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Forum Review

Reactive Oxygen Species in the Cerebral Circulation: Are They All Bad?

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ABSTRACT

Reactive oxygen species (ROS) are a diverse family of molecules generated by all cells. ROS may serve as important cell-signalling molecules in the cerebral circulation. Indeed, in contrast to systemic arteries, major products of superoxide metabolism, including hydrogen peroxide, are powerful cerebral vasodilators, raising the possibility that ROS represent important molecules for increasing local cerebral blood flow. Two major determinants of the overall effects of ROS on cerebrovascular tone are the rate of production of the parent molecule, superoxide, and its rate of metabolism by superoxide dismutases. Although the major enzymatic source of ROS in cerebral arteries has not been clarified, nicotinamide adenine dinucleotide phosphate, reduced form (NADPH)-oxidases, along with cyclooxygenases and lipoxygenases, are probably the primary sources. In cerebral arteries, activation of NADPH-oxidase elicits both an increase in superoxide production and vasodilatation. The identity of the ROS molecule responsible for the vasodilator effects may be hydrogen peroxide, generated from the dismutation of superoxide. NADPH-oxidase activity and function appears to be profoundly greater in cerebral versus systemic arteries. Furthermore, NADPH-oxidase–derived ROS partly contribute to flow-dependent dilatation and may offset angiotensin II–induced constriction of cerebral arteries, consistent with the hypothesis that NADPH-oxidase–derived ROS may play a physiologic role in the control of cerebrovascular tone. *Antioxid. Redox Signal.* 8, 1113—1120.

INTRODUCTION

Over the past decade, a growing body of evidence has implicated reactive oxygen species (ROS) as pathologic mediators in vascular disease. Indeed, it is well established that under conditions of enhanced ROS generation or impaired ROS metabolism or both, a condition called *oxidative stress* can develop. In the systemic circulation, compelling evidence exists that oxidative stress contributes to the complex functional changes that occur in the vessel wall during a variety of cardiovascular diseases (8). For example, it is well established that the parent ROS molecule, superoxide (O_2^-), can increase vascular tone via the inactivation of nitric oxide (NO) (8). In the normal physiologic state, however, the production and removal of ROS within vascular cells is tightly controlled, and under these conditions, ROS are thought to serve as important signalling molecules in both systemic and cerebral circulations (24). Indeed, it is now well established that major products of O_2^- metabolism, such as hydrogen peroxide (H_2O_2), are powerful cerebral vasodilators (31, 44, 54). These intriguing findings have raised the possibility that ROS may, surprisingly, represent important molecules in vasodilator responses within the cerebral circulation. Over the last few years, much has been written on the importance of ROS in the pathogenesis of cerebrovascular disease (11, 21, 30, 45, 53); however, little attention has been given to the potential role of ROS as physiologic vasodilators in the cerebral circulation. Thus in this brief review, we first discuss potential sources of cerebrovascular ROS and the effects of exogenous ROS on cerebrovascular tone. We then summarize current evidence supporting the relatively recent hypothesis that ROS serve as important physiologic cerebral vasodilators.

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CHEMISTRY OF REACTIVE OXYGEN SPECIES

ROS are a family of highly reactive oxygen-derived molecules generated by all mammalian cells. O_2^- is generated by a number of vascular oxidases or by autooxidation of tissue components via the one-electron reduction of molecular oxygen (Fig. 1). O_2^- is highly reactive, but because of its very short half-life and low permeability, its biologic effects are highly dependent on its subcellular localization. The most commonly studied effect of O_2^- on blood vessels is its reaction with the endothelium-derived vasodilator, NO (8). This results in the generation of another highly reactive ROS called peroxynitrite ($ONOO^-$), which can spontaneously decompose to form the hydroxyl radical (OH^-). Alternatively, O_2^- can be dismutated, either spontaneously or catalyzed by O_2^- dismutases (SOD), to form H_2O_2. Owing to its high cell-permeability and relative stability, H_2O_2 is now believed to be one of the most important ROS molecules in modulating endothelial and vascular smooth muscle (VSM) cell function (6). The dismutation of O_2^- is the main source of H_2O_2 in blood vessels; however, evidence now exists that it also can be generated directly by some enzymes such as nicotinamide adenine dinucleotide phosphate, reduced form (NADPH)-oxidases, xanthine oxidase, and glucose oxidase (6, 36) (see Regulation of Cerebrovascular ROS). H_2O_2 can be metabolized to water by either catalase or glutathione peroxidase (Fig. 1) or can undergo nonenzymatic reactions to produce other, more damaging ROS. In particular, OH^- is generated from H_2O_2 in the presence of ferrous iron or other transition metals in the metal-catalyzed Haber–Weiss or Fenton reaction. O_2^- is essential for this reaction, as it reduces the transition metal, which is then oxidized when OH^- is formed, and thus the cycle can be repeated. Alternatively, H_2O_2 can be used as a substrate by the endogenous enzyme myeloperoxidase, resulting in the formation of the highly reactive compound, hypochlorous acid (Fig. 1).

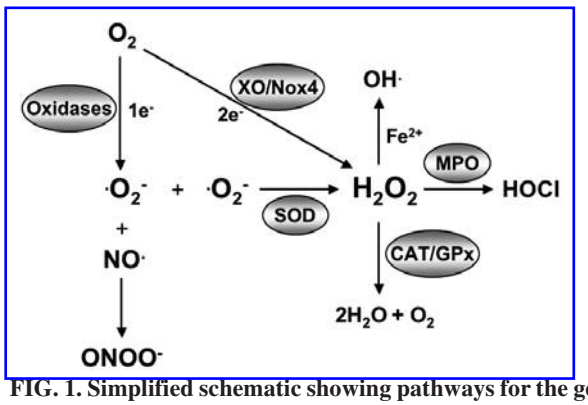

FIG. 1. Simplified schematic showing pathways for the generation of reactive oxygen species (ROS) in vascular cells. Superoxide (O_2^-) is generated by oxidases via the one-electron reduction of oxygen. O_2^- can react with endothelium-derived nitric oxide (NO), forming peroxynitrite ($ONOO^-$), or it can be broken down by O_2^- dismutases (SOD), forming the highly diffusible and relatively stable molecule, hydrogen peroxide (H_2O_2). H_2O_2 also can be generated directly from oxygen by some vascular oxidases, such as xanthine oxidase (XO), glucose oxidase (GO), and Nox4-containing NADPH-oxidases (Nox4). H_2O_2 can be metabolised by catalase (CAT) or glutathione peroxidase (GPx) to form water and oxygen or can undergo nonenzymatic reactions to generate the hydroxyl radical (OH^-) in the metal-catlayzed Haber–Weiss or Fenton reaction. Alternatively, ferrous-containing enzymes, such as myeloperoxidase (MPO), can use H_2O_2, along with the nitrogen dioxide anion (NO_2^-), as a substrate to form the nitrogen dioxide radical ($NO_2^{\cdot}$), which in turn may nitrate tyrosines (Tyr).

REGULATION OF CEREBROVASCULAR ROS

The balance between levels of individual ROS within blood vessels under physiologic conditions will ultimately be determined by the rate of O_2^- production, the metabolism rate of O_2^- by endogenous superoxide dismutases (SODs), and the removal rate of H_2O_2 by antioxidant enzymes (catalase or glutathione peroxidase) and/or involvement in Haber–Weiss or Fenton chemistry. In mammalian cells, potential enzymatic sources of O_2^- include the mitochondrial electron-transport chain, the arachidonic acid–metabolizing enzymes (cyclooxygenase and lipoxygenase), the cytochrome P450s, xanthine oxidase, NADPH-oxidases, and NO synthases. In cerebral arteries, the major enzymatic source of O_2^- remains to be fully clarified. Although likely multiple sources of ROS exist in cerebral arteries, compelling evidence suggests that vascular NADPH-oxidases and cyclooxygenases/lipoxygenases are the primary physiologic sources.

SUPEROXIDE DISMUTASES

Three isoforms of SOD are expressed in vascular cells: CuZn-containing cytosolic SOD (SOD1), Mn-containing mitochondrial SOD (SOD2), and CuZn-containing extracellular SOD (SOD3) (22). The proportion of Mn-SOD in vascular cells is lower compared with the Cu/Zn-containing isoforms (22). However, a recent study reported that levels of Mn-SOD were higher in cerebral arteries than in the carotid artery and aorta, raising the possibility that this isoform might be particularly important for the conversion of O_2^- to H_2O_2 in cerebral arteries (43). This point notwithstanding, it is generally thought that CuZn-containing isoforms are the most important for O_2^- metabolism in cerebral vascular cells (22). Indeed, Didion *et al*. (15) demonstrated that inhibition of CuZn-containing SODs by using diethyldithiocarbamate (DETCA) resulted in an ~2.5-fold increase in basal O_2^- production by rabbit basilar arteries, suggesting that endogenous CuZn-containing SODs play an essential role in limiting basal O_2^- levels in cerebral arteries. Similarly, mice lacking the gene for SOD1 have enhanced levels of O_2^- and impaired cerebral dilatations to endogenous NO (16), consistent with a role for this isoform of SOD in limiting O_2^- levels under physiologic conditions, thus preserving NO-mediated and presumably, H_2O_2-induced vasomotor effects.

VASCULAR NADPH-OXIDASES

Vascular NADPH-oxidases are similar, but not identical, in structure to the phagocytic NADPH-oxidase, which consists of a membrane-bound cytochrome *b*558 domain, up to three cytosolic subunits (p47phox, p67phox, and p40phox), and a regulatory G protein, Rac-1 or Rac-2 (4) (Fig. 2). The cytochrome *b*558 domain consists of two proteins: a small α-subunit, p22phox, and a larger, catalytic β-subunit, gp91phox (4). On assembly of the cytosolic subunits at the membrane, phagocytic NADPH-oxidase generates a large burst of O_2^- on the extracellular side of the membrane (3). This is achieved by the one-electron reduction of oxygen via the flavin containing catalytic gp91phox subunit, and by using reduced NADPH as a substrate (3). The vascular NADPH-oxidases differ from the phagocytic isoform in several aspects. First, vascular NADPH-oxidases generate continuous low levels of O_2^-, most of which is intracellular (7). Thus in contrast to the phagocytic NADPH-oxidase, it is believed that at least part of the enzyme is preassembled in vascular cells (7). Second, the vascular NADPH-oxidases have been reported to use NADH, as well as NADPH, as a substrate (14). Third, although gp91phox also appears to be an important catalytic subunit for the generation of O_2^- by vascular NADPH-oxidases, it is now thought that nonphagocytic gp91phox homologues, called *Nox proteins*, also are important for O_2^- by vascular NADPH-oxidases.

To date, four novel homologues of the phagocytic NADPH-oxidase catalytic domain have been discovered, and these are named Nox1, Nox3, Nox4, and Nox5, with gp91phox being renamed Nox2 (7). Recently, two proteins with homology to the cytosolic subunits, p47phox and p67phox, have been discovered, Nox organizer 1 and Nox activator 1, respectively (7). The precise physiologic roles of these Nox homologues and how they are activated remains to be fully clarified. Nevertheless, it has been suggested that each isoform may play a distinct role in modulating vascular function. Compelling evidence indicates that this may be ultimately dependent on the subcellular localization of each homologue and/or their coupling to agonists and/or the identity of the ROS molecule generated (26, 34). Indeed, a recent study reported that in a cultured cell expression, systemic Nox4 generates H_2O_2 rather than O_2^- (36).

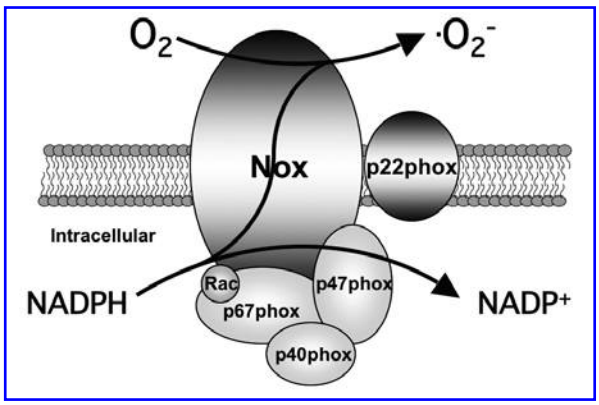

FIG. 2. Schematic diagram showing the generalized structure of vascular NADPH-oxidases. Vascular NADPH-oxidases are thought to contain a membrane domain, consisting of a catalytic subunit (Nox) and p22phox, three cytosolic subunits (p47phox, p67phox, and p40phox) and a regulatory G protein (Rac). To date, five different Nox proteins have been identified, and at present, it is unclear which cytosolic subunits are necessary for their activation. It is believed, however, that electrons (*arrows*) flow from NADPH to oxygen (O_2^-), via the flavin containing catalytic subunit, yielding superoxide (O_2^-).

Although our understanding of the role of vascular NADPH-oxidases in cerebral arteries lags behind what is understood in arteries outside of the brain, a focus has been emerging in this area over the last few years. It is now established that vascular NADPH-oxidases are expressed in cerebral arteries (1, 39, 44). We have recently reported that messenger RNA (mRNA) for Nox1, Nox2, Nox4, and p22phox, as well as the cytosolic subunit p47phox, is expressed in rat basilar arteries (44). Similarly, a recent study by Ago *et al.* (1) reported that mRNA for Nox1, Nox2, and Nox4 is found in endothelial cells from rat basilar arteries. Furthermore, they reported that Nox1 and Nox4 are more abundantly expressed than Nox2 and that both p47phox and p67phox enhance NADPH-oxidase activity (1). Of importance, evidence now exists that cerebral arteries have a differential Nox composition than arteries from the systemic circulation. Specifically, Ago *et al*. (1) reported that mRNA for Nox1 and Nox4 were greater in endothelial cells of rat basilar arteries compared with endothelial cells from the aorta. In accordance with their findings, we now have evidence that Nox4 protein expression is 10-fold higher in rat basilar arteries versus a range of systemic arteries (39). Furthermore, we have found that this differential Nox composition is associated with profound differences in NADPH-oxidase activity and function in cerebral versus systemic arteries (39) (see ROS as Endogenous Vasodilators). Compelling evidence indicates that vascular NADPH-oxidases are functionally active in endothelial, VSM, and adventitial cells of cerebral arteries (Fig. 3) and are likely to represent a major source of ROS in cerebral arteries. Application of either NADPH or NADH to intact arteries or homogenates of cerebral arteries from a variety of species elicits a large increase in O_2^- production (14, 39, 44, 46, 49). Moreover, this effect can be inhibited by a wide range of NADPH-oxidase inhibitors including apocynin, gp91ds-tat, and diphenylionodium (DPI) (14, 39, 44, 49).

CYCLOOXYGENASES AND LIPOXYGENASES

Cyclooxygenase (COX) and lipoxygenase are rate-limiting enzymes in the synthesis of prostanoids and hydroperoxides, respectively, from arachidonic acid (AA) (18). To date, two isoforms of COX have been discovered, COX-1 and COX-2. COX-1 is expressed constitutively in most cells, including cerebral vascular cells (18). COX-2, conversely, is generally associated with inflammation, as its expression can be upregulated by inflammatory mediators (18). However, some evidence indicates that COX-2 is expressed under basal conditions in cerebral microvessels, particularly during development (47, 48). Besides the synthesis of prostanoids, AA

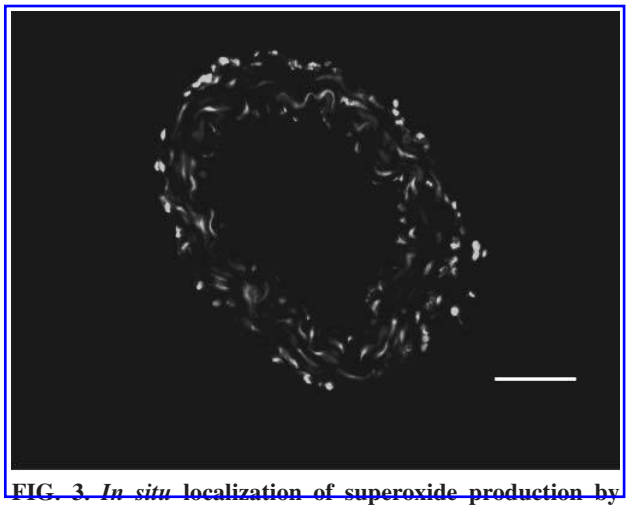

FIG. 3. *In situ* localization of superoxide production by NADPH-oxidases in a section (16 μm) of rat basilar artery by using dihydroethidium (DHE; 2 μ*M*). In the presence of O_2^-, DHE is oxidized to ethidium and oxyethidium, which intercalates between DNA, producing a nuclear fluorescent (red) staining pattern. In the presence of NADPH (100 μ*M*), O_2^- production is detected in endothelial, vascular smooth muscle, and adventitial cells, indicating that NADPH-oxidases are expressed and functionally active throughout the vessel walls of cerebral arteries. Furthermore, the fluorescent signal is abolished by application of polyethylene glycol-SOD, but not native SOD (not shown), confirming that intracellular superoxide was the major reactive oxygen species detected by DHE. Magnification, ×400. Scale bar, 50 μm.

metabolism by COX in cerebral arteries also is thought to generate ROS. Didion *et al*. (15) reported that AA increased O_2^- production by rabbit basilar arteries, and that this effect was augmented when arteries were treated with the CuZn-containing SOD inhibitor, DETCA (15). Moreover, the COX inhibitor, indomethacin, significantly attenuated this increase in O_2^- production, implying that the COX pathway is an important source of ROS generated by cerebral arteries in response to AA (15). At present, however, it is unclear whether COX is directly responsible for the generation of ROS or whether COX is merely a critical step leading to the activation of other ROS-generating enzymes.

The lipoxygenase pathway also has been reported to contribute to the increase in ROS production by cerebral arteries in response to AA (23). However, studies in noncerebral VSM cells and phagocytic cells suggest that this effect is mediated through the activation of other ROS-generating enzymes, such as NADPH-oxidase, by lipoxygenase-derived products rather than direct generation of ROS by lipoxygenase itself (52, 63).

OTHER POTENTIAL SOURCES

As discussed, NADPH-oxidases and the cyclooxygenases/lipoxygenases are now recognized as the most important sources of ROS in cerebral arteries; however, other sources could potentially contribute. The NO synthase (NOS) family of enzymes consist of endothelial NOS (eNOS), inducible NOS, and neuronal NOS. NOS is a cytochrome p450 reductase–like enzyme that catalyzes flavin-mediated electron transport from NADPH to a prosthetic heme group. The enzyme requires tetrahydrobiopterin (BH_4) to transfer electrons to a guinidino nitrogen of L-arginine to generate NO. In the absence of either L-arginine or BH_4, all three isoforms of NOS have been reported to "uncouple," resulting in the generation of O_2^- rather than NO (50, 61, 62). Moreover, NOS uncoupling has been linked to numerous cardiovascular diseases, such as atherosclerosis, hypertension, and heart failure (2, 41). However, it remains to be established whether a similar phenomenon occurs in cerebral arteries. This latter point notwithstanding, emerging evidence suggests that ROS molecules may actually play an important role in initiating NOS uncoupling. Landmesser *et al*. (33) reported that H_2O_2, derived from the dismutation of NADPH-oxidase–derived O_2^-, oxidizes BH_4 and causes uncoupling of eNOS. Furthermore, NADPH-oxidase–derived H_2O_2 mediates agonist-provoked BH_4 deficiency to increase eNOS uncoupling (9). In addition to H_2O_2, $ONOO^-$ has been shown to oxidize BH_4 and lead to direct uncoupling of eNOS (2). Thus this feed-forward mechanism could serve further to amplify ROS generation within vascular cells.

EFFECTS OF EXOGENOUS ROS ON CEREBROVASCULAR TONE

Over the past few years, it has become apparent that cerebral arteries display a different profile of vasomotor responses to ROS than do arteries from the systemic circulation. First, in systemic arteries, O_2^- is commonly regarded as a constrictor ROS molecule, whereas in cerebral arteries, it has been reported to dilate (58) as well as to constrict (10). Second, numerous investigators have shown that by-products of O_2^- metabolism, such as H_2O_2 and $ONOO^-$, are powerful cerebral dilators.

In contrast to O_2^-, H_2O_2 is uncharged, relatively longer living, and freely diffusible (including through cell membranes). Thus as for NO, these properties make H_2O_2 an ideal signalling molecule for the control of vascular tone and function. It has been demonstrated in a number of species that exogenously applied H_2O_2 is a powerful cerebral vasodilator both *in vitro* and *in vivo* (28, 44, 54, 55, 60). Moreover, it is now thought that activation of potassium channels is the major mechanism by which H_2O_2 dilates cerebral arteries. In canine large cerebral arteries, application of H_2O_2 elicits dilatation in part by activation of AA metabolism via the COX pathway with a subsequent increase in cyclic adenine monophosphate levels and activation of Ca^{2+}-activated and delayed rectifier potassium channels (28). Furthermore, a depolarizing solution of potassium chloride attenuated relaxations, suggesting that H_2O_2 may cause relaxation of canine cerebral arteries by hyperpolarizing VSM cell membranes (28). We have reported that H_2O_2-mediated dilatations of rat cerebral arterioles *in vivo* are mediated in large part via activation of Ca^{2+}-activated potassium channels (54). In contrast, adenosine triphosphate (ATP)-sensitive potassium channels have been reported to mediate dilatations to H_2O_2 in feline

cerebral arterioles (60), suggesting that the specific potassium channel activated by H_2O_2 is species dependent. In addition to its direct effects on the tone of VSM cells, evidence suggests that H_2O_2 may also modulate tone via endothelium-dependent mechanisms. Specifically, it has been described in noncerebral arteries that H_2O_2 acutely stimulates NO production by eNOS (57). Furthermore, recent studies have reported that H_2O_2 potently upregulates eNOS expression *in vitro* and *in vivo* (17). It remains to be established, however, whether H_2O_2 has similar effects on eNOS activity and expression in cerebral arteries.

A few studies demonstrated that $ONOO^-$, the product of the reaction between O_2^- and NO, can relax cerebral arteries (35, 59, 60). Topical application of $ONOO^-$ to feline cerebral arterioles *in vivo* elicited dose-dependent dilatations via ATP-sensitive potassium channels (60). However, the mechanism by which $ONOO^-$ opened ATP-sensitive potassium channels was not investigated, and thus it is possible that a by-product of $ONOO^-$, such as OH^-, could mediate the channel-opening effects of $ONOO^-$. A recent study reported that application of $ONOO^-$ to canine cerebral arteries *in vitro* also elicited powerful relaxations (35). In this study, investigators reported that $ONOO^-$-mediated relaxations involved multiple signalling pathways, including an elevation of guanosine monophosphate levels, membrane hyperpolarization via potassium channel activation, activation of myosin light-chain phosphatase activity, and interference with membrane Ca^{2+} entry (35). In contrast, other investigators have demonstrated that $ONOO^-$ contracts cerebral arteries via inhibition of basal activity of Ca^{2+}-activated potassium channels (5, 19).

ROS AS ENDOGENOUS VASODILATORS

Despite providing only limited information on the physiologic significance of ROS in cerebral arteries, studies using exogenous ROS have provided important insights into the effects of ROS on cerebrovascular tone. Indeed, as discussed, by-products of O_2^- metabolism (H_2O_2 and $ONOO^-$) are now established as powerful cerebral vasodilators, raising the possibility that these molecules may serve as important vasodilatory molecules in the cerebral circulation.

Kontos *et al*. (32) were the first to provide direct evidence that ROS may act as endogenous vasodilators in the cerebral circulation. Specifically, they reported that dilatations of feline cerebral arterioles to AA are inhibited by a combination of SOD and catalase, indicating that dilatations are partly mediated by O_2^- or H_2O_2 or both (or a downstream ROS thereof) (32). Such a role for ROS in mediating the vasoactive effects of AA has since been confirmed in cerebral arteries from a number of different species, including rat, rabbit, and mouse (20, 51, 54, 55). Consistent with observations in feline cerebral arteries, treatment of rabbit cerebral arterioles with SOD and catalase inhibits dilations to AA (20). Moreover, indomethacin inhibited the effects of AA, suggesting that COX activity is essential for ROS-mediated dilatation (20). Didion *et al*. (15) described a role for H_2O_2-induced dilatation of rabbit basilar arteries in response to AA. Moreover, they reported that inhibition of CnZn-containing SODs attenuated those dilatations, highlighting the key importance of endogenous SODs in ROS-mediated responses to some endogenous factors (15). In contrast, dilatations of mouse arterioles to AA are mediated by OH^-, because responses were attenuated by deferoxamine or SOD plus catalase (51). In rat pial arterioles *in vivo*, we have shown that AA-induced dilatations are abolished by indomethacin or catalase and are attenuated by the OH^- scavenger, deferoxamine, suggesting that COX-derived ROS (H_2O_2 and OH^-) play an important role in mediating these effects (55). Thus although all of these studies imply that endogenous ROS mediate the vasodilator effects of AA, it also is apparent that the identity of the ROS responsible may differ between species, presumably reflecting differences in the rate of O_2^- production or metabolism or both.

In addition to our studies on AA, we also reported that H_2O_2 contributes to the vasodilator effects of bradykinin in rat pial arterioles (54), suggesting that ROS may play a role in endothelium-dependent dilator responses in the cerebral circulation. ROS have since been implicated in the dilator effects of bradykinin in feline (31) and mouse cerebral arterioles (51); however, as with responses to AA, it appears that the identity of the ROS molecule responsible differs between species. Recently it has been described in both mouse (38) and human systemic arteries (37, 42) that H_2O_2 acts as an endothelium-derived hyperpolarizing factor (EDHF), mediating dilatation to acetylcholine and shear stress, respectively. As discussed, some evidence indicates that H_2O_2 hyperpolarizes VSM cells via the activation of potassium channels; however, at present, it is unclear whether H_2O_2 acts as an EDHF in cerebral arteries.

In the systemic circulation, vascular NADPH-oxidases have been studied extensively and are generally associated with blood vessel pathology rather than physiology (7, 40). It is not known what role, if any, is played by NADPH-oxidase–derived ROS in modulating vascular tone in the systemic circulation under physiologic conditions. In contrast, findings from recent studies have raised the possibility that NADPH-oxidase–derived ROS may serve as an important physiologic vasodilator mechanism in the cerebral circulation. As discussed, application of NADPH or NADH elicits a large increase in O_2^- production by cerebral arteries from a number of different species (14, 39, 44, 49). We recently reported that basal, angiotensin II-, and NADPH-stimulated O_2^- generation from NADPH-oxidase is one to two orders of magnitude greater in rat cerebral arteries (basilar and middle cerebral) than in a wide range of systemic arteries, and that this is associated with higher Nox4 expression (39). In systemic arteries, activation of NADPH-oxidase is generally thought to increase vascular tone via the inactivation of endothelium-derived NO (13, 56). Somewhat surprisingly, activation of NADPH-oxidase in cerebral arteries from rats, rabbits, and mice elicits profound cerebral vasodilatation *in vitro* and *in vivo* (14, 39, 44, 49). The identity of the NADPH-oxidase–derived ROS responsible for mediating cerebral vasodilatation to NADPH has not been fully clarified but seems likely to be H_2O_2. We have reported that NADPH-induced dilatations of rat basilar arteries *in vivo* and *in vitro* were inhibited by catalase and DETCA, suggesting that H_2O_2 (or a downstream ROS) generated from the dismutation of O_2^- by CuZn-containing SOD was responsible for the vasodilatation

(39, 44) (Fig. 4). In contrast, a recent study in mice found that O_2^-, and not H_2O_2, was partially responsible for cerebral vasodilatation to NADPH (49) (Fig. 4).

One of the most important attributes of the vascular NADPH-oxidases is not only that they are basally active, but also that their activity can be enhanced by numerous humoral (29, 39) and physical factors (12, 27), making them and their ROS products ideal secondary messengers or paracrine signalling factors or both. It has been demonstrated extensively in noncerebral arteries that the activity of NADPH-oxidase is enhanced by angiotensin II, thrombin, platelet-derived growth factor, and tumour necrosis factor-α (25). Similarly, we have found that angiotensin II potentiates vascular O_2^- generation from NADPH-oxidase in cerebral arteries (39). Thus vasodilator ROS production stimulated by angiotensin II in cerebral arteries would be expected to offset and hence protect against angiotensin II–induced cerebral vasospasm. In endothelial cells, mechanical forces, including cyclic stretch and laminar and oscillatory shear stress, also have been shown to increase NADPH-oxidase activity (12, 27). Moreover, we recently reported that NADPH-oxidase–derived ROS partly mediate flow-dependent responses of the basilar artery *in vivo* (46) (Fig. 4). Thus it is possible that NADPH-oxidase–derived ROS serve as important signalling molecules in endothelial cells of the cerebral circulation in response to endogenous stimuli. Consistent with this hypothesis, we have found that catalase can substantially and selectively augment cerebral vasoconstriction by angiotensin II (39), and that DPI and catalase can constrict the rat basilar artery *in vivo* (44) (Fig. 4).

PERSPECTIVES

Over the past decade, ROS have been increasingly linked with vascular disease and are generally considered as damaging and proconstricting molecules within blood vessels. Although their deleterious role in the development of cerebrovascular disease is at present undisputed, it is becoming increasingly apparent that this concept may be an oversimplification that masks the importance and intricacy of the functions of ROS molecules in cell signalling for the control of cerebrovascular tone under normal physiologic conditions. As discussed in this review, compelling evidence now suggests that ROS may play an important role in physiologic vasodilator responses within the cerebral circulation. Of importance, NADPH-oxidases are now emerging as a potential source of dilatory ROS molecules in the cerebral circulation under physiologic conditions. Although this concept is still quite speculative, if NADPH-oxidase is to be confirmed as a major source of beneficial vasodilator ROS in cerebral arteries, it could represent a significant dilemma for the treatment of vascular diseases commonly associated with excessive NADPH-oxidase activity.

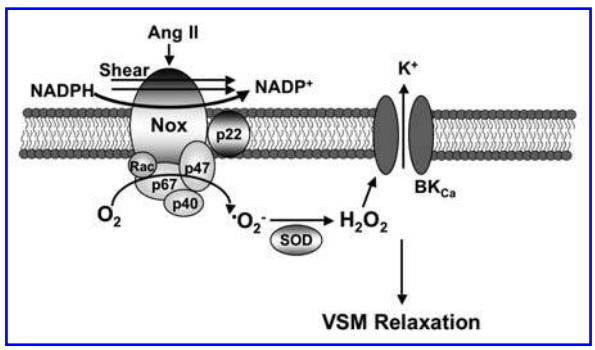

FIG. 4. Schematic diagram showing the proposed physiologic mechanism by which vascular NADPH-oxidases mediate cerebral vascular smooth muscle (VSM) cell relaxation. Activation of NADPH-oxidases by either angiotensin II (Ang II), NADPH, or increases in shear stress (Shear) enhances superoxide (O_2^-) production, which is converted to hydrogen peroxide (H_2O_2) by endogenous O_2^- dimutases (SOD). H_2O_2 then is thought to mediate VSM cell relaxation at least partly through activation of large-conductance calcium-activated potassium channels (BK_{ca}), resulting in potassium efflux.

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ABBREVIATIONS

Arachidonic acid, AA; CuZn-containing cytosolic superoxide dismutase, SOD1; CuZn-containing extracellular superoxide dismutase, SOD3; cyclooxygenase, COX; diethyldithiocarbamate, DETCA; diphenylionodium, DPI; endothelial nitric oxide synthase, eNOS; hydrogen peroxide, H_2O_2; hydroxyl, $OH^{\bullet}$; mitochondrial superoxide dismutase, SOD2; nitric oxide, NO; nitric oxide synthase, NOS; peroxynitrite, $ONOO^-$; reactive oxygen species, ROS; superoxide, O_2^-; superoxide dismutase, SOD; tetrahydrobiopterin, BH_4; VSM, vascular smooth muscle.

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