Published in final edited form as:
Curr Pharm Biotechnol. 2011 September 1; 12(9): 1334–1342.

Role of endothelial nitric oxide in cerebrovascular regulation

Dmitriy N. Atochin and **Paul L. Huang**
Cardiovascular Research Center and Cardiology Division, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 0219

Abstract

Endothelial nitric oxide (NO) plays important roles in the vascular system. Animal models that show vascular dysfunction demonstrate the protective role of endothelial NO dependent pathways. This review focuses on the role of endothelial NO in the regulation of cerebral blood flow and vascular tone. We will discuss the importance of NO in cerebrovascular function using animal models with altered endothelial NO production under normal, ischemic and reperfusion conditions, as well as in hyperoxia. Pharmacological and genetic manipulations of the endothelial NO system demonstrate the essential roles of endothelial NO synthase in maintenance of vascular tone and cerebral perfusion under normal and pathological conditions.

Keywords

cerebrovascular regulation; endothelial nitric oxide synthase; mutant mice

Introduction

Cerebral blood flow (CBF) regulated by perfusion pressure, by functional, metabolic and hormonal activity, by gases carried in the blood, and by neurogenic stimulation. NO is gaseous molecule produced by endothelial (eNOS), neuronal (nNOS) and inducible nitric oxide (iNOS) synthases. NO is involved in CBF regulation under normal conditions, due to production by endothelial cells [1], neurons [2, 3], and nitrergic perivascular nerves [2]. eNOS and nNOS are involved in constitutive basal and stimulated NO production [4], and iNOS generates NO when induced by pathophysiologic and inflammatory conditions [5].

1. eNOS regulation

Endothelial NO, constitutively produced by eNOS, participates in various processes, including maintenance of blood pressure, vascular reactivity, angiogenesis, inhibition of platelet adhesion and aggregation, suppression of smooth muscle cell proliferation, and antioxidative processes. eNOS is localized in caveolae. It binds to caveolin1 and migrates intracellulary in response to elevated concentration of calcium [6]. eNOS produces NO from L-arginine in the presence of oxygen and co-factors nicotinamide adenine dinucleotide phosphate (NADPH), tethrahydrobiopterin (BH_4), heme, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and calmodulin [1]. eNOS activity is regulated by protein-protein interactions with hsp90 and caveolin, posttranslational myristoylation and palmitoylaytion and S-nitrosylation [1, 7–9]. eNOS is activated by increased transmembrane and intracellular Ca^{2+} influx and by phosphorylation at several sites in eNOS, including serine (S1177, S617, S635) and threonine (T497) [9]. Molecular mechanisms of enhanced

Corresponding author: Paul Huang, MD, PhD, Professor of Medicine, Harvard Medical School, Massachusetts General Hospital, 149 Thirteen Street, Charlestown, MA 02129, USA, phuang1@partners.org, Tel: (617) 724–9849, Fax: (617) 726–5806.

activity of eNOS phosphorylated at S1177 inlcude increased electron flux through the reductase domain and decreased dissociation with calmodulin. Phosphorylation of eNOS induces a conformational shift of the FMN-binding domain to allow enhanced transfer of electrons, activating the synthase. Estrogens [10], statins [11, 12], leptin [12], adiponectin [13], shear stress, vascular endothelial growth factor (VEGF) and insulin all promote S1177 eNOS phosphorylation [14]. S1177 is phosphoryalted by various kinases, including Akt [6], 5′ adenosine monophosphate-activated protein (AMP) kinase [15] and protein kinases A and G [16]. eNOS uncoupling, caused by insufficiency of BH_4, leads to production of superoxide anion, resulting in hypertension and contributing to the pathophysiology of hyperlipidamemia, atherosclerosis, diabetes[17] and complications of hypoxic-ischemic brain injury [18].

2. Endothelial NO/cGMP signaling pathway

Endothelial NO functions are mediated by soluble guanylyl cyclase (sGC) and its intracellular second messenger cyclic guanosine monophosphate (cGMP). NO activates the heme-containing heterodimer sGC, resulting in cGMP synthesis, and downstream activation of potassium channels in smooth muscle cells [19]. Several cGMP-independent mechanisms of NO-induced vasorelaxation have also been identified, including S-glutathiolation, activation of sacroendoplasmic reticulum calcium adenosine triphosphatase [20], and calcium dependent activation of potassium channels [21] on vascular muscle.

3. Basal eNOS and CBF

3.1. Rats

CBF attenuation was observed in parallel with downregulation of eNOS expression in spontaneously hypertensive rats [22]. Head-down tail suspension of rats, inducing headward fluid shifts and arterial blood pressure elevation was associated with diminished middle cerebral artery eNOS protein levels, lower CBF and higher cerebral vascular resistance [23]. Intracisternal administration of adenovirus with the bovine eNOS gene resulted in transient CBF augmentation [24].

3.2. Mice (eNOS knockouts and nNOS knockout)

The relative contributions of endothelial and neuronal NO to CBF responses have been difficult to establish because nNOS is the dominant form of NOS in neurons in the brain, while eNOS is found in cerebrovascular endothelial cells. NOS inhibitors lack adequate selectivity between the NOS isoforms, limiting pharmacologic approaches to distinguish between the two. The development of eNOS and nNOS knockout mice deficient in eNOS or nNOS isoforms provided an opportunity to specifically evaluate the role of the isoforms in CBF regulation. Brain distribution of NOS in eNOS knockout and nNOS knockout mice was quantitated using (^{3}H)L-NG-nitro-arginine binding [25]. The density of the binding was significantly reduced in nNOS knockout mice, but there were no differences in the binding between wild-type (WT) and eNOS deficient mice at baseline. This demonstrates that nNOS is major source of NO in brain.

N-methyl-D-aspartate stimulated glutamate release was attenuated in the cortex of nNOS knockout mice, while N-methyl-D-aspartate-stimulated GABA release was attenuated in brain of eNOS knockout mice [26]. These results suggest that eNOS and nNOS contribute differently to the modulation of inhibitory and excitatory neurotransmission in brain.

Resting regional CBF (rCBF), as measured by hydrogen clearance method in absolute values in caudate putamen nucleus (striatum) and parietal cortex of urethane-anesthetized artificially breathing mice, was not significantly different between WT, eNOS knockout and

nNOS knockout animals. Normal basal rCBF values in the knockout mice indicate the involvement of compensatory mechanisms of CBF regulation that maintain CBF in the physiologic range after eNOS gene deletion.

3.3. eNOS inhibition

3.3.1. Rats—In rats, injected with the non-specific NOS inhibitor *N*G-nitro-L-arginine methyl ester (L-NAME), absolute CBF was decreased to 25–35% of control level in parallel with decrease in brain PO_2, demonstrating significant role of NO in maintaining of CBF resting level [27].

3.3.2. Mice (eNOS knockout and nNOS knockout)—To assess the contribution of eNOS and nNOS in regulating absolute resting rCBF, blood flow was measured in mice treated with L-NAME. 30 minutes after L-NAME injection, absolute rCBF was significantly attenuated in WT and nNOS knockout, but not in eNOS knockout mice [28]. These data are consistent with results obtained using laser-Doppler flowmetry and a closed cranial window technique [29]. In these experiments laser Doppler probe was attached to the scull of anesthetized with urethane mice and resting blood flow was measured in relative values under physiological temperature and arterial blood pressure conditions. Resting cortical CBF decreased by approximately 25 % after NG-nitro-L-arginine (L-NNA) superfusion in WT, but not in eNOS knockout mice, suggesting a key role for eNOS in baseline CBF maintenance.

So, cerebrovascular effects of NOS inhibitors were observed in rats, nNOS knockout and WT mice due to inhibition of eNOS. The results indicate that basal endothelial NO production influences cerebral vascular tone and maintains resting CBF regulation.

3.4. NO donors

3.4.1. Mice—The sensitivity of smooth muscle cells to exogenous NO depends on eNOS activity. The endothelial NO contribution to cerebrovascular reactivity to exogenous NO was studied with the NO donor [1-propamine, 3-(2 hydroxy-2-nitroso-1-propylhydrazine)] (PAPA NONOate). PAPA NONOate increased absolute rCBF in WT, eNOS and nNOS deficient mice, but the CBF responses in eNOS knockout mice were significantly attenuated [28]. These results indicate reduced responsiveness to NO in brain vessels in eNOS knockout mice. Chronic eNOS deficiency may reduce NO sensitivity of cerebral vasculature, possible due to changes in downstream guanylate cyclase effector mechanisms.

3.5. Acetylcholine (ACh)

The endothelium-dependent vasorelaxation to acetylcholine (ACh) is a physiologic vascular response to stimuli that activate NO signaling. The endothelial NO contribution to cerebrovascular reactivity was studied using a physiologic stimulator of endothelium, ACh.

3.5.1. Mice (eNOS knockout and nNOS knockout)

3.5.1.1. *Ex vivo* experiments on isolated vessels: In myograph experiments, vessels were isolated and mounted onto wires or pressurized cannulas in physiological saline aerated with 95 % oxygen and 5% CO_2 at 37°C. Vessels were constricted with phenylephrine and vascular relaxation was measured in response to increasing concentrations of ACh. The isolated aorta and carotid arteries from the eNOS deficient mice did not relax in response to ACh [30, 31] and the mice are hypertensive [30].

3.5.1.2. *In vivo* experiments: ACh significantly increased absolute CBF in WT and nNOS knockout mice, but not in eNOS deficient mice [28, 32]. These results agree with previous

cranial window experimental results, which showed that arteriolar dilation to ACh superfusion was greater in nNOS deficient mice as compared with eNOS mutant mice [33]. The arterioral relaxation of eNOS knockout mice was partially NOS dependent and attenuated by tetrodotoxin, an inhibitor of voltage-dependent sodium channels, and the NOS inhibitor L-NNA, suggesting that nNOS-dependent mechanism compensates for the deficiency of eNOS in eNOS knockout mice. Superfusion with the nNOS specific inhibitor 7-nitroindazole (7-NI) attenuated the ACh response in eNOS deficient mice, while the sGC inhibitor 1H-(1,2,4) oxadiazolo (4,3-a) qinoxalin-1-one (ODQ) attenuated the ACh relaxation in eNOS, nNOS and WT mice. These results indicate that nNOS-cGMP-dependent pathways dilate pial arterioles as compensatory mechanism after eNOS gene disruption [34]. Neuronal NO, produced by nNOS in nerves innervating blood vessels, also causes smooth muscle relaxation, vasodilation and increase of blood flow [2].

These data have been confirmed by mathematical models of contributive role of eNOS and nNOS in vascular tone. Computational models show significant effect of the nNOS on the NO levels in smooth muscle [35, 36]. Experimental results demonstrated that NO can be synthesized around arterioles by endothelial and neuronal NOS [37, 38, 39]. The computational models demonstrated that NO produced by nNOS in the perivascular nerve fibers and interstitial cells have the potential to play a significant role into the smooth muscle cells, and sGC reactions in arterioles [40].

3.6. Superoxide dismutase (SOD)

3.6.1. Rats and other studies—Superoxide directly contracts smooth muscle in cerebral arteries [41] and mediates constriction of cerebral blood vessels in models of vasospasm [42–45] and Alzheimer's disease [46, 47]. These effects may be due to direct effects on vascular muscle, the interaction of superoxide with NO, or other unknown mechanisms. NO bioavailibility in cerebral vasculature depends upon its interaction with superoxide anion, which reacts with and inactivates NO[48]. eNOS may produce superoxide and lead to hydrogen peroxide -dependent dilations to ACh in isolated mouse cerebral arteries [49]. eNOS-derived hydrogen peroxide mediates flow-dependent dilation [50]. Superoxide-mediated impairment of cerebrovascular reactivity to endothelium-dependent relaxation without significant changes in resting vascular diameter observed in models of diabetes, alcoholism, hyperhomocysteinemia, and genetic deficiency in SOD [51–55].

The interaction of superoxide with endothelial NO results in peroxynitrite production, which also can alter vascular tone. The peroxynitrite dilates cerebral arterioles *in vivo* via ATP-sensitive potassium channels [56]. Peroxynitrite also dilates cerebral arteries *in vitro* by endothelium independent [57] mechanisms. Peroxynitrite produces contraction of cerebral arteries *in vitro* via an inhibitory effect of basal activity of calcium-activated potassium channels [58].

Cu, Zn-SOD (SOD1) is located in the cytoplasm and nucleus, inactivating superoxide at the surface of the vascular endothelium. SOD1 significantly increases rCBF in rats after intravenous infusion [59].

Extracellular superoxide dismutase (SOD3) is located in the intercellular medium and prevents inactivation of NO by superoxide anion in the intercellular space of the brain, between the endothelium and smooth muscle cells. Under normal conditions, SOD3 minimized superoxide anion levels, protecting endogenous NO at a sufficient level to maintain cerebral vascular tone and reactivity [60].

3.6.2. Mice (eNOS knockout and nNOS knockout)—SOD1 significantly increases absolute CBF in WT and nNOS knockout mice, but not in eNOS knockout mice [28]. The

results indicate that the endothelial NO plays a key role in regulation of cerebrovascular tone in baseline conditions. By scavenging superoxide, SOD1 increases activity of NO and augments CBF in WT and nNOS knockout mice. Because eNOS knockout mice lack endothelial NO, enhanced scavenging of superoxide by SOD1 does not increase vascular relaxation [59]. These observations also indicate that bioavailability of endothelial NO may be regulated by superoxide anion in the cerebral vessels [61].

3.7. CBF autoregulation

CBF autoregulation is the ability of the brain vasculature to maintain constant perfusion and blood flow despite arterial blood pressure changes [62].

3.7.1. Rats and other studies—Rat cerebral arterioles demonstrated increased diameter and concentration of NO at reduced periarteriolar oxygen tension when arterial pressure was decreased [63]. After selective inhibition of nNOS with N-(4S)-(4-amino-5-[aminoethyl]aminopentyl)-N′-nitroguanidine, resting NO, oxygen tension and vessel diameters decreased, and the increase in NO during hypotension was absent. Flow-mediated dilation during occlusion of a collateral arteriole was intact after nNOS blockade and NO concentration in the vessel wall was elevated. Taken together, the results suggest that nNOS increased NO concentration during decreased periarteriolar oxygen tension during hypotension, but eNOS was the main source of NO production for flow shear mechanisms [63].

Laser Doppler flowmetry study of autoregulation in rats with a superfused closed cranial window demonstrates that NOS inhibition depressed the autoregulatory pattern. The results suggest that NO elevates CBF near the lower limit and increases the hypotensive portion of the autoregulatory curve [64].

Most studies with cortical NOS inhibition and regional CBF methods [65–69] support important roles of NO in extending the lower limit of autoregulation. 7-NI, a specific nNOS inhibitor, was ineffective in changing autoregulation [69] and the lower limit [68].

3.7.2. Mice (eNOS knockout and nNOS knockout)—CBF was relatively constant during controlled hemorarhagic hypotension until 40 mm Hg. nNOS knockout mice show no change in autoregulation [70]. At low blood pressure, the autoregulation curve of eNOS knockout mice was shifted to the right, suggesting higher resistance of brain vessels in eNOS knockout mice as compare with WT mice at lower perfusion pressure. CBF was attenuated at arterial pressure near the lower limit in eNOS knockout compared with WT mice [71].

Taken together, these studies suggest that endothelial NO is responsible for the effects of cerebral autoregulation.

3.8. Functional hyperemia (whisker stimulation, CO_2 reactivity)

3.8.1. Rats—The NOS inhibitors L-NAME and L-NNA attenuate resting CBF and hypercapnia-induced vasorelaxation. The nNOS inhibitor 7-NI significantly reduced cortical CBF and hypercapnic CBF response [72] without effects on resting CBF or on eNOS.

3.8.2. Mice (eNOS knockout and nNOS knockout)—Physiological compensation by non-cGMP-dependent mechanisms was described for CBF responses of eNOS knockout mice to hypercapnia [73] and for whisker stimulation [29, 74]. Hypercapnia (5% CO_2) augmented CBF in both WT and eNOS deficient mice. L-NNA superfusion inhibited this

increase in both strains [29]. These results suggest that the CBF response to hypecapnia depends on nNOS generated NO.

Whisker stimulation increased rCBF by similar level in WT and nNOS deficient mice in experiments with closed cranial window. L-NNA inhibited rCBF response in WT mice, whereas there was no inhibition in nNOS deficient mice. Endothelium-dependent relaxation of pial vessel in response to ACh and inhibition by L-NNA, was the same in both groups. The results suggest that endothelial NO production no mediates the rCBF coupling to neuronal activity without nNOS. NO-independent mechanisms couple rCBF and metabolism during whisker stimulation in nNOS deficient mice [29].

rCBF increased during whisker stimulation in both WT and eNOS knockout mice. The attenuation of CBF during the whisker stimulation was inhibited by nitro-L-arginine (L-NA) superfusion using closed cranial window method. That suggests that CBF coupling with increased functional activity caused by vibrissa stimulation is nNOS, but not eNOS-dependent process in cortical barrel brain areas of the mice [74].

4. Hyperbaric oxygen (HBO) and CBF regulation by eNOS

4.1. Rats

Studies on conscious rats with inhibition of NOS were used to assess the dynamics of CBF during hyperbaric oxygenation (HBO). Oxygen at a pressure of 4 ATA induced cerebral vasoconstriction in intact animals and decreased blood flow without oxygen convulsions. At 5 ATA, convulsive activity appeared and brain blood flow decreased significantly during the first 20 min, but the blood flow was significantly increased before HBO convulsions. NOS pretreatment by prior inhibition of NOS by L-NAME, or inhibition only of nNOS with 7-NI prevented the hyperemia and paroxysmal spikes on the EEG during hyperbaric oxygenation at 5 ATA. These results show that hyperbaric oxygen changes CBF and modulates oxygen neurotoxicity via eNOS and nNOS [75].

CBF decreases during HBO due to inactivation of NO by superoxide anion. SOD increased CBF in rats breathing air, but was ineffective after previous inhibition of NOS[76]. HBO induced CBF decreases, though prior SOD prevented hyperoxic vasoconstriction and increased CBF under HBO in rats. The vasodilator effect of SOD in HBO was not observed in rats pretreated with NOS inhibitor. These results suggest inactivation of NO by superoxide anion as a mechanism of hyperoxic vasoconstriction [76].

SOD3 increased the cerebrovascular relaxation by endogenous NO, neutralized superoxide anions and maintains basal NO levels in normobaric hyperoxia [60].

4.2. Mice (eNOS knockout and nNOS knockout)

eNOS and nNOS deficient mice were used to study the contributive roles of the NOS isoforms in mediating changes in cerebral vascular tone in response to hyperoxia. HBO at 5 ATA decreases rCBF over 30 minutes in WT and nNOS deficient mice, but not in eNOS knockout mice. After 60 minutes HBO, rCBF increased significantly more in WT mice than in both mutant mice. Brain NO-metabolites nitrite and nitrate (NO_x) decreased in WT and eNOS knockout mice within 30 minutes of HBO. NO_x rose above control levels in WT and eNOS deficient mice after 45 minutes of HBO, whereas they did not change in nNOS knockout mice. Brain 3-nitrotyrosine increased during HBO in WT and eNOS knockout mice but not in nNOS deficient mice. These results demonstrate that under HBO, eNOS-derived NO is responsible for the early vasoconstriction, whereas late HBO-induced vasodilation depends upon both eNOS and nNOS [28].

5. Stroke and CBF regulation by eNOS

Endothelial NO plays protective roles in vascular function during ischemia and reperfusion.

5.1. Rats

Reversible occlusion of the middle cerebral artery in rats is followed by augmented expression of eNOS in rat brain [77]. Selective eNOS inhibition in rats demonstrated worsened water maze performance in rats subjected to chronic brain hypoperfusion (CBH) as compared with rats after selective nNOS (7-NI) or iNOS (aminoguanidine) inhibition [78]. The results suggest that endothelial NO plays an important role in spatial memory function during CBH optimizing the cerebral perfusion through microvascular tone regulation and CBF.

5.2. Mice

Endothelial caveolin-1 and caveolin-2 and level of eNOS protein increased after middle cerebral artery occlusion (MCAO) in WT mice [79]. Adiponectin-deficient mice demonstrated attenuated eNOS phosphorylation in ischemic brain, decreased plasma NO metabolites, compromised CBF during ischemia and increased cerebral infarct after ischemia and reperfusion. These results suggest that adiponectin exerts a cerebroprotective action through an eNOS-dependent mechanism [80].

5.2.1. eNOS knockout mice and nNOS knockout mice—Physical exercise on treadmill apparatus and on running wheels results in attenuated cerebral infarct size and neurological deficit, improved endothelium-dependent vascular reactivity and increased CBF in WT, but not in eNOS knockout mice [81], suggesting that enhanced eNOS activity can protect against stroke injury.

eNOS deficient mice developed larger infarcts than the WT mice 24 h after permanent MCAO by filament [71]. Relative CBF in the MCA territory, measured by laser-Doppler flowmetry, was more severely reduced during occlusion and was significantly attenuated during hemorrhagic hypotension in autoregulation study. The nitro-L-arginine superfusion dilates pial arterioles of eNOS knockout mice in a closed cranial window experiments. Systemic administration of nitro-L-arginine decreased infarct size in eNOS deficient but not in the WT mice. Infarct volume did not change in eNOS knockout mice after hydralazine administration to normalize arterial blood pressure of the knockouts. These results show that endothelial NO protects brain tissue after stroke by hemodynamic mechanisms.

CBF was more severely decreased after MCAO in eNOS knockout as compare with nNOS knockout mice [70]. Infarction volume was increased when nitro-L-arginine was administrated to nNOS knockout mice, expressing only eNOS isoform. eNOS deficiency led to more severe hemodynamic deficits after focal cerebral ischemia. Hemodynamic deficit, measured by functional computer tomography scanning method in the peri-ischemic area of eNOS knockout mice was more pronounced as compare with WT mice [82]. WT and eNOS knockout mice were subjected to MCAO under halothane anesthesia and functional CT scanning was preformed to measure the cerebral transit profiles of contrast agents. The core areas were larger in eNOS knockout mice as compare with WT mice. The hemodynamic penumbra was smaller in eNOS mutant mice than in WT mice.

NO is required for ischemic preconditioning [28]. Cerebral ischemic preconditioning is neuroprotection induced by pretreatment with brief ischemic episodes. Although WT mice showed protection from ischemic preconditioning, neither eNOS nor nNOS knockout mice showed protection. Laser Doppler measurements indicated that the relative CBF decreases

in core ischemic areas were the same in all groups. Neither eNOS nor nNOS knockout mice show protection from rapid ischemic preconditioning, suggesting that both of constitutive nitric oxide may play a role in the molecular mechanisms of protection.

5.2.1.1. Rho kinase inhibitors: Rho-kinase is a serine threonine kinase and upstream negative regulator of eNOS activity. Rho-kinase negatively regulates eNOS via transcriptional and post-transcriptional mechanisms [83], subcellular translocation of eNOS due to actin cytoskeleton reorganization, and phosphorylation of eNOS at serine 1177 via Akt pathway [84]. Rho-kinase is expressed in most brain cells [85]. Hypoxic and ischemic conditions downregulate activity of eNOS via activation of Rho-kinase [86, 87, 88]. Rho-kinase down regulates eNOS activity during cerebral ischemia, worsening the CBF insufficiency [89]. Rho-kinase is activated after cerebral ischemia [90], increasing the sensitivity of smooth muscle contraction to intracellular calcium by enhancing phosphorylation of myosin light chain [91] and reducing activity of eNOS [84]. Rho-kinase activity is increased in penumbral cerebrovasculature during MCAO [92].

Rho-kinase inhibitors regulate vasomotor tone, reduce inflammation [93], NADPH oxidase and superoxide production in neutrophils [94] and activate ATP dependent potassium channels in cardiac myocytes [95], improve CBF in ischemic areas and penumbra in an eNOS-dependent fashion [89].

Inhibition of Rho-kinase decreases stroke size in WT, but not in eNOS deficient mice[89]. Rho-kinase inhibition induces vasodilation and increase CBF in ischemic brain inhibiting of smooth muscle cells contractility and activating eNOS. Because Rho-kinase inhibition by hydroxyfasudil did not improve CBF in eNOS knockout mice, it was suggested, that eNOS plays an obligatory role in this effect[89]. Because CBF increases rapidly after Rho-kinase inhibition, nontranslational eNOS activity upregulation is likely to account for this, such as S1177 phosphorylation [96].

Rho-kinase inhibitors increase CBF in ischemic cortex, but not in nonischemic brain [89]. Resting CBF decreased in non-ischemic brain due to hypotensive effect of hydroxyfasudil and Y-27632. In contrast, fasudil and hydroxyfasudil augment CBF in canine cortex [93].

Changes in the actin cytoskeleton of endothelium affect eNOS expression [97] and are mediated by guanosine-5′-triphosphate binding (GTP-binding) proteinRho [98]. The inhibition of Rho-mediated endothelial actin cytoskeleton changes ameliorates expression and activity of eNOS, increases CBF, and reduced stroke injury following cerebrovascular occlusion [97]. eNOS deficient mice do not demonstrated the neuroprotection after pretreatment with Rho inhibitor C3 transferase or the actin cytockeleton disrupter cytochalasin D. Rho modulates the actin cytockeleton, but Rho inhibition is not sufficient to up-regulate eNOS, because cytochalasin D improve eNOS expression despite increased compensatory activation of vascular Rho [97]. The similar results of the Rho inhibitor and 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors suggest that eNOS expression increased with affected endothelial actin cytoskeleton integrity. The actin cytoskeleton regulates eNOS expression by post-transcriptional stabilization of eNOS mRNA. Rho may stabilize eNOS mRNA by regulation of the cytoskeletal localization of the eNOS mRNA. Results with cytochalasin D suggest that Rho induces phosphorylation of myosin light chain and mediates eNOS expression up-regulation by focal adhesion assembly and reorganization of actin cytoskeleton.

5.2.1.2. Statins: The statins, including simvastatin, lowastatin, pravastatin, and atorvastatin, decrease serum cholesterol and the incidence of stroke. Statins elevate CBF by eNOS upregulation and NO generation [99, 100]. Statin treatment improves endothelial function

even without changes in serum cholesterol levels [101, 102]. The statins upregulate eNOS expression in vitro under cholesterol-clamped conditions [83, 88, 103].

Prophylactic pretreatment with statins increases CBF, decreases cerebral infarct volume, and improves neurological function in WT mice. These hemodynamic and neuroprotective effects were absent in eNOS knockout mice, indicating that eNOS activation by HMG-CoA reductase inhibitors is the major protective mechanism against stroke injury [99]. In addition, inhibition of platelet aggregation and leukocyte adhesion may also contribute to neuroprotection by statins. Neuroprotection by statins in WT mice lasted for up to 72 hours after MCAO.

CBF measured by laser-Doppler flowmetry was increased after L-arginine infusion in WT, but not in eNOS knockout mice. Chronic simvastatin treatment upregulates eNOS. L-arginine infusion after simvastatin treatment amplified the hyperemia, and increased absolute CBF. Simvastatin enhanced CBF within ischemic cerebral tissue. These results suggest that eNOS activity is critical for enhanced CBF during L-arginine infusion, and L-arginine administration during chronic upregulation of eNOS elevates CBF in the normal and ischemic brain [100].

Another statin, mevastatin, administered 7, 14 or 28 days before an ischemic event, upregulates eNOS and augments absolute CBF in the absence of changes in serum cholesterol levels. Mevastatin pretreatment resulted in neuroprotection after 2 hours MCAO reperfusion injury by filament and 22 hours of reperfusion [104]. eNOS deficient mice did not demonstrate neuroprotection after mevastatin treatment. Interestingly, simvastatin and atorvastatin have neuroprotective effects after embolic cerebral ischemia [105], increasing both eNOS and tissue plasminogen activator (tPA), but not plasminogen activator inhibitor type 1(PAI-1) mRNA levels. These effects are eNOS independent because eNOS knockout mice demonstrated reduced ischemic injury and improved neurological outcome after embolic blood clot stroke.

Thus, statins effect increase eNOS expression and protein level, CBF and protect against stroke injury. Vascular mechanisms of statins neuroprotective effect predominate as compare with neuronal mechanisms. Statins may also protect against myocardial infarction and attenuate tissue plasminogen activator activity in endothelium [101, 106]. The difference between statins effects depends on lipophilicity, when highest lipophilicity provides the greatest degree of eNOS upregulation and neuroprotection [104].

5.2.2. eNOS S1177 mutant mice—Modulation of the eNOS serine 1177 (S1177) phosphorylation site determines vascular reactivity and infarct size. Substitution of serine for alanine on position 1177 (S1177A) produces an eNOS unphosphorylated form, because it lacks the hydroxyl group on the side chine of the serine. Substitution of aspartate on the position (S1177D) mimics the attenuated catalytic activity caused by the phosphorylation [107]. Mutant mice expressing a phosphomimetic (S1179D) form of eNOS show better vascular reactivity, less severe stroke damage, and improved CBF during the middle cerebral artery occlusion than mice expressing an unphosphorylatable (S1179A) form [31]. Importantly, mice with increased eNOS activity on serine 1179 (S1179D mice) show better isolated carotid artery relaxation as compare to eNOS deficient mice and to mice, inactive on eNOS serine 1179 (S1179A mice [31].

Conclusion

Endothelial NO plays important roles in CBF regulation under basal resting conditions, and more importantly, under conditions of pathology and stress. The precise contributions of

endothelial and neuronal produced NO are still being defined, specifically the roles of the isoforms in resting CBF and its autoregulation.

References

1. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochem J. 2001; 357(Pt 3):593–615. [PubMed: 11463332]
2. Toda N, Okamura T. The pharmacology of nitric oxide in the peripheral nervous system of blood vessels. Pharmacol Rev. 2003; 55(2):271–324. [PubMed: 12773630]
3. Bhardwaj A, Northington FJ, Carhuapoma JR, Falck JR, Harder DR, Traystman RJ, Koehler RC. P-450 epoxygenase and NO synthase inhibitors reduce cerebral blood flow response to N-methyl-D-aspartate. Am J Physiol Heart Circ Physiol. 2000; 279(4):H1616–24. [PubMed: 11009448]
4. Toda N, Ayajiki K, Okamura T. Cerebral blood flow regulation by nitric oxide: recent advances. Pharmacol Rev. 2009; 61(1):62–97. [PubMed: 19293146]
5. Samdani AF, Dawson TM, Dawson VL. Nitric oxide synthase in models of focal ischemia. Stroke. 1997; 28(6):1283–8. [PubMed: 9183363]
6. Sessa WC. eNOS at a glance. J Cell Sci. 2004; 117(Pt 12):2427–9. [PubMed: 15159447]
7. Huang PL. Unraveling the links between diabetes, obesity, and cardiovascular disease. Circ Res. 2005; 96(11):1129–31. [PubMed: 15947251]
8. Shaul PW. Regulation of endothelial nitric oxide synthase: location, location, location. Annu Rev Physiol. 2002; 64:749–74. [PubMed: 11826287]
9. Dudzinski DM, Igarashi J, Greif D, Michel T. The regulation and pharmacology of endothelial nitric oxide synthase. Annu Rev Pharmacol Toxicol. 2006; 46:235–76. [PubMed: 16402905]
10. Hisamoto K, Ohmichi M, Kurachi H, Hayakawa J, Kanda Y, Nishio Y, Adachi K, Tasaka K, Miyoshi E, Fujiwara N, Taniguchi N, Murata Y. Estrogen induces the Akt-dependent activation of endothelial nitric-oxide synthase in vascular endothelial cells. J Biol Chem. 2001; 276(5):3459–67. [PubMed: 11044445]
11. Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJ, Sessa WC, Walsh K. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. Nat Med. 2000; 6(9):1004–10. [PubMed: 10973320]
12. Vecchione C, Maffei A, Colella S, Aretini A, Poulet R, Frati G, Gentile MT, Fratta L, Trimarco V, Trimarco B, Lembo G. Leptin effect on endothelial nitric oxide is mediated through Akt-endothelial nitric oxide synthase phosphorylation pathway. Diabetes. 2002; 51(1):168–73. [PubMed: 11756337]
13. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. J Biol Chem. 2003; 278(45):45021–6. [PubMed: 12944390]
14. Scotland RS, Morales-Ruiz M, Chen Y, Yu J, Rudic RD, Fulton D, Gratton JP, Sessa WC. Functional reconstitution of endothelial nitric oxide synthase reveals the importance of serine 1179 in endothelium-dependent vasomotion. Circ Res. 2002; 90(8):904–10. [PubMed: 11988492]
15. Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-stimulated activation of eNOS is independent of Ca2+ but requires phosphorylation by Akt at Ser(1179). J Biol Chem. 2001; 276(32):30392–8. [PubMed: 11402048]
16. Butt E, Bernhardt M, Smolenski A, Kotsonis P, Frohlich LG, Sickmann A, Meyer HE, Lohmann SM, Schmidt HH. Endothelial nitric-oxide synthase (type III) is activated and becomes calcium independent upon phosphorylation by cyclic nucleotide-dependent protein kinases. J Biol Chem. 2000; 275(7):5179–87. [PubMed: 10671564]
17. Channon KM. Tetrahydrobiopterin: regulator of endothelial nitric oxide synthase in vascular disease. Trends Cardiovasc Med. 2004; 14(8):323–7. [PubMed: 15596110]
18. Fabian RH, Perez-Polo JR, Kent TA. Perivascular nitric oxide and superoxide in neonatal cerebral hypoxia-ischemia. Am J Physiol Heart Circ Physiol. 2008; 295(4):H1809–14. [PubMed: 18676689]
19. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol. 1995; 268(4 Pt 1):C799–822. [PubMed: 7733230]

20. Cohen RA, Adachi T. Nitric-oxide-induced vasodilatation: regulation by physiologic s-glutathiolation and pathologic oxidation of the sarcoplasmic endoplasmic reticulum calcium ATPase. Trends Cardiovasc Med. 2006; 16(4):109–14. [PubMed: 16713532]
21. Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature. 1994; 368(6474):850–3. [PubMed: 7512692]
22. Jesmin S, Togashi H, Mowa CN, Ueno K, Yamaguchi T, Shibayama A, Miyauchi T, Sakuma I, Yoshioka M. Characterization of regional cerebral blood flow and expression of angiogenic growth factors in the frontal cortex of juvenile male SHRSP and SHR. Brain Res. 2004; 1030(2): 172–82. [PubMed: 15571667]
23. Wilkerson MK, Lesniewski LA, Golding EM, Bryan RM Jr, Amin A, Wilson E, Delp MD. Simulated microgravity enhances cerebral artery vasoconstriction and vascular resistance through endothelial nitric oxide mechanism. Am J Physiol Heart Circ Physiol. 2005; 288(4):H1652–61. [PubMed: 15576439]
24. Luders JC, Weihl CC, Lin G, Ghadge G, Stoodley M, Roos RP, Macdonald RL. Adenoviral gene transfer of nitric oxide synthase increases cerebral blood flow in rats. Neurosurgery. 2000; 47(5): 1206–14. discussion 1214–5. [PubMed: 11063115]
25. Hara H, Waeber C, Huang PL, Fujii M, Fishman MC, Moskowitz MA. Brain distribution of nitric oxide synthase in neuronal or endothelial nitric oxide synthase mutant mice using [3H]L-NG-nitro-arginine autoradiography. Neuroscience. 1996; 75(3):881–90. [PubMed: 8951881]
26. Kano T, Shimizu-Sasamata M, Huang PL, Moskowitz MA, Lo EH. Effects of nitric oxide synthase gene knockout on neurotransmitter release in vivo. Neuroscience. 1998; 86(3):695–9. [PubMed: 9692709]
27. Demchenko IT, Luchakov YI, Moskvin AN, Gutsaeva DR, Allen BW, Thalmann ED, Piantadosi CA. Cerebral blood flow and brain oxygenation in rats breathing oxygen under pressure. J Cereb Blood Flow Metab. 2005; 25(10):1288–300. [PubMed: 15789033]
28. Atochin DN, Demchenko IT, Astern J, Boso AE, Piantadosi CA, Huang PL. Contributions of endothelial and neuronal nitric oxide synthases to cerebrovascular responses to hyperoxia. J Cereb Blood Flow Metab. 2003; 23(10):1219–26. [PubMed: 14526232]
29. Ma J, Meng W, Ayata C, Huang PL, Fishman MC, Moskowitz MA. L-NNA-sensitive regional cerebral blood flow augmentation during hypercapnia in type III NOS mutant mice. Am J Physiol. 1996; 271(4 Pt 2):H1717–9. [PubMed: 8897969]
30. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature. 1995; 377(6546):239–42. [PubMed: 7545787]
31. Atochin DN, Wang A, Liu VW, Critchlow JD, Dantas AP, Looft-Wilson R, Murata T, Salomone S, Shin HK, Ayata C, Moskowitz MA, Michel T, Sessa WC, Huang PL. The phosphorylation state of eNOS modulates vascular reactivity and outcome of cerebral ischemia in vivo. J Clin Invest. 2007; 117(7):1961–7. [PubMed: 17557122]
32. Demchenko IT, Atochin DN, Boso AE, Astern J, Huang PL, Piantadosi CA. Oxygen seizure latency and peroxynitrite formation in mice lacking neuronal or endothelial nitric oxide synthases. Neurosci Lett. 2003; 344(1):53–6. [PubMed: 12781920]
33. Meng W, Ma J, Ayata C, Hara H, Huang PL, Fishman MC, Moskowitz MA. ACh dilates pial arterioles in endothelial and neuronal NOS knockout mice by NO-dependent mechanisms. Am J Physiol. 1996; 271(3 Pt 2):H1145–50. [PubMed: 8853353]
34. Meng W, Ayata C, Waeber C, Huang PL, Moskowitz MA. Neuronal NOS-cGMP-dependent ACh-induced relaxation in pial arterioles of endothelial NOS knockout mice. Am J Physiol. 1998; 274(2 Pt 2):H411–5. [PubMed: 9486242]
35. Kavdia M, Popel AS. Contribution of nNOS-and eNOS-derived NO to microvascular smooth muscle NO exposure. J Appl Physiol. 2004; 97(1):293–301. [PubMed: 15033959]
36. Lamkin-Kennard KA, Buerk DG, Jaron D. Interactions between NO and O2 in the microcirculation: a mathematical analysis. Microvasc Res. 2004; 68(1):38–50. [PubMed: 15219419]

37. Kashiwagi S, Kajimura M, Yoshimura Y, Suematsu M. Nonendothelial source of nitric oxide in arterioles but not in venules: alternative source revealed in vivo by diaminofluorescein microfluorography. Circ Res. 2002; 91(12):e55–64. [PubMed: 12480826]
38. Bidri M, Feger F, Varadaradjalou S, Ben Hamouda N, Guillosson JJ, Arock M. Mast cells as a source and target for nitric oxide. Int Immunopharmacol. 2001; 1(8):1543–58. [PubMed: 11515818]
39. Brown LA, Key BJ, Lovick TA. Fluorescent imaging of nitric oxide production in neuronal varicosities associated with intraparenchymal arterioles in rat hippocampal slices. Neurosci Lett. 2000; 294(1):9–12. [PubMed: 11044574]
40. Chen K, Popel AS. Vascular and perivascular nitric oxide release and transport: biochemical pathways of neuronal nitric oxide synthase (NOS1) and endothelial nitric oxide synthase (NOS3). Free Radic Biol Med. 2007; 42(6):811–22. [PubMed: 17320763]
41. Tosaka M, Hashiba Y, Saito N, Imai H, Shimizu T, Sasaki T. Contractile responses to reactive oxygen species in the canine basilar artery in vitro: selective inhibitory effect of MCI-186, a new hydroxyl radical scavenger. Acta Neurochir (Wien). 2002; 144(12):1305–10. discussion 1310. [PubMed: 12478342]
42. Kamii H, Kato I, Kinouchi H, Chan PH, Epstein CJ, Akabane A, Okamoto H, Yoshimoto T. Amelioration of vasospasm after subarachnoid hemorrhage in transgenic mice overexpressing CuZn-superoxide dismutase. Stroke. 1999; 30(4):867–71. discussion 872. [PubMed: 10187893]
43. Kontos HA, Wei EP, Marshall JJ. In vivo bioassay of endothelium-derived relaxing factor. Am J Physiol. 1988; 255(5 Pt 2):H1259–62. [PubMed: 3142279]
44. Mori T, Nagata K, Matsui T, Ishida T, Ohami H, Asano T. Superoxide anions in the pathogenesis of talc-induced cerebral vasocontraction. Neuropathol Appl Neurobiol. 1995; 21(5):378–85. [PubMed: 8632832]
45. Watanabe Y, Chu Y, Andresen JJ, Nakane H, Faraci FM, Heistad DD. Gene transfer of extracellular superoxide dismutase reduces cerebral vasospasm after subarachnoid hemorrhage. Stroke. 2003; 34(2):434–40. [PubMed: 12574556]
46. Niwa K, Porter VA, Kazama K, Cornfield D, Carlson GA, Iadecola C. A beta-peptides enhance vasoconstriction in cerebral circulation. Am J Physiol Heart Circ Physiol. 2001; 281(6):H2417–24. [PubMed: 11709407]
47. Park L, Anrather J, Forster C, Kazama K, Carlson GA, Iadecola C. Abeta-induced vascular oxidative stress and attenuation of functional hyperemia in mouse somatosensory cortex. J Cereb Blood Flow Metab. 2004; 24(3):334–42. [PubMed: 15091114]
48. Didion SP, Hathaway CA, Faraci FM. Superoxide levels and function of cerebral blood vessels after inhibition of CuZn-SOD. Am J Physiol Heart Circ Physiol. 2001; 281(4):H1697–703. [PubMed: 11557560]
49. Drouin A, Thorin-Trescases N, Hamel E, Falck JR, Thorin E. Endothelial nitric oxide synthase activation leads to dilatory H2O2 production in mouse cerebral arteries. Cardiovasc Res. 2007; 73(1):73–81. [PubMed: 17113574]
50. Drouin A, Thorin E. Flow-induced dilation is mediated by Akt-dependent activation of endothelial nitric oxide synthase-derived hydrogen peroxide in mouse cerebral arteries. Stroke. 2009; 40(5): 1827–33. [PubMed: 19286591]
51. Dayal S, Arning E, Bottiglieri T, Boger RH, Sigmund CD, Faraci FM, Lentz SR. Cerebral vascular dysfunction mediated by superoxide in hyperhomocysteinemic mice. Stroke. 2004; 35(8):1957–62. [PubMed: 15178827]
52. Didion SP, Lynch CM, Baumbach GL, Faraci FM. Impaired endothelium-dependent responses and enhanced influence of Rho-kinase in cerebral arterioles in type II diabetes. Stroke. 2005; 36(2): 342–7. [PubMed: 15637328]
53. Didion SP, Ryan MJ, Didion LA, Fegan PE, Sigmund CD, Faraci FM. Increased superoxide and vascular dysfunction in CuZnSOD-deficient mice. Circ Res. 2002; 91(10):938–44. [PubMed: 12433839]
54. Erdos B, Snipes JA, Miller AW, Busija DW. Cerebrovascular dysfunction in Zucker obese rats is mediated by oxidative stress and protein kinase C. Diabetes. 2004; 53(5):1352–9. [PubMed: 15111506]

55. Sun H, Mayhan WG. Temporal effect of alcohol consumption on reactivity of pial arterioles: role of oxygen radicals. Am J Physiol Heart Circ Physiol. 2001; 280(3):H992–H1001. [PubMed: 11179040]
56. Wei EP, Kontos HA, Beckman JS. Mechanisms of cerebral vasodilation by superoxide, hydrogen peroxide, and peroxynitrite. Am J Physiol. 1996; 271(3 Pt 2):H1262–6. [PubMed: 8853367]
57. Li J, Li W, Altura BT, Altura BM. Peroxynitrite-induced relaxation in isolated canine cerebral arteries and mechanisms of action. Toxicol Appl Pharmacol. 2004; 196(1):176–82. [PubMed: 15050419]
58. Brzezinska AK, Gebremedhin D, Chilian WM, Kalyanaraman B, Elliott SJ. Peroxynitrite reversibly inhibits Ca(2+)-activated K(+) channels in rat cerebral artery smooth muscle cells. Am J Physiol Heart Circ Physiol. 2000; 278(6):H1883–90. [PubMed: 10843885]
59. Demchenko IT, Boso AE, Bennett PB, Whorton AR, Piantadosi CA. Hyperbaric oxygen reduces cerebral blood flow by inactivating nitric oxide. Nitric Oxide. 2000; 4(6):597–608. [PubMed: 11139368]
60. Demchenko IT, Gutsaeva DR, Moskvin AN, Zhilyaev SY. Involvement of extracellular superoxide dismutase in regulating brain blood flow. Neurosci Behav Physiol. 40(2):173–8. [PubMed: 20033309]
61. Wei EP, Kontos HA, Christman CW, DeWitt DS, Povlishock JT. Superoxide generation and reversal of acetylcholine-induced cerebral arteriolar dilation after acute hypertension. Circ Res. 1985; 57(5):781–7. [PubMed: 4053309]
62. White RP, Vallance P, Markus HS. Effect of inhibition of nitric oxide synthase on dynamic cerebral autoregulation in humans. Clin Sci (Lond). 2000; 99(6):555–60. [PubMed: 11099400]
63. Bauser-Heaton HD, Bohlen HG. Cerebral microvascular dilation during hypotension and decreased oxygen tension: a role for nNOS. Am J Physiol Heart Circ Physiol. 2007; 293(4):H2193–201. [PubMed: 17630350]
64. Jones SC, Easley KA, Radinsky CR, Chyatte D, Furlan AJ, Perez-Trepichio AD. Nitric oxide synthase inhibition depresses the height of the cerebralblood flow-pressure autoregulation curve during moderate hypotension. J Cereb Blood Flow Metab. 2003; 23(9):1085–95. [PubMed: 12973025]
65. Kobari M, Fukuuchi Y, Tomita M, Tanahashi N, Takeda H. Role of nitric oxide in regulation of cerebral microvascular tone and autoregulation of cerebral blood flow in cats. Brain Res. 1994; 667(2):255–62. [PubMed: 7697363]
66. Preckel MP, Leftheriotis G, Ferber C, Degoute CS, Banssillon V, Saumet JL. Effect of nitric oxide blockade on the lower limit of the cortical cerebral autoregulation in pentobarbital-anaesthetized rats. Int J Microcirc Clin Exp. 1996; 16(6):277–83. [PubMed: 9049705]
67. Tanaka K, Fukuuchi Y, Gomi S, Mihara B, Shirai T, Nogawa S, Nozaki H, Nagata E. Inhibition of nitric oxide synthesis impairs autoregulation of local cerebral blood flow in the rat. Neuroreport. 1993; 4(3):267–70. [PubMed: 8477049]
68. Jones SC, Radinsky CR, Furlan AJ, Chyatte D, Perez-Trepichio AD. Cortical NOS inhibition raises the lower limit of cerebral blood flow-arterial pressure autoregulation. Am J Physiol. 1999; 276(4 Pt 2):H1253–62. [PubMed: 10199850]
69. Toyoda K, Fujii K, Ibayashi S, Nagao T, Kitazono T, Fujishima M. Role of nitric oxide in regulation of brain stem circulation during hypotension. J Cereb Blood Flow Metab. 1997; 17(10): 1089–96. [PubMed: 9346434]
70. Huang Z, Huang PL, Panahian N, Dalkara T, Fishman MC, Moskowitz MA. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. Science. 1994; 265(5180):1883–5. [PubMed: 7522345]
71. Huang Z, Huang PL, Ma J, Meng W, Ayata C, Fishman MC, Moskowitz MA. Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. J Cereb Blood Flow Metab. 1996; 16(5):981–7. [PubMed: 8784243]
72. Wang Q, Pelligrino DA, Baughman VL, Koenig HM, Albrecht RF. The role of neuronal nitric oxide synthase in regulation of cerebral blood flow in normocapnia and hypercapnia in rats. J Cereb Blood Flow Metab. 1995; 15(5):774–8. [PubMed: 7545691]

73. Irikura K, Huang PL, Ma J, Lee WS, Dalkara T, Fishman MC, Dawson TM, Snyder SH, Moskowitz MA. Cerebrovascular alterations in mice lacking neuronal nitric oxide synthase gene expression. Proc Natl Acad Sci U S A. 1995; 92(15):6823–7. [PubMed: 7542777]
74. Ayata C, Ma J, Meng W, Huang P, Moskowitz MA. L-NA-sensitive rCBF augmentation during vibrissal stimulation in type III nitric oxide synthase mutant mice. J Cereb Blood Flow Metab. 1996; 16(4):539–41. [PubMed: 8964791]
75. Moskvin AN, Zhilyaev SY, Sharapov OI, Platonova TF, Gutsaeva DR, Kostkin VB, Demchenko IT. Brain blood flow modulates the neurotoxic action of hyperbaric oxygen via neuronal and endothelial nitric oxide. Neurosci Behav Physiol. 2003; 33(9):883–8. [PubMed: 14969426]
76. Zhilyaev SY, Moskvin AN, Platonova TF, Gutsaeva DR, Churilina IV, Demchenko IT. Hyperoxic vasoconstriction in the brain is mediated by inactivation of nitric oxide by superoxide anions. Neurosci Behav Physiol. 2003; 33(8):783–7. [PubMed: 14635993]
77. Veltkamp R, Rajapakse N, Robins G, Puskar M, Shimizu K, Busija D. Transient focal ischemia increases endothelial nitric oxide synthase in cerebral blood vessels. Stroke. 2002; 33(11):2704–10. [PubMed: 12411665]
78. de la Torre JC, Aliev G. Inhibition of vascular nitric oxide after rat chronic brain hypoperfusion: spatial memory and immunocytochemical changes. J Cereb Blood Flow Metab. 2005; 25(6):663–72. [PubMed: 15703700]
79. Jasmin JF, Malhotra S, Singh Dhallu M, Mercier I, Rosenbaum DM, Lisanti MP. Caveolin-1 deficiency increases cerebral ischemic injury. Circ Res. 2007; 100(5):721–9. [PubMed: 17293479]
80. Nishimura M, Izumiya Y, Higuchi A, Shibata R, Qiu J, Kudo C, Shin HK, Moskowitz MA, Ouchi N. Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase dependent mechanisms. Circulation. 2008; 117(2):216–23. [PubMed: 18158361]
81. Endres M, Gertz K, Lindauer U, Katchanov J, Schultze J, Schrock H, Nickenig G, Kuschinsky W, Dirnagl U, Laufs U. Mechanisms of stroke protection by physical activity. Ann Neurol. 2003; 54(5):582–90. [PubMed: 14595647]
82. Lo EH, Hara H, Rogowska J, Trocha M, Pierce AR, Huang PL, Fishman MC, Wolf GL, Moskowitz MA. Temporal correlation mapping analysis of the hemodynamic penumbra in mutant mice deficient in endothelial nitric oxide synthase gene expression. Stroke. 1996; 27(8):1381–5. [PubMed: 8711806]
83. Laufs U, Liao JK. Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. J Biol Chem. 1998; 273(37):24266–71. [PubMed: 9727051]
84. Ming XF, Viswambharan H, Barandier C, Ruffieux J, Kaibuchi K, Rusconi S, Yang Z. Rho GTPase/Rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase B/Akt in human endothelial cells. Mol Cell Biol. 2002; 22(24): 8467–77. [PubMed: 12446767]
85. Noma K, Oyama N, Liao JK. Physiological role of ROCKs in the cardiovascular system. Am J Physiol Cell Physiol. 2006; 290(3):C661–8. [PubMed: 16469861]
86. Fagan KA, Oka M, Bauer NR, Gebb SA, Ivy DD, Morris KG, McMurtry IF. Attenuation of acute hypoxic pulmonary vasoconstriction and hypoxic pulmonary hypertension in mice by inhibition of Rho-kinase. Am J Physiol Lung Cell Mol Physiol. 2004; 287(4):L656–64. [PubMed: 14977625]
87. Takemoto M, Sun J, Hiroki J, Shimokawa H, Liao JK. Rho-kinase mediates hypoxia-induced downregulation of endothelial nitric oxide synthase. Circulation. 2002; 106(1):57–62. [PubMed: 12093770]
88. Liao JK, Zulueta JJ, Yu FS, Peng HB, Cote CG, Hassoun PM. Regulation of bovine endothelial constitutive nitric oxide synthase by oxygen. J Clin Invest. 1995; 96(6):2661–6. [PubMed: 8675632]
89. Shin HK, Salomone S, Potts EM, Lee SW, Millican E, Noma K, Huang PL, Boas DA, Liao JK, Moskowitz MA, Ayata C. Rho-kinase inhibition acutely augments blood flow in focal cerebral ischemia via endothelial mechanisms. J Cereb Blood Flow Metab. 2007; 27(5):998–1009. [PubMed: 17033691]
90. Rikitake Y, Kim HH, Huang Z, Seto M, Yano K, Asano T, Moskowitz MA, Liao JK. Inhibition of Rho kinase (ROCK) leads to increased cerebral blood flow and stroke protection. Stroke. 2005; 36(10):2251–7. [PubMed: 16141422]

91. Amano M, Fukata Y, Kaibuchi K. Regulation and functions of Rho-associated kinase. Exp Cell Res. 2000; 261(1):44–51. [PubMed: 11082274]
92. Yagita Y, Kitagawa K, Sasaki T, Terasaki Y, Todo K, Omura-Matsuoka E, Kaibuchi K, Hori M. Rho-kinase activation in endothelial cells contributes to expansion of infarction after focal cerebral ischemia. J Neurosci Res. 2007; 85(11):2460–9. [PubMed: 17526023]
93. Satoh S, Utsunomiya T, Tsurui K, Kobayashi T, Ikegaki I, Sasaki Y, Asano T. Pharmacological profile of hydroxy fasudil as a selective rho kinase inhibitor on ischemic brain damage. Life Sci. 2001; 69(12):1441–53. [PubMed: 11531167]
94. Arai M, Sasaki Y, Nozawa R. Inhibition by the protein kinase inhibitor HA1077 of the activation of NADPH oxidase in human neutrophils. Biochem Pharmacol. 1993; 46(8):1487–90. [PubMed: 8240400]
95. Terzic A, Kurachi Y. Actin microfilament disrupters enhance K(ATP) channel opening in patches from guinea-pig cardiomyocytes. J Physiol. 1996; 492 (Pt 2):395–404. [PubMed: 9019537]
96. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC. Regulation of endothelium-derived nitricoxide production by the protein kinase Akt. Nature. 1999; 399(6736):597–601. [PubMed: 10376602]
97. Laufs U, Endres M, Stagliano N, Amin-Hanjani S, Chui DS, Yang SX, Simoncini T, Yamada M, Rabkin E, Allen PG, Huang PL, Bohm M, Schoen FJ, Moskowitz MA, Liao JK. Neuroprotection mediated by changes in the endothelial actin cytoskeleton. J Clin Invest. 2000; 106(1):15–24. [PubMed: 10880044]
98. Hall A. Rho GTPases and the actin cytoskeleton. Science. 1998; 279(5350):509–14. [PubMed: 9438836]
99. Endres M, Laufs U, Huang Z, Nakamura T, Huang P, Moskowitz MA, Liao JK. Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. Proc Natl Acad Sci U S A. 1998; 95(15):8880–5. [PubMed: 9671773]
100. Yamada M, Huang Z, Dalkara T, Endres M, Laufs U, Waeber C, Huang PL, Liao JK, Moskowitz MA. Endothelial nitric oxide synthase-dependent cerebral blood flow augmentation by L-arginine after chronic statin treatment. J Cereb Blood Flow Metab. 2000; 20(4):709–17. [PubMed: 10779015]
101. O'Driscoll G, Green D, Taylor RR. Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. Circulation. 1997; 95(5):1126–31. [PubMed: 9054840]
102. Williams JK, Sukhova GK, Herrington DM, Libby P. Pravastatin has cholesterol-lowering independent effects on the artery wall of atherosclerotic monkeys. J Am Coll Cardiol. 1998; 31(3):684–91. [PubMed: 9502654]
103. Laufs U, Fata VL, Liao JK. Inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase blocks hypoxia-mediated down-regulation of endothelial nitric oxide synthase. J Biol Chem. 1997; 272(50):31725–9. [PubMed: 9395516]
104. Amin-Hanjani S, Stagliano NE, Yamada M, Huang PL, Liao JK, Moskowitz MA. Mevastatin, an HMG-CoA reductase inhibitor, reduces stroke damage and upregulates endothelial nitric oxide synthase in mice. Stroke. 2001; 32(4):980–6. [PubMed: 11283400]
105. Asahi M, Huang Z, Thomas S, Yoshimura S, Sumii T, Mori T, Qiu J, Amin-Hanjani S, Huang PL, Liao JK, Lo EH, Moskowitz MA. Protective effects of statins involving both eNOS and tPA in focal cerebral ischemia. J Cereb Blood Flow Metab. 2005; 25(6):722–9. [PubMed: 15716855]
106. Essig M, Nguyen G, Prie D, Escoubet B, Sraer JD. Friedlander G3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells. Role of geranylgeranylation and Rho proteins. Circ Res. 1998; 83(7):683–90. [PubMed: 9758637]
107. McCabe TJ, Fulton D, Roman LJ, Sessa WC. Enhanced electron flux and reduced calmodulin dissociation may explain "calcium-independent" eNOS activation by phosphorylation. J Biol Chem. 2000; 275(9):6123–8. [PubMed: 10692402]