzymes in normal cellular functions as well as in the pathogenesis of many diseases, our knowledge about the specific mechanisms of activation and subsequent functional consequences of activating specific Nox enzymes is limited. In the CNS, NADPH oxidases are essential for diverse processes, from memory to neurocardiovascular homeostasis, and dysregulation of the enzymes result in neurodegenerative and neurogenic cardiovascular diseases. As shown in Fig. 3, multiple Nox-containing NADPH oxi-

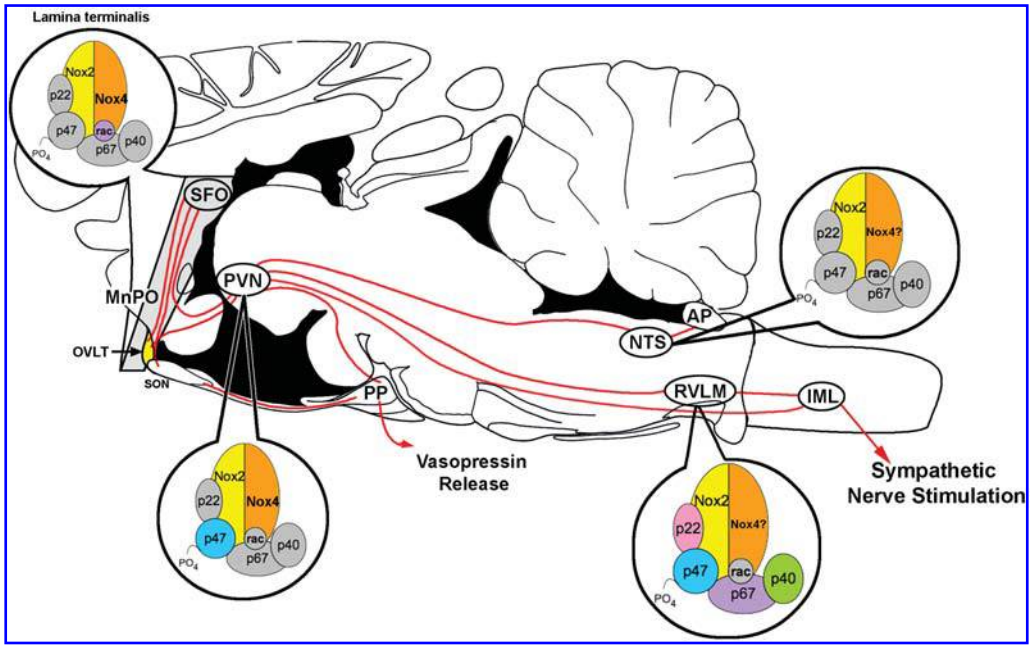

FIG. 3. Schematic summarizing central cardiovascular regulatory pathways and identified NADPH oxidase subunits which may contribute to redox signaling in these networks. A signaling role for ROS has been established in mediating Ang-II-driven sympathoexcitation. Circulating Ang-II can interface with the CNS via blood–brain barrier lacking circumventricular organs such as the SFO, OVLT, and AP (*yellow*), which subsequently project to hypothalamic and hindbrain structures, including PVN, NTS, RVLM, and IML. ROS signaling is implicated in these regulatory circuits, and recently a role for NADPH has emerged in some of these nuclei. For example, Ang-II-stimulated ROS generation in the SFO depends on a Rac1/NADPH oxidase, likewise, central Ang-II stimulation increases $O_2^{\bullet-}$ and expression of NADPH oxidase subunits. Furthermore, pharmacological or peptide inhibitors of the NADPH oxidase inhibit Ang-II-stimulated neuronal firing and intracellular signaling in brainstem nuclei. Immunohistochemical staining has been used to detect subunits for which antibodies are available, and we have used real-time PCR to examine Nox homologue expression in some of these brain nuclei. NADPH oxidase subunits which have been detected or inferred are shown in *color*, *gray shaded* subunits have not been found as of yet. It is important to note multiple Nox homologues have been found in certain nuclei, suggesting multiple levels of regulation in the local oxidative environment. AP, area postrema, IML, interomedial lateral cell column of the spinal cord, MnPO, median preoptic nucleus, NTS nucleus tractus solitarius, OVLT, organum vasculosum of the lamina terminalis, PP, posterior pituitary, PVN, paraventricular nucleus, RVLM, rostral ventrolateral medulla, SFO, subfornical organ, SON, supraoptic nucleus.

dases are likely involved in the central control of cardiovascular function, and evidence is emerging that such diversity in oxidant generation applies to other CNS functions as well. Similar to peripheral organ systems, a better understanding of the expression patterns, regulation, and functional significance of specific Nox enzymes in CNS neurons and other cell types is needed.

Molecular tools to evaluate Nox function in the CNS have been relatively limited. Pharmacological and peptide inhibitors of NADPH oxidase can implicate this enzyme complex in normal or disease processes, yet the lack of selectivity necessary to unravel individual Nox homologue activation has slowed progress. Likewise, the Nox2 transgenic knockout model has been useful in studying cardiovascular physiological and pathophysiological roles of this enzyme (101, 102, 106); however, global null mutations have the potential to lead to confounding compensatory increases in expression of other Nox homologues.

In general, dissection of gene function in the mammalian brain is challenging due to the broad expression of many genes functioning in multiple neural circuits. Analysis of the molecular underpinnings of certain CNS processes, including regulation of cardiovascular function, has posed an even greater challenge because of the lack of known regulatory sequences that restrict gene expression to specific brain nuclei (20). Recently, a number of laboratories have turned to brain site-directed gene transfer using recombinant viral vectors to circumvent this issue, and the strategy has been very useful in defining specific molecular pathways in particular circuits (20, 21, 48, 76). Coupled with conditional gene knockout using the Cre-loxP system (71, 92), and more recently gene silencing with siRNA (10, 15, 109), the potential exists for functionally mapping the roles of individual Nox enzymes and related molecules in specific CNS networks.

In addition to defining the spatiotemporal expression and activation of NADPH oxidase enzymes in the CNS under normal and pathological conditions, the cellular consequences of a chronically dysregulated oxidative environment must also be examined. It is well established that long-lasting alterations in CNS function in the setting of both physiological (*e.g.,* long-term memory) and pathophysiological (*e.g.,* neurodegeneration) processes require changes in gene expression (64, 67). A major mechanism by which this occurs is through consolidation of transient extracellular signals and activation of inducible transcription factors (2). In the context of oxidant mechanisms, redox-sensitive transcription factors such as NF-κB and AP-1 are important proteins involved in modulating long-lasting

changes in signal transduction cascades (58) and can alter gene expression in CNS neurons (67, 70). For example, recently we and others have begun to identify ROS-dependent transcription factor activation in specific brain circuits that may be linked to long-term regulation of cardiovascular responses. Using *in vivo* bioluminescence, a technology based on photocounting of a light-producing chemical reaction inside a living organism without the need for an excitation light (19), we have been able to track transcription factor activity in cardiovascular regulatory centers within the CNS during the pathogenesis of cardiovascular disease. Determining which transcription factors are involved in chronic manifestations of cardiovascular and other CNS-driven diseases will provide new strategies for the development of clinical treatments for these pathologies.

It is difficult to comprehend an enzyme so complexly regulated that it can function to promote cell growth and cell death, long-term potentiation and neurodegeneration, cardiovascular homeostasis and cardiovascular disease. Considering its myriad functions and diversity, it is not surprising that so little is known about the regulatory mechanisms of Nox homologues in the CNS. It is important to note that despite this broad range of functions in which it is implicated, the whole complex is still only capable of reducing molecular oxygen. This fact stresses not only the importance of Nox oxidase regulation, but also the cellular milieu in which activation takes place. Defining the consequences of an altered oxidative state in different cell types and tissues will provide essential understanding of disease pathologies and provide new treatments for these diseases. Considering the effort and progress that has been made over the past few years, such novel therapies might not be far away.

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ABBREVIATIONS

Ang-II, angiotensin II; AP, area postrema; BBB, blood–brain barrier; CNS, central nervous system; CVO, circumventricular organ; Duox, dual oxidase; EC-SOD, extracellular superoxide dismutase; eNOS, endothelial nitric oxide synthase; ERK 1/2, extracellular signal-related protein kinase 1/2; H_2O_2, hydrogen peroxide; HF, heart failure; ICV, intracerebroventricular; iNOS, inducible nitric oxide synthase; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; MCAO, middle cerebral artery occlusion; MOXCON, moxonidine congestive heart failure trial; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO, nitric oxide; Nox, NADPH oxidase; Nox2, $gp91^{phox}$; NTS, nucleus tractus solitarius; $O_2^{\bullet -}$, superoxide; OVLT, organum vasculosum of the lamina terminalis; PVN, paraventricluar nucleus; ROS, reactive oxygen species; RVLM, rostral ventrolateral medulla; RSNA, renal sympathetic nerve activity; SFO, subfornical organ; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase; WKY, Wistar–Kyoto.

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