Oxidative Stress and Brain Endothelial Cells 86

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Abstract

In addition to their role in maintaining the anatomical and functional integrity of the blood–brain barrier (BBB) and hemodynamic regulation of blood flow, brain microvascular endothelial cells critically contribute to brain development and homeostasis. Thus, injuries to the brain microvasculature can have deleterious

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I. Laher (ed.), *Systems Biology of Free Radicals and Antioxidants*,
DOI 10.1007/978-3-642-30018-9_93, © Springer-Verlag Berlin Heidelberg 2014

consequences on the neurovascular unit and play an important role in the pathogenesis of several neurological disorders. Reactive oxygen species (ROS) and proinflammatory lipid mediators released either from endothelial cells or from the surrounding brain parenchyma are implicated in endothelial cell injury and vascular damage as well as in regulation of endothelial cell physiological responses. While ROS can initiate an inflammatory response and target membrane lipids, inflammatory lipid mediators can exacerbate oxidative stress by increasing ROS production or can alternatively limit cell damage by homeostatic feedback reactions. This chapter will review the current information pertaining to the role and mechanisms of ROS-dependent regulation of brain endothelial cell function and dysfunction. The role of arachidonic acid, a biologically active and oxidant sensitive polyunsaturated fatty acid, whose levels dramatically increase during inflammation, on ROS generation and subsequent brain vascular damage, will be discussed. Emphasis will be placed on the signaling cooperation by which ROS and arachidonic acid influence brain endothelial cell responses during oxidative stress and inflammation. The clinical significance of ROS- and arachidonic acid-dependent cellular interactions in regulation of brain endothelial cell responses and the therapeutic implications of targeting their signaling effectors will be discussed.

Keywords

Arachidonic acid • Brain endothelial cells • Inflammation • Oxidative stress • ROS

Abbreviations

ASK-1	Apoptosis signal-regulating kinase-1
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
cPLA2	Cytosolic phospholipase A_2
ERK	Extracellular signal-regulated kinase
JNK	c-Jun-N-terminal kinase
NO	Nitric oxide
NO2	Nitric dioxide
PGE_2	Prostaglandin E_2
PGG_2	Hydroperoxy endoperoxide prostaglandin G2
sPLA2	Secreted phospholipase A_2
VEGF	Vascular endothelial growth factor

Introduction

The close anatomical and functional relationship between brain endothelial cells and the remaining neural cells within the neurovascular unit underscores the importance of the cerebral endothelium in brain homeostasis and disease (Park et al. 2003).

Brain endothelial cells release neurotrophic factors essential for neuronal survival and function, while their physical interactions with astrocytes and pericytes regulate endothelium vascular tone and BBB function, ensuring the proper metabolic exchange between blood and the brain parenchyma (Ward and Lamanna 2004; Hawkins and Davis 2005; Iadecola and Nedergaard 2007; Hamel 2006; Abbott et al. 2006). Emerging evidence also indicates a crucial role for brain endothelial cells in regulating neural stem cell functions. In the vascular niches of the brain, the direct contact between neural stem cells and brain endothelial cells, together with a continuous supply of endothelial-derived growth factors, is essential for neural stem cell self-renewal and differentiation (Shen et al. 2004; Kim et al. 2004). Given that the finely tuned communication between endothelial cells and other components of the neurovascular unit ensures the anatomical and functional integrity of the brain, injuries to brain endothelial cells can have dramatic clinical consequences and contribute to the pathogenesis of diverse neurological disorders (Rizzo and Leaver 2010). Thus, acute disruption of the anatomical integrity of the BBB, due to endothelial cell damage or death, results in vasogenic edema and tissue damage (Hatashita and Hoff 1990), while chronic injuries to the endothelium appear to have a critical role in the pathogenesis of neurodegeneration (Zipfel et al. 2009; Bell and Zlokovic 2009). Moreover, an injured and dysfunctional brain endothelium can interfere with the formation of new blood vessels and therefore delay the recovery of neurological functions after cerebral ischemia or traumatic brain injury (Beck and Plate 2009; Xiong et al. 2010). On the other hand, stimulation of brain endothelial cell angiogenic responses is a peculiar and essential component of malignant brain tumor development and progression (Plate et al. 1992).

Oxidative stress, a condition induced when ROS production overcomes antioxidant cellular defenses, plays a critical role in the pathogenesis of brain endothelial cell injury and vascular damage; although when appropriately regulated and controlled, ROS production contributes to brain endothelial cell homeostatic functions (Chrissobolis et al. 2011; Dröge 2001). Brain endothelial cells are especially susceptible to oxidative stress-induced injuries because of intrinsic features as well as environmental factors. Compared to endothelial cells from the periphery, brain endothelial cells are enriched in mitochondria and therefore are a potent source of ROS, which in turn can act upon the surrounding brain cellular environment inducing damage or regulating homeostatic functions (Oldenford et al. 1977; Adam-Vizi 2005). On the other hand, the high number of mitochondria renders brain endothelial cells an easy target of endogenously produced ROS (Adam-Vizi 2005). Additionally, brain endothelial cells lie in a microenvironment especially favorable for ROS-dependent cell damage. The brain parenchyma is enriched in highly reactive transition metal ions, such as iron and copper, which catalyze several oxidation-reduction reactions involved in generation of ROS (Jomova and Valko 2011). An additional factor is the availability of polyunsaturated fatty acids, in which neural cell membranes are especially enriched (Hazel and Williams 1990). While some polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA) can have specific protective effects on the brain, others such as arachidonic acid are not only targets of ROS attack but also substrates for enzymatic generation of ROS and therefore

contribute, potentiate, and exacerbate ROS-induced vascular damage or alternatively regulate, in a context-specific manner, endothelial cell homeostatic functions (Zhang and Bazan 2010; Calandria and Bazan 2010; Rapoport 2008).

Increased production of arachidonic acid has been linked to neuroinflammation (Rapoport 2008; Rao et al. 2011). Activation of the phospholipase A_2 enzyme family by proinflammatory cytokines, hypoxia, or ROS results in the release of arachidonic acid, which either directly or via metabolic oxidation to eicosanoids, induce endothelial cell injury and deterioration of vascular functions (Rosa and Rapoport 2009; Adibhatla and Hatcher 2007; Phillis et al. 2006). Interestingly, while in the peripheral vascular system, the transformation of arachidonic acid into prostacyclin is the main mechanism maintaining physiological vasodilation and ensuring delivery of nutrients and oxygen to tissues, brain microvessels produce mainly PGE_2, and both COX-1 and COX-2 are expressed in neonatal brain microvascular endothelial cells under basal conditions and after cytokine challenge (Dusting et al. 1977; Ellis et al. 1979; Parfenova et al. 2002). Thus, while certain arachidonic acid metabolites are involved in regulation of brain microvasculature physiological functions, such as control of vascular tone and blood flow (Niwa et al. 2001; Medhora et al. 2007), arachidonic acid-derived ROS are mostly relevant to cerebral vascular pathology (Armstead 2003).

Evidence obtained from other organs points to a significant degree of cross-talk and cross-regulation between oxidative stress and inflammation (Spychalowicz et al. 2012). Thus, oxidative stress exacerbates endothelial dysfunction caused by inflammation and can contribute to the production of arachidonic acid via several mechanisms, including a direct effect on $cPLA_2$ phosphorylation and $sPLA_2$ release (Goldman et al. 1992; Buschbeck et al. 1999; Jensen et al. 2009). On the other hand, inflammation not only increases the production of ROS but also impairs the antioxidant system further damaging the endothelium (Spychalowicz et al. 2012). Given the relevance of the brain endothelium to brain homeostasis and disease processes and the lack of effective treatments for vascular inflammation, a clearer understanding of the signaling integration and cross-talk between mediators of oxidative stress and inflammation, such as ROS and arachidonic acid, may improve the identification of new signaling targets that can be exploited to prevent or control the clinical consequences of vascular damage in a wide range of neurological disorders.

Detection and Source of ROS in Brain Endothelial Cells

Due to their transient nature and complex cellular distribution and pathophysiology, ROS participating in oxidative stress in the brain microvasculature, as well as in other cellular systems, are difficult to quantitate, and most studies employ downstream markers to monitor oxidative stress (Halliwell and Chirico 1993; Morrow et al. 1990). However, advances in imaging and analytical techniques, including spin trap techniques and mass spectrometry and the development of novel animal models, have improved identification of ROS and their metabolites in brain endothelial cells in situ (Yamato et al. 2003; Sparvero et al. 2010; Maier et al. 2006).

ROS generated by or targeting brain microvascular endothelial cells include oxygen radicals such as superoxide anion, hydroxyl and peroxyl radicals, and non-radicals such as hydrogen peroxide (Miller et al. 2006). Moreover, superoxide reaction with NO generates peroxynitrites, which have direct cytotoxic effects while also inducing cellular damage by decreasing NO availability (Miller et al. 2006).

While production of ROS was initially viewed as an inevitable consequence of cell metabolic activity, the discovery of several enzyme complexes involved in generation of ROS led to the current notion that ROS production is a highly regulated process. In the brain endothelium, NADPH oxidase, the mitochondrial respiratory chain, and xanthine oxidase play a major role in production of ROS (Chrissobolis and Faraci 2008; Zhang and Gutterman 2007; Terada et al. 1991). It should, however, be emphasized that the damaging vascular consequences of ROS are a function of several factors, including their concentrations, their cellular compartmentalization, the activity of the cell detoxification systems, and the surrounding microenvironment (Sies 1993). When defenses against ROS, including superoxide dismutase (which converts superoxide into hydrogen peroxide) and catalase (which converts hydrogen peroxide to water and molecular oxygen), together with other intracellular and extracellular antioxidants such as glutathione and ascorbic acid are functioning, the free radical attack is limited, and resistance and repair mechanisms against oxidative damage are enhanced (Sies 1993). Recent evidence also indicates that endogenously expressed heme oxygenase-2 plays an important role in protecting brain endothelial cells against oxidative stress-induced injuries (Parfenova and Leffler 2008). Therefore, in the presence of a functioning detoxification system, ROS are maintained at low concentrations and as such serve as physiological regulators of endothelial cell functions. On the other hand, when the amount of ROS overcomes the antioxidant system, as during inflammation, ischemia-reperfusion, tumor growth, and γ-radiation-induced cellular stress, endothelial cell injury can occur leading ultimately to tissue damage and organ failure.

Contribution of Arachidonic Acid to ROS Production and ROS-Dependent Cellular Targets

During inflammation, oxidation of arachidonic acid via cyclooxygenases and lipoxygenases contributes to ROS production by the endothelium or by inflammatory and immune cells (Virdis et al. 2005; Cho et al. 2011; Leaver et al. 1995). In the cyclooxygenase reaction cycle, the derived alkoxyl radical intermediate PGG_2 can oxidize a large number of substrates including NADPH to generate superoxide, which can be rapidly transformed by tissue dismutases to hydrogen peroxide (Kukreja et al. 1986; Marnett et al. 1999). Moreover, in a reaction catalyzed by iron, superoxide reacts to produce hydroxyl radicals (Halliwell and Chirico 1993). Importantly, ROS can also be generated by COX-2 via epoxidation reactions that generate cytotoxic peroxyl radicals without the requirement of cyclooxygenase activity and PGG_2 formation (Im et al. 2006). Generation of superoxide from arachidonic acid oxidation into prostaglandins constitutes a relevant source of

ROS in the brain during pathological processes, as shown by the increase of PGE_2 in experimental models of brain injury (Easton and Fraser 1998; Easton and Abbott 2002) or during age-induced impairment of vascular functions (Armstead 2003). Products of 5-lipooxygenase are also involved in mediating arachidonic acid-induced cerebral edema (Yen and Lee 1987).

The involvement of other enzyme complexes in arachidonic acid-induced ROS production in the cerebral vasculature is not well characterized. However, in phagocytes, arachidonic acid is an essential cofactor of superoxide production due to its ability to directly activate NADPH oxidase (Shiose and Sumimoto 2000; Leaver et al. 1995). Interestingly, emerging evidence indicates that oxidative products of arachidonic acid metabolism are direct activators of NADPH oxidase, thus providing additional possibilities for arachidonic acid-dependent contribution and amplification of oxidative stress (Cho et al. 2011). Given the emerging role of endothelial NADPH oxidase in generation of ROS, activation of NADPH oxidase by arachidonic acid could constitute a relevant source of ROS in the brain microvasculature and a point of cross-talk between ROS- and arachidonic acid-dependent cellular responses.

In addition to its contribution to ROS generation, arachidonic acid is a critical target of ROS. Generation of lipid peroxides from ROS attack on arachidonic acid constitutes a major mechanism of ROS-induced cytotoxicity (Halliwell and Chirico 1993; Fraser 2011). Brain endothelial cells are particularly sensitive to lipid peroxides. 4-Hydroxynonenal, a lipid peroxidation product whose levels increase during hypoxia-reperfusion, increases brain endothelial cell vascular permeability and induces endothelial cell apoptosis (Mertsch et al. 2001; Kunstmann et al. 1996). These highly cytotoxic lipid peroxides are produced enzymatically or via auto-oxidation of arachidonic acid (Jian et al. 2005). This latter process is enhanced in the presence of iron and could constitute an important mechanism of lipid peroxidation-induced damage in vivo during hemorrhagic stroke, for example, because of the availability of hemoglobin-derived iron (Wang and Lo 2003).

ROS-mediated peroxidation of arachidonic acid generates iso-eicosanoids, including isoprostanoids and isoleukotrienes. Among isoprostanoids, F2-isoprostane, which is generated independently of cyclooxygenase, is not only the best characterized product of lipid peroxidation but also a useful marker of lipid peroxidation in vivo (Morrow et al. 1990). The isoprostane 8-iso-PGF2α has been shown to cause endothelial cell injury and impairment of neovascularization (Benndorf et al. 2008). Additional isoprostanes, such as 8-iso-PGE_2, which is derived from PGE_2, can also mediate oxidative stress-induced vascular damage during inflammation (Huber et al. 2003). Isomers of leukotrienes, such as B4-isoleukotrienes, are also generated, at least in vitro, via free radical-induced oxidation of arachidonic acid or arachidonate-containing glycerophospholipids (Harrison and Murphy 1995). While these complex molecules act as potent agonists of calcium mobilization in neutrophils (Harrison and Murphy 1995), their relevance to cerebral vascular damage remains to be determined.

Additional free radicals that have harmful consequences on the brain microvasculature are generated from isomerization of arachidonic acid by NO2. These compounds, known as trans-arachidonic acid (TAA) metabolites, are produced

in vivo under a variety of pathological conditions (Jiang et al. 1999). There is substantial evidence in support of a relevant role of TAA metabolites in inducing cytotoxicity of the brain vasculature (Kooli et al. 2008). In a recent study, exposure of newborn pups to hypercapnia was associated with production of TAA metabolites and subsequent degeneration and death of the cerebral microvasculature. Importantly, damage to the cerebral microvasculature resulted in impairment of brain growth (Honoré et al. 2010). The damaging-inducing effects of TAA products are mediated by the production of the proapoptotic and antiangiogenic factor thrombospondin-1 (Kermorvant-Duchemin et al. 2005) (Fig. 86.1).

ROS and Arachidonic Acid in Regulation of Brain Endothelial Cell Physiological Functions

Under normal physiological conditions, ROS are constantly produced and detoxified in the brain environment. There is substantial evidence that ROS produced at low concentrations are required for normal physiological functions of the brain microvasculature acting as signaling molecules and impinging upon homeostatic, prosurvival, and cytoprotective pathways (Luczak et al. 2004; Chan et al. 2009; Lassègue and Griendling 2010) (Fig. 86.1). Studies performed in vivo or in isolated cerebral vessels indicate that exogenously generated or endogenously produced ROS play a critical role as regulators of vascular tone (Wei et al. 1996). This cellular response is of particular importance in the brain where the lack of glucose storage makes this organ uniquely vulnerable and dependent on blood flow for nutrients. The mechanisms involved in physiological regulation of cerebral vessel vascular tone by ROS such as hydrogen peroxide and peroxynitrite include regulation of calcium-sensitive or ATP-sensitive potassium channel opening (Sobey et al. 1997; Fraser 2011) (Fig. 86.1).

Studies of Kontos et al. provided the first evidence that ROS produced endogenously by cerebral vessels are important mediators of physiological vasodilation. Endogenous production of ROS appears also to mediate the vasodilation effects of arachidonic acid and bradykinin in the cerebral arteries in a manner dependent on arachidonic acid metabolism (Kontos et al. 1984). Consistent with this possibility, other studies have shown a role for COX-mediated arachidonic acid metabolism in cerebral vessel vasodilation (Kanu et al. 2006). Moreover, arachidonic acid-induced vasodilation was reduced in a COX-1 knockout mouse model, further indicating that oxidative metabolites of arachidonic acid play a regulatory role in physiological vasodilation (Niwa et al. 2001). The mechanisms of arachidonic acid and PGE_2-induced vasodilation include the release of carbon monoxide in a manner independent of ROS generation (Kanu et al. 2006; Kanu and Leffler 2011). It is important, however, to emphasize that ROS and arachidonic acid-induced responses in the cerebral vasculature are under multicellular control, as the underlying pericytes and associated astrocytes critically contribute to modulate capillary vasoactivity either directly or via the production of soluble factors (Abbott et al. 2006; Hamel 2006; Shimizu et al. 2012).

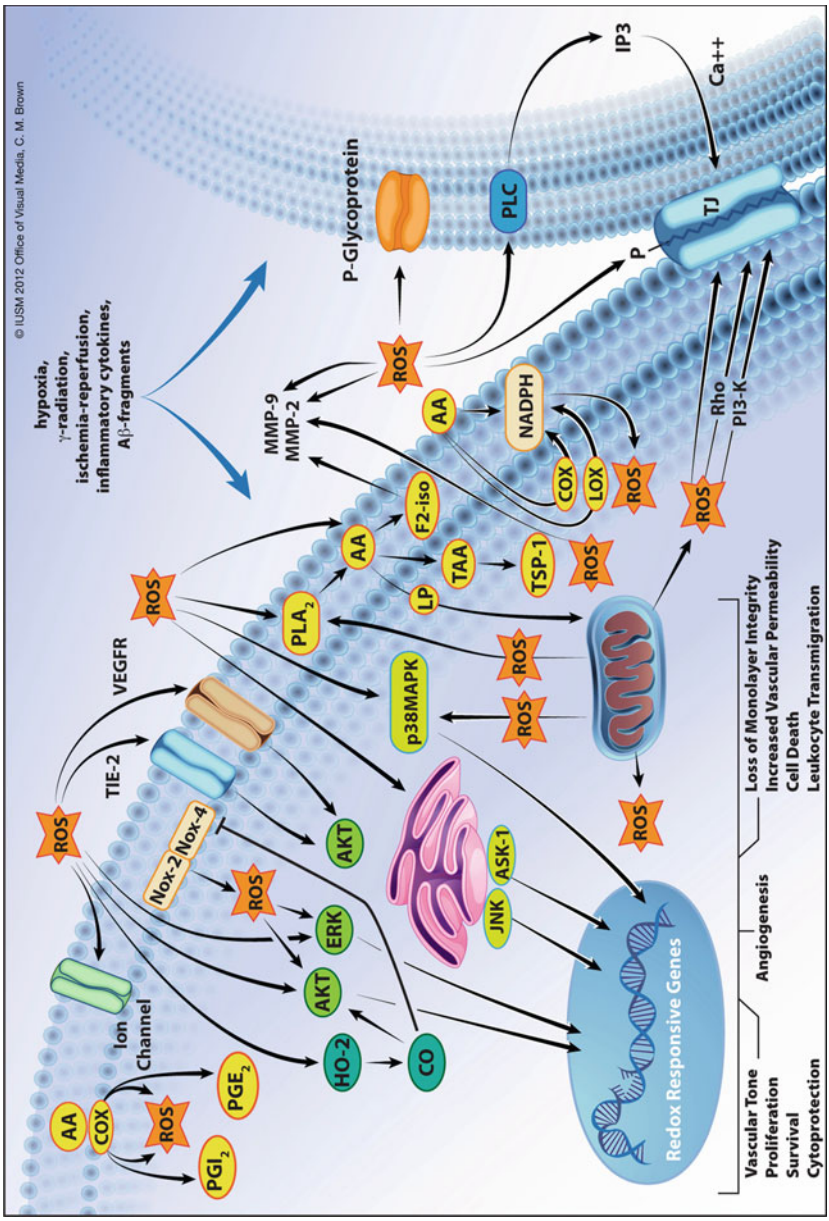

Fig. 86.1 (continued)

In addition to vasodilation, ROS participate in regulation of endothelial cell survival acting as signaling molecules that impact prosurvival or antiapoptotic signaling networks with the outcome of protecting and maintaining the anatomical and functional integrity of the brain endothelium against inflammatory insults. Consistent with this possibility, Basuroy et al. recently demonstrated that production of ROS during TNF-α-induced brain endothelial cell injury resulted in activation of heme oxygenase-2 and subsequent production of the antioxidant carbon monoxide, which in turn protected brain endothelial cell from ROS-induced death by inhibiting Nox4 via AKT-dependent suppression of ERK and p38-MAPK (Basuroy et al. 2011). These intriguing findings underscore a novel role of ROS as signaling effectors that exert not only cytotoxic effects but activate also feedback systems able to restore redox homeostasis and possibly to initiate repair responses.

The intracellular source of ROS responsible for stimulating endothelial cell functions under physiological conditions is not well defined, possibly because it is under the dynamic control and regulation of several factors, including stimuli and environmental conditions. However, the NADPH oxidase system appears to constitute a major source of ROS under physiological conditions in endothelial cells (Chan et al. 2009), while ROS derived from the mitochondrial respiratory chain and generated during inflammation and other cellular insults are mostly linked to endothelial cell damage (Babior 2000). Consistent with a homeostatic role of NADPH, oxidase-derived ROS are the findings showing that expression of NOX2 and NOX4 in brain endothelial cells has been linked to endothelial cell proliferation (Van Buul et al. 2005; Luczak et al. 2004). However, there is

Fig. 86.1 ROS- and arachidonic acid-sensitive signaling interactions and feedback loops in regulation of brain endothelial cell responses. Exogenously or endogenously generated by a variety of agonists, ROS target the brain endothelium either directly or through their interactions with arachidonic acid (AA) and activate signaling pathways that ultimately mediate their consequences on brain endothelial cell function and dysfunction. ROS-induced regulation of ion channels, activation of AKT and ERK, and heme oxygenase–2 (HO–2)–dependent release of carbon monoxide (CO) transmit signals that regulate vascular tone, survival, and cytoprotection. ROS-dependent modulation of angiogenic growth factor receptors, such as Tie-2 and VEGFR, activates downstream effectors responsible for stimulation of angiogenesis. ROS-induced activation of JNK, ASK-1, and p38-MAPK and mitochondrial perturbations result in endothelial cell death. Multiple signaling pathways, including P-glycoprotein upregulation, phospholipase C (PLC)-dependent intracellular calcium mobilization, tyrosine phosphorylation of the tight junction (TJ) complexes and subsequent activation of PI3 kinase and Rho, and activation of matrix metalloproteases (MMP-9, MMP-2) mediate ROS-induced changes in cell shape, loss of monolayer integrity, and TJ opening resulting in BBB breakdown and increases in vascular permeability. ROS can interact with AA at several levels. ROS can activate PLA_2 and release AA, which in turn can serve as a target of ROS attack and generate lipid peroxides (LP), isoprostanes (F2-iso), and trans-arachidonic acid (TAA). These ROS-AA derivatives can then affect downstream signaling effectors responsible for cell death and disruption of monolayer integrity. On the other hand, AA can function as a supply of ROS by activating NADPH oxidase either directly, at least in phagocytes, or via COX- or LOX-dependent mechanisms. These signaling interactions potentiate ROS and AA-deleterious cellular consequences and contribute to brain endothelial cell dysfunction

evidence that NADPH oxidase also mediates ROS-induced vascular damage, suggesting that the environmental context under which the injury occurs dictates the involvement and cellular outcome of certain ROS-producing enzymes (Lassègue and Griendling 2010).

ROS and Arachidonic Acid in Brain Endothelial Cell Injury

Among the deleterious consequences of oxidative stress in the cerebral vascular system is disruption of BBB endothelial monolayer integrity with subsequent increase of vascular permeability, vasogenic edema, and tissue damage (Yang and Rosenberg 2011). Furthermore, enhanced permeability of the BBB allows the transmigration of leukocytes thus exacerbating the inflammatory process (Van der Goes et al. 2001). The signaling pathways mediating the deleterious effects of ROS and arachidonic acid-derived ROS in brain endothelial cells are not fully characterized. However, several pathways that target cellular effectors implicated in regulating the passage of molecules through the BBB are affected by ROS in cooperation with arachidonic acid, as shown in Fig. 86.1. Studies of Haorah et al. (2007) demonstrated that ROS-induced tyrosine phosphorylation of the BBB tight junctional complexes results in intracellular gap formation and increased vascular permeability. Alterations of the tight junctional complexes responsible for disruption of the BBB are also elicited by ROS-dependent phospholipase C activation and changes in the intracellular levels of calcium (Van der Goes et al. 2001). Moreover, using an in vitro model of BBB, Scheibert et al. (2007) demonstrated that ROS-induced stress fiber formation and increases in transmigration of monocytes were mediated by a signaling pathway involving activation of Rho kinase, AKT, and PI3 kinase. An additional mechanism by which ROS affect the BBB function is via the multidrug transporter P-glycoprotein, which was found to be upregulated following exposure of cultured brain endothelial cells to oxidative stress (Felix and Barrand 2002).

Loss of adherence to the matrix substratum and subsequent disruption of endothelial monolayer integrity also result in increases of BBB permeability. Recent studies have shown that matrix metalloproteases (MMP) are significant targets of ROS signaling and ROS attack on arachidonic acid (Lehner et al. 2011). Both mediators cooperate in targeting MMPs as indicated by recent studies in which ROS and arachidonic acid-derived lipid peroxides mediated ammonia-induced disruption of the BBB via activation of MMP-9 and MMP-2 in an in vivo model of liver failure (Skowrońska et al. 2012). Additionally, isoprostanes exert potent cytotoxic effects on the brain microvasculature that result in vasoconstriction, increased vascular permeability, and monocyte adhesion to the endothelium (Brault et al. 2003; Huber et al. 2003). Thus, disruption of the BBB constitutes an example of cooperation between ROS and arachidonic acid as arachidonic acid-derived lipid peroxide and ROS generated by the COX or LOX pathways are especially relevant to increase in vascular permeability and cerebral edema (Yen and Lee 1987; Fraser 2011).

Additional mechanisms mediating ROS-induced increases of BBB vascular permeability involve the release of angiogenic growth factors, such as VEGF.

In a model of ischemia-reperfusion, ROS stimulated the release of VEGF, which in turn induced alteration of BBB integrity and increased vascular permeability via activation of ERK (Narasimhan et al. 2009). How ERK mediates VEGF-induced vascular permeability is not fully understood. However, changes in cytoskeletal or junctional proteins are possibly involved.

Endothelial cell survival is essential to the maintenance of the BBB monolayer integrity. Increased production of ROS, derived from the mitochondrial respiratory chain, can negatively affect endothelial cell survival via activation of proapoptotic signaling pathways (Zhang and Gutterman 2007). High levels of free fatty acids have been linked to brain endothelial cell apoptosis via a ROS-mediated mechanism (Zhou et al. 2009). Stress kinases, including JNK and ASK-1, play an important role in mediating ROS and arachidonic acid death-inducing signals (Hsu et al. 2007; Shen and Liu 2006; Rizzo and Carlo-Stella 1996). ASK-1-dependent activation of p38-MAPK has been implicated in apoptosis of brain endothelial cells induced by Aβ-fragments (Hsu et al. 2007). The involvement of p38-MAPK in brain endothelial cell death responses was also reported in a model of ischemia-reperfusion or following in vitro exposure of brain microvascular endothelial cells to free arachidonic acid (Lee and Lo 2003; Rizzo et al. unpublished observations).

While ROS involved in brain endothelial cell injury are produced within the brain, it should be noted that ROS or arachidonic acid and its metabolites produced by the peripheral vasculature can target brain endothelial cells, especially in the presence of a permissive BBB as it occurs, for example, during the acute phase of inflammation. Thus, peripherally generated ROS or ROS sources such as arachidonic acid and its metabolites can target the brain endothelium and exacerbate brain endothelial cell dysfunction during system inflammation (Davidson et al. 2001; Skowrońska et al. 2012).

Regulation of Brain Endothelial Cell Angiogenic Responses by ROS and Arachidonic Acid

Angiogenesis, the formation of new blood vessels from preexisting capillaries, occurs in the brain during embryonic development in concert with vasculogenesis, the formation of new blood vessels from hemangioblasts (Mancuso et al. 2008; Conway et al. 2001). In adult brain, where growth and development are complete, endothelial cells become quiescent being mainly involved in preserving the BBB integrity (Plate 1999). However, in response to inflammatory, traumatic, neoplastic, or degenerative insults to the brain, the balance between inhibitors and stimulators of angiogenesis, which under normal conditions prevent angiogenesis, is shifted in favor of neovascularization (Lee et al. 2009; Rizzo and Leaver 2010). Under these conditions, the release of proangiogenic protein and lipid growth factors, together with increased metabolic demands of the brain, results in stimulation of endothelial cell angiogenic responses and neovascularization (Plate et al. 1992; Rush et al. 2007; Rizzo and Leaver 2010).

Several lines of evidence indicate that ROS regulate brain endothelial cell angiogenic responses. Several angiogenic growth factors, such as VEGF and angiopoietin-1, in addition to hypoxia and products of arachidonic acid metabolism, activate NADPH oxidase. Work from the laboratory of Ushio-Fukai has elegantly characterized ROS-dependent signaling networks that regulate endothelial cell angiogenic responses. ROS-dependent stimulation of VEGF receptor autophosphorylation and downstream activation of AKT mediates VEGF-induced neovascularization, while ROS production through activation of Rac-1 subunit of NADPH oxidase mediates angiopoietin-1-induced angiogenesis (Ushio-Fukai 2006). While information on the cellular source of ROS production during brain angiogenesis is scanty, the relevance of NADPH oxidase in ROS-induced angiogenesis has been supported by studies demonstrating upregulation of Nox4 expression in new brain capillaries following ischemia-reperfusion (Vallet et al. 2005). PLA_2 and products of arachidonic acid metabolism also contribute to endothelial cell angiogenic responses in vitro and in vivo (Rizzo et al. 2000; Nie et al. 2000). The extent of ROS involvement in these responses is not known. Moreover, these studies were performed in endothelial cells from peripheral organs. Therefore, their relevance to the brain microvasculature remains to be established.

Superoxide and hydrogen peroxide, produced during ischemia-reperfusion or hypoxia, can promote angiogenesis via upregulation of hypoxia- and redox-sensitive transcription factors and their target genes (Ushio-Fukai 2006). On the other hand, they can stimulate apoptotic pathways and thus impair endothelial cell angiogenic responses. Similarly, isoprostanes have been shown to selectively promote necrosis of brain microvascular endothelial cells during ischemia (Brault et al. 2003).

Rationale for Targeting ROS and Arachidonic Acid-Sensitive Signaling Pathways in the Brain Endothelium

Brain endothelial cell dysfunction contributes to several neurological disorders, while interfering with brain endothelial cell angiogenic responses can affect response to therapy in brain tumors. Substantial evidence indicates that ROS contribute to the progression of malignant gliomas by targeting the endothelium and triggering the development of structurally disorganized and highly permeable neovascular structures, the presence of which correlates with a more aggressive tumor phenotype (Lee et al. 2009). Whether these tumor-associated angiogenic responses are stimulated by ROS produced endogenously by the endothelial cells or by ROS derived from tumor cells or from other components of the tumor microenvironment is not entirely clear. However, it is clear that intratumoral hypoxia, which is a potent stimulus for the angiogenic switch in gliomas, is also a potent stimulator of ROS production, suggesting that tumor-derived ROS are involved in targeting the endothelium (Richard et al. 1999). Importantly, enzymes participating in the oxidation of arachidonic acid during tumorigenesis, such as COX-2, are also upregulated by hypoxia in glioma cells and contribute to stimulation of tumor-associated angiogenesis (Rizzo 2011). Thus, ROS generation and

upregulation of COX-2 during hypoxia constitute an important point of functional cross-talk influencing several aspects of tumor biological behavior. Understanding the signaling integration between ROS and arachidonic acid metabolism during glioma-associated neovascularization may provide new therapeutic opportunities for these very aggressive and clinically challenging tumors.

The interplay between ROS and arachidonic acid may be significant also in nonneoplastic disorders of the brain, including ischemia, traumatic brain injuries, and neurodegeneration. Emerging evidence indicates a crucial contribution of ROS and arachidonic acid-derived lipid peroxides to neurodegeneration. Deposition of oligomeric Aβ fragments in cerebral vessel walls induces ROS-mediated apoptosis (Hsu et al. 2007). On the other hand, activation of the PLA_2 family and subsequent release of arachidonic acid by Aβ peptides could initiate ROS-dependent cytotoxic responses leading to cerebrovascular dysfunction (Shelat et al. 2008; Moses et al. 2006). Intriguingly, patients with Alzheimer's disease have increased amount of arachidonic acid and lipid peroxidation (Esposito et al. 2008). Studies in $cPLA_2$ knockout animals have provided evidence in support for a role of PLA_2, arachidonic acid, and ROS in cerebral ischemia and associated edema (Tabuchi et al. 2003). Additional studies in these areas of brain pathology should provide useful insights of the interplay between ROS and arachidonic acid that could translate into clinical benefits.

Concluding Remarks

In summary, cooperation and cross-talk between oxidative stress and inflammation play an important role in regulating brain endothelial cell responses. While physiological fluctuations in certain ROS play a role in homeostasis, oxidative stress can also have drastic effects on the cerebral vasculature, promoting and/or exacerbating pathologies ranging from stroke to neurodegenerative disease and oncogenesis. Similarly, while arachidonic acid contributes to vascular homeostasis, generation of ROS via arachidonic acid metabolism or generation of lipid peroxides may result in endothelial cell injury and vascular damage.

The signaling pathways of ROS-induced damage of the brain microvasculature and the contribution of arachidonic acid to ROS-dependent networks are, however, not well characterized, partly because of limitations to the detection of transiently formed ROS and arachidonic acid oxidative products in complex cellular systems. Moreover, the majority of the information on ROS-dependent signaling pathways in the vasculature derives mostly from studies performed with endothelial cells isolated from peripheral organs. Such knowledge cannot readily apply to the brain microvasculature because of its unique morphological and biochemical characteristics. Therefore, studies using brain endothelial cells and new experimental models that better represent the multicellular nature of the brain vasculature together with improved in vivo imaging techniques are needed to provide further insights into the functional and signaling interactions between ROS and arachidonic acid and allow the development of better therapeutic strategies to modulate ROS production and

enhance mechanisms of repair and cytoprotection. Identification of new oxidative and inflammatory cellular targets and molecular profiling within the brain microvasculature should also facilitate the development of new noninvasive biomarkers than can be introduced into clinical practice.

Given that inflammatory, traumatic, degenerative, and neoplastic disorders of the brain constitute a major threat and burden to public health worldwide, understanding the mechanisms that control brain endothelial cell function and dysfunction may aid the development of targeted vascular therapies aimed at restoring the anatomical and functional integrity of the neurovascular unit.

Acknowledgments We are grateful to Dr. Dino Rotondo for advice on eicosanoid vasoactivity, Professor Ian Whittle for discussion of clinical edema, and Chris Brown for artwork. We regret having to omit the work of many colleagues because of space limitations. The original research findings from our laboratories discussed in this chapter were supported by the American Heart Association, the Showalter Foundation and Institutional Funds to M.T.R., and the Chest, Heart and Stroke Foundation (Scotland) to H.A.L.

References

Abbott NJ, Rönnbäck L, Hansson E (2006) Astrocyte–endothelial interactions at the blood-brain barrier. Nature Rev Neurosci 7:41–53

Adam-Vizi V (2005) Production of reactive oxygen species in brain mitochondria: contribution by electron transport chain and non-electron transport chain sources. Antioxid Redox Signal 7:1140–1149

Adibhatla RM, Hatcher JF (2007) Secretory phospholipase A2 IIA is upregulated by TNF-α and IL-1α/β after transient focal cerebral ischemia in rats. Brain Res 1134:199–205

Armstead WM (2003) Cyclooxygenase-2-dependent superoxide generation contributes to age-dependent impairment of G protein-mediated cerebrovasodilatation. Anesthesiology 98:1378–1383

Babior BM (2000) The NADPH oxidase of endothelial cells. IUBMB Life 50:267–269

Basuroy S, Tcheranova D, Bhattacharya S, Leffler CW, Parfenova H (2011) Nox4 NADPH oxidase-derived reactive oxygen species, via endogenous carbon monoxide, promote survival of brain endothelial cells during TNF-α-induced apoptosis. Am J Physiol 300: C256–C265

Beck H, Plate KH (2009) Angiogenesis after cerebral ischemia. Acta Neuropathol 117:481–496

Bell RD, Zlokovic BV (2009) Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. Acta Neuropathol 118:103–113

Benndorf RA, Schwedhelm E, Gnann A, Taheri R, Kom G, Didié M, Steenpass A, Ergün S, Böger RH (2008) Isoprostanes inhibit vascular endothelial growth factor-induced endothelial cell migration, tube formation, and cardiac vessel sprouting in vitro, as well as angiogenesis in vivo via activation of the thromboxane A(2) receptor: a potential link between oxidative stress and impaired angiogenesis. Cir Res 103:1037–1046

Brault S, Martinez-Bermudez AK, Marrache AM, Gobeil F Jr, Hou X, Beauchamp M, Quiniou C, Almazan G, Lachance C, Roberts J 2nd, Varma DR, Chemtob S (2003) Selective neuromicrovascular endothelial cell death by 8-Iso-prostaglandin F2alpha: possible role in ischemic brain injury. Stroke 343:776–782

Buschbeck M, Ghomashchi F, Gelb MH, Watson SP, Börsch-Haubold AG (1999) Stress stimuli increase calcium-induced arachidonic acid release through phosphorylation of cytosolic phospholipase A2. Biochem J 344:359–366

Calandria JM, Bazan NG (2010) Neuroprotectin D1 modulates the induction of pro-inflammatory signaling and promotes retinal pigment epithelial cell survival during oxidative stress. Adv Exp Med Biol 664:663–670

Chan EC, Jiang F, Peshavariya HM, Dusting GJ (2009) Regulation of cell proliferation by NADPH oxidase-mediated signaling: potential roles in tissue repair, regenerative medicine and tissue engineering. Pharmacol Ther 122:97–108

Cho KJ, Seo JM, Kim JH (2011) Bioactive lipoxygenase metabolites stimulation of NADPH oxidases and reactive oxygen species. Mol Cells 32:1–5

Chrissobolis S, Faraci FM (2008) The role of oxidative stress and NADPH oxidase in cerebrovascular disease. Trends Mol Med 14:495–502

Chrissobolis S, Miller AA, Drummond GR, Kemp-Harper BK, Sobey CG (2011) Oxidative stress and endothelial dysfunction in cerebrovascular disease. Front Biosci 16:1733–1745

Conway ME, Collen D, Carmeliet P (2001) Molecular mechanisms of blood vessel growth. Cardiovasc Res 49:507–521

Davidson J, Abul HT, Milton AS, Rotondo D (2001) Cytokines and cytokine inducers stimulate prostaglandin E2 entry into the brain. Pflugers Arch 4424:526–533

Dröge W (2001) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95

Dusting GJ, Mancada S, Vane JR (1977) Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. Prostaglandins 13:3–15

Easton AS, Abbott NJ (2002) Bradykinin increases permeability by calcium and 5-lipoxygenase in the ECV304/C6 cell culture model of the blood-brain barrier. Brain Res 953:157–169

Easton AS, Fraser PA (1998) Arachidonic acid increases cerebral microvascular permeability by free radicals in single pial microvessels of the anaesthetized rat. J Physiol 507:541–547

Ellis EF, Wei EP, Kontos HA (1979) Vasodilation of cat cerebral arterioles by prostaglandins D2, E2, G2, and I2. Am J Physiol 237:H381–H385

Esposito G, Giovacchini G, Liow JS, Bhattacharjee AK, Greenstein D, Schapiro M, Hallett M, Herscovitch P, Eckelman WC, Carson RE, Rapoport SI (2008) Imaging neuroinflammation in Alzheimer's disease with radiolabeled arachidonic acid and PET. J Nucl Med 49:1414–1421

Felix RA, Barrand MA (2002) P-glycoprotein expression in rat brain endothelial cells: evidence for regulation by transient oxidative stress. J Neurochem 80:64–72

Fraser PA (2011) The role of free radical generation in increasing cerebrovascular permeability. Free Radicals Biol Med 51:967–977

Goldman R, Ferber E, Zou U (1992) Reactive oxygen species are involved in activation of phospholipase A2. FEBS Lett 309:190

Halliwell B, Chirico S (1993) Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr 57:715S–725S

Hamel E (2006) Perivascular nerves and the regulation of the vascular tone. J Appl Physiol 100:1059–1064

Haorah J, Ramirez SH, Schall K, Smith D, Pandya R, Persidsky Y (2007) Oxidative stress activates protein tyrosine kinase and matrix metalloproteinases leading to blood-brain barrier dysfunction. J Neurochem 101:566–576

Harrison A, Murphy RC (1995) Isoleukotrienes are biologically active free radical products of lipid peroxidation. J Biol Chem 270:17273–17278

Hatashita S, Hoff JT (1990) Brain edema and cerebrovascular permeability during cerebral ischemia in rats. Stroke 21:582–588

Hawkins BT, Davis TP (2005) The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev 57:173–185

Hazel JR, Williams EE (1990) The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Prog Lipid Res 29:167–227

Honoré JC, Kooli A, Hou X, Hamel D, Rivera JC, Picard E, Hardy P, Tremblay S, Varma DR, Jankov RP, Mancini JA, Balazy M, Chemtob S (2010) Sustained hypercapnia induces cerebral

microvascular degeneration in the immature brain through induction of nitrative stress. Am J Physiol Regul Integr Comp Physiol 298:R1522–R1530

Hsu M-J, Hsu CY, Chen BC, Chen M-C, Ou G, Lin C-H (2007) Apoptosis signal-regulating kinase 1 in amyloid β peptide-induced cerebral endothelial cell apoptosis. J Neurosci 27:5719–5729

Huber J, Bochkov VN, Binder BR, Leitinger N (2003) The isoprostane 8-iso-PGE2 stimulates endothelial cells to bind monocytes via cyclic AMP- and p38 MAP kinase-dependent signaling pathways. Antioxid Redox Signal 5:163–169

Iadecola C, Nedergaard M (2007) Glia regulation of the cerebral microvasculature. Nat Neurosci 10:1369–1376

Im J, Kim D, Paik SG, Han PL (2006) Cyclooxygenase-2-dependent neuronal death proceeds via superoxide anion generation. Free Rad Biol Med 41:960–972

Jensen MD, Sheng W, Simonyi A, Johnson GS, Sun AY, Sun GY (2009) Involvement of oxidative pathways in cytokine-induced secretory phospholipase A2-IIA in astrocytes. Neurochem Int 55:362–368

Jian W, Arora JS, Oe T, Shuvaev VV, Blair IA (2005) Induction of endothelial cell apoptosis by lipid hydroperoxide-derived bifunctional electrophiles. Free Radic Biol Med 39:1162–1176

Jiang H, Kruger N, Lahiri DR, Wang D, Vatèle JM, Balazy M (1999) Nitrogen dioxide induces *cis-trans*-isomerization of arachidonic acid within cellular phospholipids. J Biol Chem 274:16235–16241

Jomova K, Valko M (2011) Advances in metal-induced oxidative stress and human disease. Toxicology 283:65–87

Kanu A, Leffler CW (2011) Arachidonic acid and prostaglandin E2-induced cerebral vasodilation is mediated by carbon monoxide independent of reactive oxygen species in piglets. Am J Physiol Heart Circ Physiol 301:H2482–H2487

Kanu A, Gilpin D, Fedinec AL, Leffler CW (2006) Cyclooxygenase products stimulate carbon monoxide production by piglet cerebral microvessels. Exp Biol Med 231:181–185

Kermorvant-Duchemin E, Sennlaub F, Sirinyan M, Brault S, Andelfinger G, Kooli A, Germain S, Ong H, d'Orleans-Juste P, Gobeil F Jr, Zhu T, Boisvert C, Hardy P, Jain K, Falck JR, Balazy M, Chemtob S (2005) Trans-arachidonic acids generated during nitrative stress induce a thrombospondin-1-dependent microvascular degeneration. Nat Med 11:1339–1345

Kim H, Li Q, Hempstead BL, Madri JA (2004) Paracrine and autocrine functions of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in brain-derived endothelial cells. J Biol Chem 279:33538–33546

Kontos HA, Wei EP, Povlishock JT, Christman CW (1984) Oxygen radicals mediate the cerebral arteriolar dilation from arachidonate and bradykinin in cats. Circ Res 3:295–303

Kooli A, Kermorvant-Duchemin E, Sennlaub F, Bossolasco M, Hou X, Honoré JC, Dennery PA, Sapieha P, Varma D, Lachapelle P, Zhu T, Tremblay S, Hardy P, Jain K, Balazy M, Chemtob S (2008) Trans-arachidonic acids induce a heme oxygenase-dependent vasorelaxation of cerebral microvasculature. Free Radic Biol Med 44:815–825

Kukreja RC, Kontos HA, Hess ML, Ellis EF (1986) PGH synthase and lipooxygenase generate superoxide in the presence of NADH or NADPH. Cir Res 49:612–619

Kunstmann S, Mertsch K, Blasig IE, Grune T (1996) High metabolic rates of 4-hydroxynonenal in brain capillary endothelial cells during hypoxia/reoxygenation. Brain Res 740:353–355

Lassègue B, Griendling KK (2010) NADPH oxidases: functions and pathologies in the vasculature. Arterioscler Thromb Vasc Biol 30:653–661

Leaver HA, Yap PL, Rogers P, Wright I, Smith G, Williams PE, France AJ, Craig SR, Walker WS, Prescott RJ (1995) Peroxides in human leucocytes in acute septic shock: acute phase changes and mortality. Eur J Clin Invest 25:777–783

Lee SR, Lo EH (2003) Interactions between p38-Mitogen-Activated Protein Kinase and caspase-3 in cerebral endothelial cell death after hypoxia-reoxygenation. Stroke 34:2704–2709

Lee HS, Han J, Bai HJ, Kim KW (2009) Brain angiogenesis in developmental and pathological processes: regulation, molecular and cellular communication at the neurovascular interface. FEBS J 276:4622–4635

Lehner C, Gehwolf R, Tempfer H, Krizbai I, Hennig B, Bauer HC, Bauer H (2011) Oxidative stress and blood-brain barrier dysfunction under particular consideration of matrix metalloproteinases. Antioxid Redox Signal 15:1305–1323

Luczak K, Balcerczyk A, Soszynski M, Bartosz G (2004) Low concentration of oxidant and nitric oxide donors stimulate proliferation of human endothelial cells in vitro. Cell Biol Int 28:483–486

Maier CM, Hsieh L, Crandall T, Narasimhan P, Chan PH (2006) A new approach for the investigation of reperfusion-related brain injury. Biochem Soc Trans 34:1366–1369

Mancuso MR, Kuhnert F, Kuo CJ (2008) Developmental angiogenesis of the central nervous system. Lymphat Res Biol 6:3–4

Marnett LJ, Rowlinson SW, Goodwin DC, Kalgutkar AS, Lanzo CA (1999) Arachidonic acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition. J Biol Chem 274:22903–22906

Medhora M, Dhanasekaran A, Gruenloh SK, Dunn LK, Gabrilovich M, Falck JR, Harder DR, Jacobs ER, Pratt PF (2007) Emerging mechanisms for growth and protection of the vasculature by cytochrome P450-derived products of arachidonic acid and other eicosanoids. Prostaglandins Other Lipid Mediat 82:19–29

Mertsch K, Blasig I, Grune T (2001) 4-Hydroxynonenal impairs the permeability of an in vitro rat blood–brain barrier. Neurosci Lett 314:135–138

Miller AA, Drummond GR, Sobey CG (2006) Novel isoforms of NADPH-oxidase in cerebral vascular control. Pharmacol Ther 111:928–948

Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd (1990) A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci USA 87:9383–9387

Moses GS, Jensen MD, Lue LF, Walker DG, Sun AY, Simonyi A, Sun GY (2006) Secretory PLA2-IIA: a new inflammatory factor for Alzheimer's disease. J Neuroinflammation 3:28

Narasimhan P, Liu J, Song YS, Massengale JL, Chan PH (2009) VEGF Stimulates the ERK 1/2 signaling pathway and apoptosis in cerebral endothelial cells after ischemic conditions. Stroke 40:1467–1473

Nie D, Tang K, Diglio C, Honn KV (2000) Eicosanoid regulation of angiogenesis: role of endothelial arachidonate 12-lipoxygenase. Blood 95:2304–2311

Niwa K, Haensel C, Ross ME, Iadecola C (2001) Cyclooxygenase-1 participates in selected vasodilator responses of the cerebral circulation. Circ Res 88:600–608

Oldenford WH, Cornford ME, Brown WJ (1977) The large apparent work capability of the blood-brain barrier a study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. Ann Neurol 1:409–417

Parfenova H, Leffler CW (2008) Cerebroprotective functions of HO-2. Curr Pharm Design 14:443–453

Parfenova H, Levine V, Gunther WM, Pourcyrous M, Leffler CW (2002) COX-1 and COX-2 contributions to basal and IL-1 beta-stimulated prostanoid synthesis in human neonatal cerebral microvascular endothelial cells. Pediatr Res 52:342–348

Park JA, Choi KS, Kim SY, Kim KW (2003) Coordinated interaction of the vascular and nervous systems: from molecule- to cell-based approaches. Biochem Biophys Res Commun 311:247–253

Phillis JW, Horrocks LA, Farooqui AA (2006) Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders. Brain Res Rev 52:201–243

Plate KH (1999) Mechanisms of angiogenesis in the brain. J Neuropathol Exp Neurol 58:313–320

Plate KH, Breier G, Weich HA, Risau W (1992) Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 359:845–848

Rao JS, Kim HW, Kellom M, Greenstein D, Chen M, Kraft AD, Harry GJ, Rapoport SI, Basselin M (2011) Increased neuroinflammatory and arachidonic acid cascade markers, and reduced synaptic proteins, in brain of HIV-1 transgenic rats. J Neuroinflammation 8:101

Rapoport SI (2008) Arachidonic acid and the brain. J Nutr 138:2515–2520
Richard DE, Berra E, Pouysségur J (1999) Angiogenesis: how a tumor adapts to hypoxia. Biochem Biophys Res Commun 266:718–722
Rizzo MT (2011) Cyclooxygenase-2 in oncogenesis. Clin Chim Acta 412:671–687
Rizzo MT, Carlo-Stella C (1996) Arachidonic acid mediates interleukin-1 and tumor necrosis factor-alpha-induced activation of the c-jun amino-terminal kinases in stromal cells. Blood 88:3792–3800
Rizzo MT, Leaver HA (2010) Brain endothelial cell death: modes, signalling pathways and relevance to neural development, homeostasis and disease. Mol Neurobiol 42:52–63
Rizzo MT, Nguyen E, Aldo-Benson M, Lambeau G (2000) Secreted phospholipase A(2) induces vascular endothelial cell migration. Blood 96:3809–3815
Rosa AO, Rapoport SI (2009) Intracellular- and extracellular-derived Ca^{2+} influence phospholipase A_2-mediated fatty acid release from brain phospholipids. Biochim Biophys Acta (Mol Cell Biol Lipids) 1791:697–705
Rush S, Khan G, Bamisaiye A, Bidwell P, Leaver HA, Rizzo MT (2007) c-jun amin-terminal kinase and mitogen activated protein kinase mediate hepatocyte growth factor-induced migration of brain endothelial cells. Exper Cell Res 313:121–132
Schreibelt G, Kooij G, Reijerkerk A, van Doorn R, Gringhuis SI, van der Pol S, Weksler BB, Romero IA, Couraud PO, Pointek J, Blasig IE, Dijkstra CD, Ronken E, de Vries HE (2007) Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, P13 kinase and PKB signaling. FASEB J 21:3666–3676
Shelat PB, Chalimoniuk M, Wang JH, Strosznajder JB, Lee JC, Sun AY, Simonyi A, Sun GY (2008) Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons. Neurochem 106:45–55
Shen HM, Liu Z (2006) JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and nitrogen species. Free Radical Biol Med 40:928–939
Shen Q, Goderie SQ, Jin L, Karanth N, Sun Y, Abramova N, Vincent P, Pumiglia K, Temple S (2004) Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science 304:1338–1340
Shimizu F, Sano Y, Saito K, Abe MA, Maeda T, Haruki H, Kanda T (2012) Pericyte-derived glial cell line-derived neurotrophic factor increase the expression of claudin-5 in the blood-brain barrier and the blood-nerve barrier. Neurochem Res 37:401–409
Shiose A, Sumimoto H (2000) Arachidonic acid and phosphorylation synergistically induce a conformational change of p47phox to activate the phagocyte NADPH oxidase. J Biol Chem 275:13793–13801
Sies H (1993) Strategies of antioxidant defense. Eur J Biochem 215:213–219
Skowrońska M, Zielińska M, Wójcik-Stanaszek L, Ruszkiewicz J, Milatovic D, Aschner M, Albrecht J (2012) Ammonia increases paracellular permeability of rat brain endothelial cells by a mechanism encompassing oxidative/nitrosative stress and activation of matrix metalloproteinases. J Neurochem. doi:10.1111/j.1471-4159.2012.07669
Sobey CG, Heistad DD, Faraci FM (1997) Mechanisms of bradykinin-induced cerebral vasodilatation in rats: evidence that reactive oxygen species activate K1 channels. Stroke 28:2290–2294
Sparvero LJ, Moscato AA, Kochanek M, Pitt BR, Kagan VE, Bayır H (2010) Mass-spectrometry based oxidative lipidomics and lipid imaging: applications in traumatic brain injury. J Neurochem 115:1322–1336
Spychalowicz A, Wilk G, Sliwa T, Ludew D, Guzik TJ (2012) Novel therapeutic approaches in limiting oxidative stress and inflammation. Curr Pharm Biotechnol (ahead of print)
Tabuchi S, Uozumi N, Ishii S, Shimizu Y, Watanabe T, Shimizu T (2003) Mice deficient in cytosolic phospholipase A2 are less susceptible to cerebral ischemia/reperfusion injury. Acta Neurochir Suppl 86:169–172
Terada LS, Willingham IR, Rosandich ME, Leff JA, Kindt GW, Repine JE (1991) Generation of superoxide anion by brain endothelial cell xanthine oxidase. J Cell Physiol 148:191–196

Ushio-Fukai M (2006) Redox signaling in angiogenesis: role of NADPH oxidase. Cardiovascular Res 71:226–235

Vallet P, Charnay Y, Steger K, Ogier-Denis E, Kovari E, Herrmann F, Michel JP, Szanto I (2005) Neuronal expression of the NADPH oxidase NOX4, and its regulation in mouse experimental brain ischemia. Neuroscience 132:233–238

Van Buul JD, Fernandez-Borja M, Anthony EC, Hordijk PL (2005) Expression and localization of NOX2 and NOX4 in primary human endothelial cells. Antioxid Redox Signal 7:308–317

Van der Goes A, Wouters D, Van der Pol SM, Hizinga R, Ronken E, Adamson P, Greenwood J, Dijkstra CD, de Vries HE (2001) Reactive oxygen species enhance the migration of monocytes across the blood-barrier in vitro. FASEB J 15:1852–1854

Virdis A, Colucci R, Fornai M, Blandizzi C, Duranti E, Pinto S, Bernardini N (2005) Cyclooxygenase-2 inhibition improves vascular endothelial dysfunction in a rat model of endotoxic shock: role of inducible nitric-oxide synthase and oxidative stress. J Pharmacol Exp Ther 312:945–953

Wang X, Lo EH (2003) Triggers and mediators of hemorrhagic transformation in cerebral ischemia. Mol Neurobiol 28:229–244

Ward NL, Lamanna JC (2004) The neurovascular unit and its growth factors: coordinated response in the vascular and nervous systems. Neurol Res 26:870–883

Wei EP, Kontos HA, Beckman JS (1996) Mechanisms of cerebral vasodilation by superoxide, hydrogen peroxide, and peroxynitrite. Am J Physiol 271:H1262–H1266

Xiong Y, Mahmood A, Chopp M (2010) Angiogenesis, neurogenesis and brain recovery of function following injury. Curr Opin Investig Drugs 11:298–308

Yamato M, Egashira T, Utsumi H (2003) Application of in vivo ESR spectroscopy to measurement of cerebrovascular ROS generation in stroke. Free Radic Biol Med 35:1619–1631

Yang Y, Rosenberg GA (2011) Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. Stroke 42:3323–3338

Yen MH, Lee SH (1987) Effects of cyclooxygenase and lipoxygenase inhibitors on cerebral edema induced by freezing lesions in rats. Eur J Pharmacol 144:369–373

Zhang C, Bazan NG (2010) Lipid-mediated cell signaling protects against injury and neurodegeneration. J Nutr 140:858–863

Zhang DX, Gutterman DD (2007) Mitochondrial reactive oxygen species-mediated signaling in endothelial cells. Am J Physiol Heart Circ Physiol 292:H2023–H2031

Zhou H, liu X, Liu L, Yang Z, Zhang S, Tang M, Tang Y, Dong Q, Hu R (2009) Oxidative stress and apoptosis of human brain microvascular endothelial cells induced by free fatty acids. J Int Med Res 37:1897–1903

Zipfel GJ, Han H, Ford AL, Lee J-M (2009) Cerebral amyloid angiopathy: progressive disruption of the neurovascular unit. Stroke 40:S16–S19