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Reactive Oxygen Species Cerebral Autoregulation in Health and Disease

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Abstract

Superoxide and other oxygen radicals (ROS) derived from the oxidative metabolism of L-arginine influence cell signaling and gene expression. In some tissue ROS stimulate structural changes, such as proliferation, hypertrophy, or remodeling. ROS production within the developing CNS stimulates excitation and in vascular tissue causes contraction. The effect of ROS-induced autoregulatory failure in the CNS following hypoxic-ischemic encephalopathy, seizures, trauma, or stroke in children leads to acute mortality and chronic morbidity.

Production and effects of ROS on the cerebral vasculature

Regulation of nutritive blood flow to metabolically active tissue is a vital process supplying substrate for enzymatic production of intermediates to maintain cellular homeostasis. In the brain, neuronal metabolism relies almost exclusively on oxidative pathways requiring adequate delivery of oxygen and glucose. Under normal ranges of physiological blood pressure, cerebral blood flow to the brain remains constant despite fluctuations in transmural pressure. This autoregulation is largely a function of signaling events in the vessel wall such that increasing arterial pressure depolarizes and activates arterial muscle keeping flow relatively constant (1–4).

Reactive oxygen species are believed to be involved in cellular signaling in blood vessels in both normal and pathological states. The major pathway for the production of ROS is by way of the one-electron reduction of molecular oxygen to form an oxygen radical, the superoxide anion ($O_2^{\cdot -}$). Within the vasculature, there are several enzymatic sources of $O_2^{\cdot -}$, including xanthine oxidase, the mitochondrial electron transport chain, and nitric oxide (NO) synthases (5). Studies in recent years, however, suggest that the major contributor to $O_2^{\cdot -}$ levels in vascular cells is the membrane-bound enzyme NADPH-oxidase (6). Produced $O_2^{\cdot -}$ can react with other radicals, such as NO, or spontaneously dismutate to produce hydrogen peroxide (H_2O_2) (7). In cells, the latter reaction is an important pathway for normal $O_2^{\cdot -}$ breakdown and is usually catalyzed by the enzyme superoxide dismutase (SOD). Once formed, H_2O_2 can

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undergo various reactions, both enzymatic and non-enzymatic. The anti-oxidant enzymes catalase and glutathione peroxidase act to limit ROS accumulation within cells by breaking down H_2O_2 to H_2O. Metabolism of H_2O_2 can also produce other, more damaging ROS (8). For example, the endogenous enzyme myeloperoxidase uses H_2O_2 as a substrate to form the highly reactive compound hypochlorous acid. Alternatively, H_2O_2 can undergo Fenton or Haber-Weiss chemistry, reacting with Fe^{2+}/Fe^{3+} ions to form toxic hydroxyl radicals ($^{-}\cdot OH$) (8).

ROS are involved in oxidation of lipoproteins, modulation of apoptosis, upregulation of adhesion molecule expression, and activation of the processes involved in vascular remodeling, such as enhancement of vascular smooth muscle growth and activation of matrix metalloproteinases (9). In addition, one of the most powerful acute effects of ROS in the vasculature is the alteration of vascular smooth muscle tone. There is evidence that $O_2^{\cdot -}$ can not only constrict (10), but can also dilate cerebral arteries (11). Moreover, several studies have demonstrated clearly that small cerebral arterioles relax in response to the $O_2^{\cdot -}$ metabolite H_2O_2 (12,13). H_2O_2 thus causes powerful dilatation of cerebral arterioles whether applied exogenously or generated endogenously within the vascular wall in response to agonist, such as bradykinin or arachidonic acid (14,15). These dilator responses seem to be mediated primarily by cyclooxygenase-derived ROS, which open potassium channels in the vascular smooth muscle cell membrane to cause hyperpolarization and thus relaxation (14–16). In the cerebral circulation, therefore, ROS such as $O_2^{\cdot -}$ and H_2O_2 could possibly either dilate or even exert opposing effects on vascular tone. The balance between $O_2^{\cdot -}$ and H_2O_2 in the vascular wall is regulated by the expression and activity of endogenous SOD.

The role of cytochrome P450 enzymes for the production of ROS

The cytochrome P450 (CYP) enzymes are membrane-bound, heme-containing terminal oxidases that are bound in organism from Archaebacteria to humans. These enzymes are responsible for the metabolic activation or inactivation of most type of drugs and toxins. CYP enzymes are capable of metabolizing endogenous arachidonic acid (AA) into vasoreactive products and are therefore are often referred to as the third pathway of AA metabolism (cyclooxygenases and lipooxygenases being the other two pathways). Much attention thus have been focused on the role of CYP enzymes in vascular homeostasis (17). In addition to the production of vasoreactive AA metabolites, CYP enzymes also generate ROS, such as $O_2^{\cdot -}$, H_2O_2. For example, released free AA following stimulation of astrocytes with glutamate is converted to epoxyeicosatrienoic acid (EETs) by microsomal epoxygenases (CYP 2C11) or PGI2/Tx by cyclooxygenases. Both these processes generate $O_2^{\cdot -}$, which is further metabolized to H_2O_2 by superoxide dismutase (SOD). Similarly, stimulation of vascular smooth muscle cell by pressure, stretch, flow and so forth, triggers the release of AA, which is metabolized to 20-HETE by CYP 4A enzyme that can also generate $O_2^{\cdot -}$, which is further metabolized to H_2O_2 by SOD. ROS thus are produced during metabolism of AA by CYP enzymes and may play an important role in regulation of the tyrosine kinase pathways. Additionally, another recent report provides strong evidence that $O_2^{\cdot -}$ participates in the endothelium-derived hyperpolarizing factor (EDHF) response of CYP-derived EETs, suggesting that an endothelial epoxygenase homologous to human CYP 2C8/9 is the source of $O_2^{\cdot -}$ in coronary arteries (17). Release of inhibitory radical species would be expected to inhibit pressure-induced myogenic tone. On the other hand, $O_2^{\cdot -}$ has been shown to inhibit NO production, which would enhance myogenic tone (18). Given the potential sources of ROS formation in the form of NADPH P450 oxidases in the cerebral arterial wall, it is important that we define the role of ROS on myogenic mechanisms in the cerebral circulation.

Action of ROS on ion channels

Maintenance of cellular ionic gradient is essential to cell survival and function. Apart from maintaining osmotic equilibrium, ion channels are co-transporters that mediate the movement of ions against electrical and concentration gradients and regulate plasma and mitochondrial membrane potential. Plasma membrane potential controls many cell-specific processes. In the brain, the membrane potential controls the active state of arterial muscle, release of paracrine substances from vascular and capillary endothelial cells, neuronal activity, and multiple processes in astrosytes. With respect to arteriolar muscle there are four major K^+ channel isoforms: Ca^{2+}-activated K^+ channels, delayed rectifier K^+ channels, inwardly rectifying K^+ channels, and ATP-sensitive K^+ channels (19). Upon patch clamping freshly isolated cerebral arteriolar muscle the major K^+ channel isoform present is K_{Ca}. If analogy with other excitable cells is assumed, it is the inwardly rectifying K^+ channel that is largely responsible for setting the level of membrane potential (19,20). Inhibition of K^+ channels depolarizes cerebral vascular muscle as defined by the K^+ equilibrium potential, however (21,22). The major voltage sensitive Ca^{2+} channel in cerebral arterial muscle is the L-type Ca^{2+} channel (23). To date we have very little evidence for rapidly inactivating T-type Ca^{2+} channels in cerebral arterial muscle. There are also several Cl^- channels in cerebral arteriolar muscle, and there is increased interest in them with respect to regulation of membrane potential and other cellular processes (24). In this review wefocus on K_{Ca} channel.

Free radicals exert direct and indirect actions on ion channels. The redox status of channel proteins has been hypothesized to affect the ion channel activity in arterial muscle (25,26). This hypothesis states that the balance between oxygen and its reactive species functions as an oxygen sensor through actions on ion channels (26,27). In this regard K^+ channels have been shown to be sensitive to H_2O_2 (26,28–31). H_2O_2 has been demonstrated to hyperpolarize arterial muscle by way of activation of maxi K_{Ca} by direct and indirect mechanisms (15,26, 28–31). In cat, H_2O_2 has been shown to activate ATP sensitive K^+ channels (11). A recent report provides evidence that H_2O_2 can function as an EDHF (31). ROS repeatedly have been demonstrated to modulate $[Ca^{2+}]_i$ on agonist stimulation of L-type Ca^{2+} channels, which could be either the primary or secondary target (32). The literature on free radicals and direct action on ion channels as determined by patch clamp or direct measurement of membrane potential is not extensive. Given the importance of membrane potential on cellular control mechanisms, however, this is an area of active investigation.

There are many indirect actions of ROS on vascular membrane potential. Activation/inhibition of ion channels to a large extent depends on channel protein phosphorylation. Both upstream regulators of protein/tyrosine kinases and direct effects on their translocation and activation have been shown to be sensitive to reactive radical species. Tyrosine kinases and phosphatases are targets of H_2O_2 as activators and inhibitors (33). H_2O_2 can activate ERK1/2 and p38 MAPK in the presence of agents such as angiotensin (9,34). H_2O_2 also can mediate EGF-induced activation of phospholipase C (PLC) (35). In general, phospholipases are targets of ROS. PLC activity regulates diacilglycerol (DAG) levels, which in turn activates and induces translocation of protein kinase C (PKC) (36). Recent literature demonstrates that ion channel phosphorylation is mediated by kinase activity (37,38). Alteration of PKC, either through DAG or directly, and tyrosine kinase activities induced by ROS would be expected to modulate ion channel activity, resulting in membrane potential responses with downstream modulation of cell functions under membrane potential influence. Similarly, modification of PLC activity changes levels of intracellular inositol triphosphate (IP_3) affecting Ca^{2+} release from internal stores.

Action of ROS on K_{Ca} channel activity in cerebral arterial muscle cells

Activation of arterial smooth muscle is regulated by the level of membrane potential. The plasma membrane potential is set by unequal distribution of ions. Ion species with high relative conductance set the membrane potential in accordance to specific charge and concentration gradients (ie, Nernst potential); in vascular muscle cells K^+ is the dominant species in this regard. Vascular smooth muscle membrane potential is a major influence in defining the level of activation, and in cerebral arterial muscle cells the major determinant of activation and contraction. Indeed, a 1.0 mV reduction in membrane potential initiates a significant increase in active tension with a correlation coefficient relating change in membrane potential to change in active tension of 0.98 (2,3). The mechanisms by which ROS modulate ion channels, including K_{Ca} channel activity in vascular muscle remain largely unexplored. We have shown that the K_{Ca} is a target for $O_2^{\cdot -}$ (39). At this time we do not know how $O_2^{\cdot -}$ enhance K_{Ca} activity; it could act directly on channel proteins or on second messengers, which include PKC and tyrosine kinase, that mediate phosphorylation of K_{Ca} channels. Generation of H_2O_2 also seems to activate K_{Ca} (Debebe Gebremedhin, PhD, and David R. Harder, PhD, unpublished data, 2005). Both H_2O_2 and $O_2^{\cdot -}$ thus act to increase single-channel K_{Ca} activity in cerebral vascular muscle cells.

ROS in the brain: actions on functional hyperemia

The source of ROS in the brain may require CYP enzyme activity and is supported further by data obtained recently in our laboratory using samples of cerebrospinal fluid (CSF) containing the spin trap N-tert-butyl-hydroxylamine, which is selective for $O_2^{\cdot -}$ (Debebe Gebremedhin, PhD, and David R. Harder, PhD, unpublished data, 2005) and support a recent report that an endothelial CYP isoform (C8/C9) generate $O_2^{\cdot -}$ in coronary arteries (40). Given the many cell type in the brain, it is certain that there is no single source of ROS. Whatever the sources of ROS, these free radicals appear to impinge chronically on mechanisms regulating blood flow in the brain; For example, infusion of scavengers, such as SOD, into the CSF increased blood flow as measured by laser-Doppler flowmetry (Debebe Gebremedhin, PhD, and David R. Harder, PhD, unpublished data, 2005)). The effect of SOD on baseline blood flow is most likely attributed to removal of $O_2^{\cdot -}$, which potentially enhances nitric oxide. The increase in blood flow was reversed after the infusion was completed (Debebe Gebremedhin, PhD, and David R. Harder, PhD, unpublished data, 2005). The literature would suggest that the source of cerebral $O_2^{\cdot -}$ produced would be by way of nitric oxide synthase (NOS) through NAD(P)H dependent reductase activity. The CYP NAD(P)H reductase, however, is virtually the same as NOS (NOS is a heme containing protein similar to CYP enzyme system). In summary, scavenging of $O_2^{\cdot -}$ by infusion of SOD modulates the cerebral blood flow in vivo (Debebe Gebremedhin, PhD, and David R. Harder, PhD, unpublished data, 2005), demonstrating that these molecules participate in the regulation of nutritive blood flow in the brain. Even more dramatic is the action of 30 minute subdural infusion of a cocktail of xanthine/xanthine oxidase/catalase (X/XO/Cat, 0.2 mM xanthine/20 mU xanthine oxidase/500 U catalase). We have shown that under control conditions there is significant autoregulation of CSF; however, there is complete inhibition of CSF autoregulation on elevation of arterial pressure following infusion of a cocktail designed to generate excess $O_2^{\cdot -}$ as shown by increased fluorescent intensity of ethidium bromide produced by $O_2^{\cdot -}$ from dihydroethidium (39).

When a membrane-enriched homogenate is exposed to H_2O_2 (xanthine/XO) there is a marked and significant reduction in 20-HETE formation by way of CYP ω-hydroxylase (Debebe Gebremedhin, PhD, and David R. Harder, PhD, unpublished data, 2005), which is one of the primary mediators of pressure-induced activation of cerebral arteries (41,42). The action of $O_2^{\cdot -}$ in inhibiting 20-HETE production in a membrane-enriched solution in which all

conditions are optimized is most likely attributable to a direct action on CYP ω-hydroxylase activity.

Possible pathogenetic role of intracisternally generated ROS

Subarachnoid hemorrhage (SAH) results in a high mortality rate; 15% of patients who have SAH die before reaching the hospital and 30 % die within 24 hours of onset (43). Patients who survive the initial hemorrhage and overcome vasospasms frequently experience persistent cognitive deficits, psychosocial impairments, and a decrease in quality of life as a result of acute brain injury (44). Most of the research has focused on the late phase, however, when vasospasm occurs, whereas the mechanisms of acute brain injury are poorly understood.

Lipid peroxidation and other consequences of increased levels of ROS have been implicated in the cause of cerebral vasospasm after SAH (45,46). The primary contributor to ROS production after SAH is the autooxidation within the subarachnoid space of oxyhemoglobinto met-Hb (47). As a direct product of this redox reaction, $O_2^{\cdot -}$ is converted to highly reactive hydroxide anion ($^{-}{\cdot}OH$) through the metal-catalyzed Haber-Weiss and Fenton reactions (48, 49). In support of this theory that ROS are primary pathogens for SAH, various antioxidants have been shown to attenuate cerebral vasospasm in animals and humans (50–54). It also has been shown that intracisternal overproduction of $O_2^{\cdot -}$ may initiate or mediate cerebral arterial vasoconstriction and subsequent structural damage (46). Moreover, administration of ferrous (Fe^{2+}) or ferric (Fe^{3+}) iron chelators was shown to mitigate against cerebral vasospasm, providing evidence that the iron-catalyzed Haber-Weiss and Fenton reactions are involved in the mechanism of ROS generation leading to the occurrence of cerebral vasospasm (55,56). These studies further support the pathogenic role of ROS in cerebral vasospasm after SAH.

In summary, cerebral blood flow is maintained at a constant rate despite fluctuations in arterial pressure. The ability of the cerebral vasculature to autoregulate is primarily the function of the activities of native K_{Ca} channel. The K_{Ca} channel is also a target for several paracrine factors and various physical forces, which alter cerebral tone. Astrocytes are intermediary cell types that function to increase cerebral blood flow to match the metabolic demand of activated neurons. Channelophathy coupled with functional alteration of the mechanisms regulating cerebral blood flow could lead to stroke or cerebral vasospasm. Knowledge of the mechanism by which the functions of the cerebral circulation are regulated will help to develop new therapies for the treatment of pediatric patients suffering from hypoxic injury, trauma, stroke and other cerebral disorders, including acute infections such as meningitis and encephalitis (57).

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