Reactive oxygen species: influence on cerebral vascular tone

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Reactive oxygen species (ROS) have multiple effects on vascular cells. Defining the sources and the impact of reactive oxygen species within the vessel wall has emerged as a major area of study in vascular biology. Oxygen-derived free radicals are a subgroup of ROS that contain one or more unpaired electrons and include species such as superoxide radical (superoxide anion) and hydroxyl radical. Included within the larger group of ROS are also some species that are not radicals such as hydrogen peroxide (H$_2$O$_2$). Recent work suggests that H$_2$O$_2$ may be an important signaling molecule within blood vessels. In addition to ROS, there are also reactive nitrogen species, which include peroxynitrite and the much-studied nitric oxide.

Superoxide is formed from molecular oxygen and is a precursor for several ROS and reactive nitrogen species (Fig. 1). There are multiple sources of superoxide within vascular cells, including mitochondria, cyclooxygenases, NAD(P)H oxidases, xanthine oxidases, lipoxygenases, and, under special circumstances, nitric oxide synthases (37, 42).

This review will focus on select findings related to effects of ROS on cerebral vascular tone. In addition to highlighting effects of different ROS, the consequences of the molecular interaction of superoxide with nitric oxide and arachidonic acid will also be discussed.

**SUPEROXIDE**

The effects of superoxide on vascular tone are complex. The radical may have direct and indirect effects on vascular muscle, and available data suggest that both relaxation and contraction of vascular muscle may occur but are dependent on the model and the circumstances. For example, superoxide (generated by using xanthine and xanthine oxidase in the presence of catalase to remove H$_2$O$_2$) produces dilation of cerebral arterioles (58). Similarly, NADH or NADPH [used to activate NAD(P)H oxidase] increases formation of superoxide and produces relaxation of cerebral vessels, which is inhibited by scavengers of superoxide but not by catalase (11, 47). Relaxation of these blood vessels in response to superoxide appears to be mediated by potassium channels (11, 58), a major dilator mechanism in the cerebral circulation (21, 22). Generation of even higher concentrations of superoxide with NADPH or greater concentrations of NADH produces contraction of cerebral arteries (11), raising the possibility that responses to superoxide might be biphasic, with relaxation at lower concentrations and contraction of vascular muscle in response to high levels of the radical. Consistent with this possibility, generation of superoxide using acetaldehyde and xanthine oxidase produces dilation of cerebral arterioles at low substrate concentrations but vasoconstriction at higher substrate concentrations (48).

In addition to potential direct effects, superoxide may affect the tone of blood vessels via its interaction with nitric oxide, a potent vasodilator. Nitric oxide reacts with superoxide at a rate three times faster than the dismutation of superoxide by superoxide dismutases (SODs) (20). Because superoxide reacts with nitric oxide more efficiently than with any other known molecule, the local concentration of superoxide is a key determinant of the biological half-life of nitric oxide. The reaction with and inactivation of nitric oxide by superoxide results in the loss of the vasodilator influence of nitric oxide on vascular tone.
Because the tone of cerebral blood vessels is influenced by nitric oxide under resting conditions, the loss of nitric oxide bioavailability produces vasoconstriction (21). In addition, the reaction of nitric oxide with superoxide produces a new molecular species, peroxynitrite, which may have its own effect on vascular tone (see below) (Fig. 1).

Few studies have examined direct effects of superoxide on cerebral vascular muscle using vessels without endothelium and in the absence of other ROS. A recent report using pyrogallol to generate superoxide described contraction of basilar arteries (without endothelium) that was sensitive to SOD but not catalase (55). These findings suggest that superoxide has the ability to directly contract smooth muscle in cerebral arteries.

There is some evidence for superoxide-mediated constriction of cerebral blood vessels in models of vasospasm (for examples, see Refs. 29, 31, 39, 57) and Alzheimer’s disease (43, 46). To what extent these effects are due to the direct effect of superoxide on vascular muscle, the interaction of superoxide with nitric oxide, or other mechanisms has not been fully defined.

Superoxide may also affect endothelium-dependent relaxation while having little or no detectible effect on resting vascular diameter (vascular tone) in vivo. For example, there is superoxide-mediated impairment of responses of cerebral blood vessels to agonists that produce endothelium-dependent relaxation (in the absence of significant changes in resting vascular diameter) in models of diabetes, alcoholism, hyperhomocysteinemia, and genetic deficiency in SOD (9, 13, 14, 17, 54). Precisely how superoxide may impair endothelial function without an alteration in resting vascular tone is unclear. One possibility might be concentration-dependent differences in the effects of superoxide on baseline vessel tone vs. endothelium-dependent relaxation (a nitric oxide-mediated response). Perhaps higher concentrations of superoxide are needed to affect baseline vessel diameter than are needed to impair endothelium-dependent relaxation. Another possibility could relate to differences in the subcellular localization and thus the impact of superoxide on signaling within or between vascular cells.

H2O2

Of the various ROS, the effects of H2O2 on vascular tone have been the most fully studied. Exogenously applied H2O2 produces relaxation of cerebral arteries in vitro (23, 27, 63, 64). In vivo, application of H2O2 produces dilation of the basilar artery of the rat (45). The mechanism that accounts for this vasodilator response is not entirely clear, however, as multiple divergent explanations have been proposed. Relaxation of cerebral arteries in response to H2O2 has been reported to be endothelium dependent (63), to be unaffected by removal of endothelium (23), and to occur only in the absence of endothelium (27). The latter response was described in canine basilar arteries in which H2O2 produced endothelium-dependent contraction in intact arteries (30) but marked relaxation in arteries without endothelium (27, 30). Even within the same vessel, the reports are not consistent (30, 63). When direct relaxation effects of H2O2 on vascular muscle have been observed, there is strong evidence that the response is mediated by potassium channels (27). Direct electrophysiological recordings have shown that H2O2 can increase activity of calcium-activated potassium channels in cerebrovascular muscle (7).

In contrast to the more divergent responses seen in large cerebral arteries in vitro, in vivo studies have consistently described dilator responses of small cerebral arteries and arterioles to H2O2 (35, 52, 58, 59, 62). Very high concentrations of H2O2 can produce vasoconstriction followed by vasodilation (35), reminiscent of the biphasic effects of superoxide on vascular tone (see SUPEROXIDE). The pharmacological profile obtained suggests that dilator responses of cerebral blood vessels to H2O2 are mediated by activation of potassium channels (45, 52, 58).

REACTIVE OXYGEN SPECIES AS ENDOTHELium-DErIVED RELAXING FACTORS

In vivo, H2O2 is formed primarily from superoxide as a result of activity of the various SODs (Fig. 1). The importance of H2O2 within the vessel wall is becoming increasingly apparent (5). For example, H2O2 may function as an endothelium-derived hyperpolarizing factor (EDHF) in some blood vessels (3, 51, 56). In other arteries, H2O2 may function as an endothelium-derived relaxing factor without producing hyperpolarization of underlying vascular muscle (i.e., without functioning as an EDHF) (6).

Experiments performed both in vitro and in vivo demonstrate that exogenously applied H2O2 can produce relaxation of cerebral blood vessels (see H2O2). Studies of ROS are sometimes criticized for the use of high concentrations of H2O2, and the question of whether such levels of H2O2 can be produced endogenously is not clear. For example, some experiments have used millimolar levels of H2O2 (35). In normal cells, antioxidants such as glutathione peroxidases and catalases are thought to keep subcellular levels of H2O2 relatively low. In addition, increasing evidence suggests that the effects of ROS may be compartmentalized, so the application of exogenous
H$_2$O$_2$ to the outside of cells may not precisely mimic effects seen in vivo. Thus studies using stimuli that produce endogeneous formation of ROS are critical in understanding the functional importance of ROS.

Both arachidonic acid and bradykinin produce dilation of cerebral arterioles that is mediated by endogeneous formation of ROS (33, 34, 52, 53, 62). Studies using catalase suggest that the mediator of these responses is H$_2$O$_2$ (34, 52, 53, 62). This vasodilatation to bradykinin is increased by SOD (which enhances formation of H$_2$O$_2$) (62) and impaired by a pharmacological inhibitor of SOD (which would reduce formation of H$_2$O$_2$) (12). These findings are all consistent with the concept that H$_2$O$_2$ is the mediator of the response. Because bradykinin-induced dilation of cerebral vessels is endothelium dependent (18, 21, 49), the data suggest that H$_2$O$_2$ is the relaxing factor that mediates the response (Fig. 2). Dilation of cerebral vessels to arachidionate also appears to be endothelium dependent (44) and is mediated by H$_2$O$_2$ (53) (Fig. 2). Relaxation of vascular muscle to H$_2$O$_2$ is produced by activation of potassium channels (34, 52, 53) (Fig. 2). These findings are important because they suggest that responses to an endothelium-dependent agonist (bradykinin) is mediated by endogenously produced H$_2$O$_2$ in cerebral arterioles.

As noted above, H$_2$O$_2$ is formed primarily from superoxide as a result of activity of the various SODs. In blood vessels, there are isoforms of SOD: cytosolic or copper-zinc SOD (CuZn-SOD), manganese SOD localized in mitochondria (Mn-SOD), and an extracellular form of CuZn-SOD (EC-SOD) (20). It will be of interest to establish which isoform(s) of SOD is the primary source of H$_2$O$_2$ that affects cerebral vascular tone. A recent study of mesenteric arteries suggested that CuZn-SOD is an important source of H$_2$O$_2$ produced in response to acetylcholine (40).

In contrast to bradykinin and arachidonic acid, dilation of cerebral vessels to acetylcholine (32, 38) and ATP (64) is not affected by SOD and catalase, suggesting no involvement of H$_2$O$_2$ or other ROS. Thus the role of H$_2$O$_2$ as an endothelium-derived relaxing factor is selective for only some vasoactive stimuli.

Although the studies outlined above suggest that H$_2$O$_2$ functions as an EDHF in the cerebral microcirculation (34, 52), it has been reported that hydroxyl radical is a mediator of endothelium-dependent responses to bradykinin in cerebral arterioles (50). This latter possibility is more difficult to envision as a normal signaling mechanism if some degree of selectivity is to be achieved. Hydroxyl radical is very short lived and extremely reactive, making it unlikely that this radical is able to diffuse intact across membranes of one cell type to another before initiating relaxation in vascular muscle.

**PEROXYNITRITE**

Although the number of studies that provide evidence that peroxynitrite is produced in the brain or cerebral blood vessels under various pathophysiological conditions continues to increase, relatively few have examined what direct effects peroxynitrite may have on vascular tone. Wei et al. (58) first demonstrated that relatively low concentrations of peroxynitrite produce dilation of cerebral arterioles in vivo. The pharmacological profile obtained suggested that this response was mediated by ATP-sensitive potassium channels (58). Peroxynitrite can also dilate cerebral arteries in vitro (10, 36), and experiments using cerebral arteries suggest that this response is endothelium independent (36). In contrast, other studies reported that peroxynitrite produces contraction of cerebral arteries in vitro via an inhibitory effect of basal activity of calcium-activated potassium channels (4, 16).

In addition to direct effects on vascular tone, peroxynitrite may alter responses to other vasoactive stimuli. For example, peroxynitrite impairs myogenic responses of cerebral arteries as well as vasodilation to calcitonin gene-related peptide and cromakalim (10), all stimuli thought to produce dilation of cerebral vessels via activation of ATP-sensitive potassium channels (22). Thus the effects of peroxynitrite are potentially complex and poorly understood. Although peroxynitrite may dilate cerebral vessels via activation of potassium channels, it also may impair responses that are mediated by the same or other potassium channels.

Hopefully, future studies will provide insight into the effect of physiological concentrations of peroxynitrite in the appropriate subcellular compartment. However, as noted above for ROS and H$_2$O$_2$, it is not clear what concentrations of peroxynitrite are produced endogenously within vascular cells. Presently, few data exist that address the functional importance of endogenously produced peroxynitrite on cerebral vascular tone. Whereas some studies employing ebselen as a scavenger of peroxynitrite have implicated peroxynitrite in the regulation of cerebrovascular tone (61), this drug is known to have additional effects that make definitive conclusions difficult.

**ISOPROSTANES**

Isoprostanes are a group of compounds formed primarily from the nonenzymatic interaction and peroxidation of arachidonic acid by oxygen-derived free radicals, including superoxide (Fig. 1) (8). The most studied of these arachidonic acid metabolites is the group known as the F$_2$ isoprostanes. Isopros-
tanes are now being commonly measured as markers of oxidative stress (41). Some have biological activity and may also be mediators of vascular disease (41). An example of one of the most studied isoprostanes is 8-iso-PGF_2α (or 15-F_2-isop) (8, 41).

Some isoprostanes produce constriction of cerebral microvessels (24–26). Although this effect is mediated by a thromboxane-like receptor, it may be mediated by a distinct receptor for isoprostanes (25, 26). Effects of isoprostane on vascular tone may also be indirect and mediated by release of thromboxane from endothelium (25, 26). Thus responses to isoprostanes may be endothelium dependent and mediated by an endothelium-derived contracting factor.

In summary, exogenously applied and endogenously produced ROS have significant effects on cerebral vascular tone. Both direct effects of ROS on vascular muscle as well as indirect effects on other cell types have been described. It is important to recognize that relatively low concentrations of ROS may function as signaling molecules (15) and may be involved with normal regulation of cerebral vascular tone and structure. Although an increasing number of studies address the impact of ROS on vascular tone in models of disease, many studies on direct effects of ROS have been performed using normal blood vessels, not blood vessels with disease.

Mechanisms by which ROS affect vascular tone may be quite complex. For example, recent work suggests that ROS generated within mitochondria can produce vasodilation via formation of calcium sparks in vascular muscle (60). In contrast, ROS also have the potential to inhibit calcium sparks (28). The interaction of ROS with other molecules such as nitric oxide and arachidonic acid can also alter vascular tone. Although this discussion has focused on the acute regulation of vascular tone, ROS may also have more chronic effects within the vessel wall by influencing gene expression and inflammation (19), vascular permeability (1), and vascular structure (2).

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REFERENCES

ROS AND THE CEREBRAL CIRCULATION

Invited Review


