THE ROBERT M. BERNE DISTINGUISHED LECTURE

Protecting against vascular disease in brain

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Faraci FM. Protecting against vascular disease in brain. Am J Physiol Heart Circ Physiol 300: H1566–H1582, 2011. First published February 18, 2011; doi:10.1152/ajpheart.01310.2010.—Endothelial cells exert an enormous influence on blood vessels throughout the circulation, but their impact is particularly pronounced in the brain. New concepts have emerged recently regarding the role of this cell type and mechanisms that contribute to endothelial dysfunction and vascular disease. Activation of the renin-angiotensin system plays a prominent role in producing these abnormalities. Both oxidative stress and local inflammation are key mechanisms that underlie vascular disease of diverse etiology. Endogenous mechanisms of vascular protection are also present, including antioxidants, anti-inflammatory molecules, and peroxisome proliferator-activated receptor-γ. Despite their clear importance, studies of mechanisms that underlie cerebrovascular disease continue to lag behind studies of vascular biology in general. Identification of endogenous molecules and pathways that protect the vasculature may result in targeted approaches to prevent or slow the progression of vascular disease that causes stroke and contributes to the vascular component of dementia and Alzheimer’s disease.

cardiovascular risk factors; angiotensin II; endothelium; oxidative stress; nitric oxide; cerebral blood flow; peroxisome proliferator-activated receptor-γ

THE BRAIN ACCOUNTS FOR 20% of the body’s oxygen consumption and 25% of total glucose utilization, despite being only 2% of body weight. Because the brain lacks energy reserves, normal cell function depends upon a level of perfusion that provides optimal nutrient delivery. Cerebral blood flow is highly regulated, involving multiple coordinated mechanisms. This regulation includes the integration of both regional and segmental changes in vascular tone, as well as major interactions between different cell types. This review focuses on the impact of endothelial cells under normal conditions and how endothelial dysfunction contributes to cerebrovascular disease.

Endothelial cells exert diverse effects within the vessel wall and on nearby nonvascular target cells in the brain (Fig. 1). Endothelial dysfunction is a term commonly used to describe cell-based abnormalities that promote vasoconstriction, inflammation, increased permeability, atherosclerosis, and thrombosis. Endothelial dysfunction is perhaps the earliest event in the initiation of vascular disease (257) but continues to play a key role throughout the disease process. In brain, functional changes in endothelial cells contribute to reductions in resting blood flow (hypoperfusion), impairment of vasodilator responses, and subsequent cellular injury. Modest, but chronic reductions in cerebral blood flow (which do not produce ischemia) have cerebral consequences (127, 131, 141, 181).

In addition to cerebrovascular disease and stroke, cardiovascular risk factors increase the likelihood of developing dementia and Alzheimer’s disease (130). Evidence continues to accumulate supporting the link between vascular abnormalities and cognitive decline (130, 131, 141, 181). Despite its clear importance, studies of mechanisms that underlie cerebrovascular disease continue to lag behind studies of vascular biology in general, and the unique features of the cerebral circulation make it difficult to extrapolate findings from peripheral blood vessels.

This review highlights features of cerebral endothelium and its role in regulation of cerebrovascular biology. The contributions of oxidative stress, inflammation, and angiotensin II (ANG II) in the pathogenesis of cerebrovascular disease are summarized. Finally, an overview of select mechanisms that protect against cerebrovascular disease is presented. Despite advances in prevention and treatment, the incidence of stroke and dementia continues to rise (201). Identification of endogenous molecules or pathways that protect the vasculature may lead to more specific cell-based therapeutic approaches to prevent or slow the progression of vascular disease that causes stroke and contributes to the vascular component of dementia and Alzheimer’s disease.

Unique Features of Cerebral Blood Vessels

The cerebral circulation has many distinctive elements (51, 84, 131). In the context of this review, a few key features will be highlighted initially. The circulation of the brain is unique...
because large cerebral arteries and cerebral arterioles on the surface of the brain (pial vessels) collectively account for 50–60% of total cerebral vascular resistance (Fig. 2). Prior to the point where pial arterioles dive into the parenchyma, local intravascular pressure is approximately half of systemic blood pressure in the cerebral cortex (Fig. 2). Thus cerebral arteries and pial arterioles make major contributions to the regulation of cerebral blood flow and local microvascular perfusion pressure (93). In common experimental models as well as in humans, large cerebral arteries have substantial tone and react to diverse vasodilator stimuli including nitric oxide (NO), endothelium-dependent agonists, increases in blood flow, and hypercapnia (94, 103, 148, 248).

About one-third of vascular resistance resides in small blood vessels within the brain parenchyma (arterioles, capillaries, and small venules) (93, 118, 119) (Fig. 2). These vessels are components of what is often referred to as the neurovascular unit. In blood vessels both on the brain surface and within the parenchyma, multiple interactions between cell types, including endothelium, smooth muscle, and local neurons (intrinsic neurons or perivascular innervation from extrinsic nerves), occur (16, 51, 94, 107, 132). In addition, astrocytes and cells comprising the glia limitans interact with nearby vessels (16, 132, 258). This segmental arrangement of vascular resistance necessitates well-coordinated integration between different vascular segments for optimal regulation of local microvascular pressure and blood flow (93, 103). Although clearly important, mechanisms that control communication along vascular segments, including between upstream and downstream blood vessels, remain poorly defined (103, 133).

The blood-brain barrier (BBB) is formed by a continuous layer of cerebral endothelial cells anchored to each other by an array of tight junction proteins that limit the entry of circulating cells and most blood-borne substances into brain (1, 51). The BBB is a unique structure with very specific functional characteristics (1). Although often discussed in relation to capillaries, the BBB is also functional in cerebral arteries and arterioles as well as cerebral venules (35, 51, 192, 205). Thus endothelial dysfunction can also involve loss of BBB integrity and function (205).

In addition to intracranial vessels, studies of the carotid artery are important in relation to defining mechanisms that underlie vascular disease and stroke. Although the carotid arteries are not major resistance vessels, these vessels are a primary site for development of atherosclerosis (carotid artery disease). Carotid artery disease is a major risk factor for ischemic stroke, being the cause of more than half of all strokes of this type (64, 172) (http://www.nhlbi.nih.gov/health/). Endothelial dysfunction is a fundamental component of mechanisms that initiate and contribute to the progression of atherosclerosis (227, 257). Carotid artery disease is a strong predictor of future vascular events, and quantification of disease in this vascular segment is used widely in both clinical and epidemiological studies to access the progression or regression of atherosclerosis (64, 172). Thus carotid arteries are studied clinically and are used in experimental models to define mechanisms that promote or protect against carotid artery disease.

Vascular Endothelium: One Cell to Rule Them All

The study of endothelial cells and their impact within the vessel wall continues to occupy a major area in the study of vascular biology (100). Much of the influence of endothelium on other cells is mediated by release of diffusible factors (8, 33, 94, 269). A key mechanism by which endothelium communicates with target cells is via the production of NO by endothelial nitric oxide synthase (eNOS) (89, 94, 269) (Fig. 3). Through this mechanism, endothelium normally exerts a chronic dilator influence on vessels throughout the brain, ranging from large cerebral arteries to small arterioles within the parenchyma (3, 24, 53, 54, 94, 105, 269). A primary molecular target of NO is soluble guanylate cyclase (GC). Downstream effects of cGMP from GTP. Downstream effects of cGMP are largely mediated by cGMP-dependent protein kinase I, which produces reductions in intracellular calcium, decreases in vascular tone, and changes in gene expression (102). Most vascular effects of NO require activation of soluble guanylate cyclase (68, 98, 185, 217, 254, 299).

In addition to influencing resting tone, endothelial cells mediate vasodilator responses to a diverse group of stimuli including neurotransmitters (e.g., acetylcholine and substance P), products released by platelets (e.g., ADP) or astrocytes, as well as mediators of injury or inflammation (e.g., bradykinin and histamine) (94, 204, 269). Vasodilator responses to many physiological stimuli as well as agents used therapeutically (e.g., corticosteroids and statins [3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors]) are mediated by en-
Mechanisms regulating production of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS) as well as signaling molecules activated by NO in vascular muscle. In response to receptor-mediated agonists such as acetylcholine acting on type 5 muscarinic receptors (M5) and other stimuli, NO is produced by eNOS from the substrate L-arginine (L-Arg). Some studies have concluded that increased shear stress also promotes endothelial- and NO-dependent vasodilation. These processes require tetrahydrobiopterin (BH4). Activity of eNOS is reduced by the endogenous inhibitor asymmetric dimethylarginine (ADMA). Levels of ADMA are dependent on activity of dimethylarginine dimethylaminohydrolase (DDAH). By scavenging superoxide (O2•−), superoxide dismutase (SOD) protects NO-mediated signaling. Within vascular muscle, NO activates soluble guanylate cyclase (sGC), which produces cGMP from GTP. A key molecular target for cGMP is cGMP-dependent protein kinase 1 (cGKI), which produces relaxation of vascular muscle via several mechanisms including activation of Ca2⁺-sensitive large-conductance potassium channels (BK), inhibition of rho kinase (not shown), and other mechanisms (102). Activation of BK channels produces local hyperpolarization, which inhibits voltage-dependent Ca2⁺ channels (VDCC). Steady-state levels of cGMP are also determined by activity of type 5 phosphodiesterase (PDE5).

Endothelium-derived NO (3, 80, 82, 94, 96, 98, 126, 160, 166, 208, 219, 264, 269). Finally, neurovascular coupling in some brain regions (e.g., basal forebrain) is mediated by neuronally released acetylcholine acting on endothelium with activation of eNOS (295). The basal forebrain is a key region in relation to the development of dementia and Alzheimer’s disease (234).

Substances such as acetylcholine are used commonly in both experimental models and humans as tools to examine endothelium-dependent regulation of vascular tone (94, 100). As this field of study has progressed, it has become apparent that this approach provides insight into the overall “health” of the endothelium as well. Impairment of endothelium-dependent vasodilation is predictive of clinical events and stroke, independent of differences in arterial pressure (100, 116, 276, 281). Genetic links between impaired endothelium-dependent vasodilation and stroke have been established (276). Thus, while studies of endothelium-dependent vasodilation are important in defining determinants of vascular tone, they also have broad implications for mechanisms that promote cerebrovascular disease and its contribution to stroke and dementia (Fig. 1).

Endothelium and eNOS do much more than influence vascular tone in the brain (Fig. 1). eNOS inhibits vascular hypertrophy (increase in cross-sectional area of the vessel wall) (24), promotes maintenance of native collateral vessels during maturation (58), and is required for normal vascular remodeling in response to changes in blood flow (236). eNOS-derived NO affects local neuronal transmission (104) and inhibits amyloidogenic processing of amyloid precursor protein (17). Endothelial cells play a supportive role in neurogenic regions by promoting neurogenesis (161, 251). Endothelial cells also promote survival and growth of oligodendrocyte precursor cells (9). eNOS-deficient mice have larger infarcts, impaired angiogenesis, as well as impaired neurogenesis and recovery of neuronal function after stroke (44, 169). Endothelial dysfunction and loss of trophic support by endothelium-derived signaling molecules may contribute to the decline in neurogenesis that occurs with aging (161) or in the presence of other cardiovascular risk factors that produce oxidative stress in endothelium. Activity of eNOS suppresses local inflammation and associated complications during meningitis (153). Thus endothelial dysfunction in brain produces deleterious vascular effects but can also have other negative consequences that promote the progression of Alzheimer’s disease and impair recovery after stroke and other forms of brain injury (Fig. 1).

In addition to eNOS, other endothelium-dependent mechanisms influence vasomotor tone (8, 33, 99, 274). Activation of calcium-sensitive potassium channels or transient receptor potential channels results in the release of a diffusible endothelium-derived hyperpolarizing factor (EDHF) that relaxes underlying smooth muscle (8, 33, 81, 105, 291). The exact identity of EDHF(s) remains controversial and may consist of a family of relaxing factors. Some studies have implicated K⁺ and hydrogen peroxide (H₂O₂) in this role (8, 88, 159, 198). A key related mechanism involves endothelium-dependent hyperpolarization of vascular muscle as the result of direct intercellular communication between cells via myoendothelial gap junctions (8, 272).

While there has been little direct evidence that EDHF or an “EDHF-like” mechanism affects basal tone in cerebral arteries, recent work suggests that such a mechanism influences resting tone in parenchymal arterioles (54). In the context of endothelial dysfunction, it is noteworthy that EDHF can compensate for the loss of NO-mediated responses during disease or brain injury (8, 33, 54, 111). Because most studies of EDHF have been performed in normal blood vessels, the overall impact of EDHF or EDHF-related mechanisms during cerebrovascular disease and particularly in vivo remains poorly defined.

How Common is Endothelial Dysfunction?

Vascular disease almost invariably includes end-organ damage or dysfunction at the level of the endothelial cell. Impairment of endothelium-dependent regulation of vascular tone has been described in diverse experimental models and in cerebral arteries from humans (94) (Table 1). All major cardiovascular risk factors are associated with endothelial dysfunction in brain. Although rarely studied, it is likely that combinations of cardiovascular risk factors (hypertension with aging, for example) interact with each other and accelerate endothelium-based cerebrovascular abnormalities.

In some models, endothelial dysfunction occurs earlier in the disease process or is larger in magnitude in the cerebral circulation than in blood vessels outside of the brain. Examples of such augmented vascular dysfunction have been described during aging (32, 199) as well as in models of hypertension (74, 76, 95, 197), diabetes (150), hyperhomocysteinemia (60),...
and high-fat diet feeding (27). Thus, although endothelium-based abnormalities can develop throughout the entire vascular tree, the circulation of the brain may be particularly susceptible to this key element of vascular disease.

Oxidative Stress: Cornerstone of Vascular Dysfunction

Oxidative stress occurs as the result of a shift in balance that favors the generation of oxygen-derived free radicals or reactive oxygen species (ROS) over various antioxidant defense mechanisms (92, 279). ROS have direct effects on vascular tone but also impair vasomotor responses to other stimuli (90, 92). The effects of ROS are prominent in the cerebral circulation. Cerebral blood vessels have the capacity to generate high levels of superoxide compared with peripheral blood vessels and are particularly sensitive to the effects of ROS (22, 48, 88, 197, 213). In the majority of the studies listed in Table 1, impairment of endothelium-dependent vasodilation was mediated by ROS.

The species of ROS produced by many enzymatic sources is superoxide anion (Fig. 4). Superoxide has direct cellular effects but is also the precursor for other ROS and some reactive nitrogen species (Fig. 4). There are multiple sources of superoxide in the vasculature, including mitochondria, NADPH oxidase, cyclooxygenase (COX), and xanthine oxidase (48, 164). Mitochondria generate superoxide during oxidative phosphorylation. Cerebral endothelial cells are metabolically active, and the number of mitochondria present in cerebral endothelium is high compared with other cells (215). It is well established that COX is an important source of superoxide in the cerebral circulation (67, 156), and COX has been implicated as an important source of ROS in humans with essential hypertension (275). After oxidation of the cofactor tetrahydrobiopterin or disruption of its zinc-thiolate center, eNOS may reduce molecular oxygen, instead of l-arginine, producing superoxide instead of NO (88, 92). While a few studies provide evidence that eNOS “uncoupling” contributes to vascular dysfunction in models of disease (87, 261), the overall importance of this mechanism for cerebrovascular dysfunction remains to be defined.

Many studies have focused on the importance of NADPH oxidase in vascular disease. The catalytic domain of NADPH oxidase resides in its Nox subunit. Depending on species, cell type, and condition, different homologs of Nox are expressed in vascular cells, including Nox1, Nox2, Nox4, and Nox5 (48, 200). Impairment of endothelial function and neurovascular coupling is restored to normal with scavengers of superoxide, pharmacological inhibitors of NADPH oxidase, or genetic deletion of the Nox2 component of NADPH oxidase in models of aging, hypertension, hypercholesterolemia, sickle-cell disease, and Alzheimer’s disease (109, 136, 144, 196, 220–222, 249, 283). Constriction in cerebral arteries in response to ANG II is dependent on expression of Nox2 (65). Collectively, these findings support the concept that NADPH oxidases are key promoters of oxidative stress. To date, most studies have focused on the role of the Nox2-containing NADPH oxidase. Cerebral arteries also express relatively high levels of Nox4 (197), which is thought to produce H2O2 rather than superoxide and thus may elicit different effects (91, 200). Other Nox-containing enzymes and Nox-dependent effects have been described (48, 200), but little is known regarding their importance in the cerebral circulation.

Once it is produced, how does superoxide affect vascular function? Superoxide can adversely affect mitochondrial and vascular function by the inactivation of proteins containing iron-sulfur centers (e.g., aconitase) (92). A key mechanism that has received considerable attention involves the interaction between NO and superoxide (Fig. 4). The first evidence that ROS impair endothelium-dependent vasodilation came from studies by Wei and Kontos and colleagues (280) demonstrating that endothelial dysfunction in pial arterioles following acute hypertension was mediated by ROS. NO reacts with superoxide and thus may elicit different effects (91, 200). Other Nox-containing enzymes and Nox-dependent effects have been described (48, 200), but little is known regarding their importance in the cerebral circulation.

Table 1. Disease states, conditions, or stimuli that produce endothelial dysfunction in carotid artery and cerebral circulation

<table>
<thead>
<tr>
<th>Model</th>
<th>References</th>
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<tbody>
<tr>
<td>Acute hypertension</td>
<td>280</td>
</tr>
<tr>
<td>Aging</td>
<td>121, 187, 188, 199, 220</td>
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<tr>
<td>Alcohol</td>
<td>260–262</td>
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<tr>
<td>Alzheimer’s disease</td>
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<td>Angiotensin II</td>
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<tr>
<td>Atherosclerosis</td>
<td>67, 264</td>
</tr>
<tr>
<td>Ceramide</td>
<td>66</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>76, 95, 108, 144, 152, 276, 288</td>
</tr>
<tr>
<td>Cigarette smoke</td>
<td>129</td>
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<tr>
<td>Cigarette smoke extract</td>
<td>294</td>
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<tr>
<td>Diabetes</td>
<td>10, 11, 73, 150, 270</td>
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<tr>
<td>Endothelin</td>
<td>142</td>
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<tr>
<td>High-salt diet</td>
<td>263</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>136, 151, 196, 214, 287</td>
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<tr>
<td>Hyperhomocysteinemia</td>
<td>60, 233</td>
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<tr>
<td>Hypoxia</td>
<td>286</td>
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<tr>
<td>Inflammation</td>
<td>70, 124</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>85, 86</td>
</tr>
<tr>
<td>Intermittent hypoxia</td>
<td>224</td>
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<tr>
<td>Ischemia with reperfusion</td>
<td>53, 54, 207</td>
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<tr>
<td>Metabolic syndrome</td>
<td>225</td>
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<tr>
<td>Methionine synthase deficiency</td>
<td>61</td>
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<tr>
<td>Microgravity</td>
<td>298</td>
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<tr>
<td>Nicotine</td>
<td>87, 186</td>
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<tr>
<td>PPARγ interference</td>
<td>26, 27</td>
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<tr>
<td>Sickle-cell disease</td>
<td>283</td>
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<tr>
<td>SOD deficiency</td>
<td>22, 75, 96, 198</td>
</tr>
<tr>
<td>Subarachnoid hemorrhage</td>
<td>138, 218</td>
</tr>
<tr>
<td>Traumatic brain injury</td>
<td>157</td>
</tr>
</tbody>
</table>

PPARγ, peroxisome proliferator-activated receptor-γ; SOD, superoxide dismutase.
Fig. 4. Interactions between NO and reactive oxygen species (ROS). eNOS-derived NO signals via sGC, cGMP, and cGKI. By oxidizing BH4 or disrupting the zinc-thiolate center of the enzyme, peroxynitrite (ONOO\(^-\)) produces uncoupling of eNOS, thus reducing NO production. Under these conditions, eNOS produces superoxide (O\(^2-\)) instead of NO (not shown). Activity of eNOS can also be reduced by increased ADMA following ROS-mediated inhibition of DDAH. After activation of AT\(_1\) receptors (AT1-R) by angiotensin II (ANG II), NADPH oxidase (Nox) produces superoxide. Once formed, superoxide can directly produce injury (not shown), can be converted by SOD into H\(_2\)O\(_2\), or can react with NO to form ONOO\(^-\). The NADPH oxidase containing Nox4 may produce H\(_2\)O\(_2\) directly. In addition to being an important signaling molecule, H\(_2\)O\(_2\) can form hydroxyl radical (OH\(^\bullet\)), a highly reactive mediator of cellular injury, in the presence of Fe\(^2+\). H\(_2\)O\(_2\) can also be degraded by glutathione peroxidase (GPx). Once formed, peroxynitrite inhibits activity of SOD and prostacyclin synthase (PGIS) and activates poly(ADP-ribose) polymerases (PARP). See text for additional details.

sensory cortex is dependent on nNOS-derived NO, while essentially the entire response is mediated by nNOS in the cerebellum (107). Like endothelium-dependent vasodilation, neurovascular coupling is impaired by superoxide (107, 144, 220–222). Reductions in resting blood flow and impairment of vasodilator responses as a result of oxidative stress may result in a mismatch between energy requirements, substrate delivery, and clearance of cellular by-products. Thus the loss of bioavailability of either eNOS- or nNOS-derived NO has far-reaching implications contributing to both short- and long-term consequences of vascular disease (48, 89, 130, 181).

Activity of rho kinase is a key determinant of vascular tone with cell-specific effects in the vasculature (170, 212, 250). In endothelium, rho kinase inhibits activity and expression of eNOS (34, 170). In vascular muscle, intracellular levels of calcium and sensitivity of contractile proteins to calcium determine the degree of actomyosin activation. Because of effects on calcium sensitivity and phosphorylation/dephosphorylation of the regulatory myosin light chain, rho kinase is a major determinant of vascular tone (34, 170). Rho kinase interacts with both NO and ROS. Via effects on cGMP-dependent protein kinase I, NO and cGMP inhibit rho kinase in vascular muscle (170, 242) (Fig. 3). As a consequence, reductions in NO increase the activity of rho kinase. Inhibition of NO production rapidly increases the influence of rho kinase on vascular tone (73).

As noted above, peroxynitrite is produced by the reaction of superoxide with NO (Fig. 4). Peroxynitrite is vasoactive (90, 178, 179) but also promotes cellular injury and impairs regulation of vascular tone via activation of poly(ADP-ribose) polymerases (PARP) and other effects (139, 178, 179) (Fig. 4). Activation of PARP contributes to endothelial dysfunction in models of disease and aging (10, 71, 110, 199). Oxidation of soluble guanylate cyclase by peroxynitrite reduces its responsiveness to NO, causing further loss of NO signaling (255) (Fig. 4). Other detrimental effects of peroxynitrite include reduced activity of prostacyclin synthase and superoxide dismutase (SOD; see below) as a result of nitration of tyrosine residues (90, 92) (Fig. 4).

Pleiotropic Effects of Angiotensin II

The renin-angiotensin system (RAS) plays a major role in vascular biology, particularly in relation to vascular disease (175, 180, 194, 230, 278). This system is a key contributor to pathophysiology in many models of hypertension and is a primary therapeutic target in patients with essential hypertension (2, 230). Chronic hypertension with activation of the RAS is associated with more cardiovascular events than other forms of hypertension (4).

The cerebral circulation is particularly sensitive to ANG II. Cerebral blood vessels are exposed to ANG II from both circulating and local (ANG II formed locally within the vessel wall and brain) sources. ANG II-mediated effects occur as a result of activation of specific receptors, and most of the detrimental effects of the peptide are mediated by AT\(_1\) receptors (Fig. 5) (11, 95, 109, 144, 175, 199, 238, 278).

ANG II produces diverse effects including vasoconstriction, oxidative stress, inflammation, endothelial dysfunction, increases in permeability, impairment of neurovascular coupling, and altered vascular structure (5, 7, 23, 25, 37–39, 47, 49, 50, 59, 69, 72, 74, 76, 95, 101, 144, 158, 175, 240, 249, 266, 278, 296) (Fig. 5). In addition to its effects on upstream resistance vessels, ANG II affects the contractile state of pericytes (143) and thus may regulate local capillary blood flow in vivo. Effects of ANG II on superoxide formation, vasomotor tone, endothelial function, and neurovascular coupling are sex dependent (48, 65, 95, 108). Vascular effects of ANG II are much larger in male compared with female mice and are dependent on the phase of the estrus cycle in female mice (37, 49, 65, 95).

In addition to hypertension, ANG II and activation of AT\(_1\) receptors contribute to vascular disease under conditions without coexisting hypertension, including aging (199), diabetes (11, 270), atherosclerosis (59, 227), and in response to cigarette smoking (129). Increases in superoxide as well as impairment
of neurovascular coupling and autoregulation are reduced by AT1 receptor inhibition in a model of Alzheimer’s disease (266). This same study suggests that cognitive impairment in Alzheimer’s disease may be mediated in part by ANG II (266). Similarly, loss of autoregulation following fluid-percussion injury in brain is restored toward normal after inhibition of AT1 receptors (18). Subarachnoid hemorrhage and focal cerebral ischemia increase both expression and functional effects of AT1 receptors in the vasculature (83). Inhibition of AT1 receptors improves local cerebral perfusion after cerebral ischemia (83, 137, 211). Autoantibodies against the AT1 receptor are thought to contribute to the pathogenesis of preeclampsia (125). After isolation from preeclamptic patients, these antibodies contract cerebral arteries via activation of AT1 receptors (289).

Because ANG II is a key contributor to vascular disease of diverse etiology, studies have focused on defining mechanisms that promote vascular dysfunction as well as endogenous mechanisms that protect against vascular abnormalities in ANG II-dependent models (Fig. 6). Experimental models used for these purposes include 1) testing direct effects of ANG II on isolated vessels or vascular cells, 2) testing effects of chronic administration of nonpressor or pressor doses of ANG II (administered systemically with osmotic minipumps), 3) studies of transgenic animals expressing components of the RAS, and 4) examining the role of endogenously produced ANG II in models of disease (e.g., spontaneously hypertensive rats).

The ability of ANG II to promote oxidative stress has received considerable study in blood vessels outside the brain (194, 230, 278). In relation to the brain, increases in superoxide in response to ANG II are much larger in cerebral arteries than in carotid arteries or aorta (74, 76, 95). In genetically hypertensive rats, inhibition of angiotensin-converting enzyme or AT1 receptors reduces the levels of superoxide and inflammation, restores endothelium-dependent responses, and improves autoregulation (7, 158, 240, 288).

Significant progress has been made in relation to defining how ANG II contributes to vascular disease (Fig. 6). Contributions of ANG II to oxidative stress are due in large part to activation of NADPH oxidase. ANG II increases vascular expression of components of NADPH oxidase and levels of superoxide (72). ANG II-induced endothelial dysfunction is prevented by inhibition or genetic deletion of AT1 receptors (109, 238), pharmacological scavengers of superoxide (49, 72, 95, 144), or genetic deletion of Nox2 (65, 109, 144, 249).

Members of the calcitonin family (calcitonin gene-related peptide, intermedin, and adrenomedullin) exert antioxidant effects on vascular cells in culture including inhibitory effects on NADPH oxidase (168, 246, 290). Signaling by these peptides depends upon the expression of receptor activity-modifying proteins (RAMPs) (226). Overexpression of RAMP2 protects against vascular inflammation and hypertrophy in a model of ANG II-dependent hypertension (165), while overexpression of RAMP1 protects against ANG II-induced oxidative stress and vascular dysfunction (50). Thus, although details of their interactions are unclear, other peptide and protein families modulate oxidative stress following activation of AT1 receptors.

A key feature of oxidative stress is that once it is initiated, ROS or peroxynitrite can feed forward, promoting additional oxidative stress. Several mechanisms may contribute to these effects (Fig. 4). First, angiotensinogen is cleaved by renin to produce ANG I (the precursor to ANG II). Oxidized angiotensinogen is more readily cleaved than the nonoxidized form (300). Thus, in an oxidative environment, the oxidized form of angiotensinogen may be more prevalent so ANG II-induced oxidative stress may feed forward, promoting further produc-

![Diagram](image-url)
vascular remodeling increases minimal resistance, resulting in chronically hypertensive animals (190). In contrast, inward mechanism protects the BBB during acute hypertension in vascular dysfunction when local pressure rises (Fig. 4). Such a lation from full transmission of pressure, thus attenuating remodeling of upstream vessels protects the distal microcircu-
ting from these effects (300). Second, once produced, superoxide can be converted by SODs to H$_2$O$_2$, which then activates NADPH oxidase in vascular cells (88). Thus H$_2$O$_2$ can stimulate formation of additional superoxide (Fig. 4). Third, as noted above, peroxynitrite can uncouple eNOS (92, 203) (Fig. 4). Fourth, oxidative and nitrosative stress can reduce activity of SOD through nitrosylation or other effects (92) (Fig. 4).

Additional mechanisms may contribute to ANG II-induced vascular dysfunction. ANG II can reduce synthesis of NO by causing mislocalization and tyrosine phosphorylation of eNOS (106, 171). Formation of endothelium-derived contracting factors (EDCFs) has been described in cerebral arteries in re-
response to ANG II, in models of vascular disease, and in hypertensive humans (76, 94, 177, 191, 275). In many cases, EDCF has been suggested to be a prostanoid produced by COX (274). Consistent with this concept, a COX-derived EDCF and activation of thromboxane receptors contribute to impaired endothelial function in spontaneously hypertensive rats and a genetic model of ANG II hypertension (76, 94, 191). In humans with essential hypertension, COX-derived prostanoids and ROS contribute to endothelial dysfunction (275). An EDCF appears to be functionally important in vertebral arteries from hypertensive humans (43). The powerful vasoconstrictor endothelin-1 is another EDCF that impairs endothelial function and produces marked reductions in blood flow and vasospasm (13, 56, 94, 142, 275). Interactions between endothelin and ANG II have been described (154). For example, ANG II stimulates endothelin-1 production, and some effects of ANG II can be mediated by endothelin-1 (154). Interactions between formation of EDCF and oxidative stress exist. Both increased levels of ROS and reductions in NO bioavailability enhance EDCF-mediated responses (274). Activation of thromboxane receptors can increase endothelial production of superoxide and peroxynitrite through stimulation of NADPH oxidase and uncoupling of eNOS (297). In addition, COX-1 and prostaglandin E$_2$ EP1 receptors can also contribute to ANG II-induced cerebrovascular dysfunction (38).

Impact of Angiotensin II on Vascular Structure

Hypertension is associated with hypertrophy in aorta and large conduit arteries but inward remodeling (with or without increases in vessel cross-sectional area) in smaller resistance vessels (20, 23). Inward remodeling represents a rearrangement of the vessel wall around a smaller lumen (20, 23). This form of remodeling has been described in cerebral vessels in some models of hypertension and in hypertensive people (20, 23, 231). Both inward remodeling and vascular hypertrophy (if it encroaches on the vessel lumen) impair vasodilator capacity. Of these two changes, inward remodeling has the greatest impact on lumen diameter (20). Inward vascular remodeling can have either detrimental or protective effects, depending upon the circumstances (Fig. 7). During hypertension, inward remodeling of upstream vessels protects the distal microcirculation from full transmission of pressure, thus attenuating vascular dysfunction when local pressure rises (Fig. 7). Such a mechanism protects the BBB during acute hypertension in chronically hypertensive animals (190). In contrast, inward vascular remodeling increases minimal resistance, resulting in reduced vasodilator responses, lower levels of perfusion pressure, and impaired blood flow in collateral-dependent regions (Fig. 7). For these reasons, inward vascular remodeling increases the susceptibility to focal ischemia. Importantly, inward remodeling has emerged as a potential risk factor for cardiovascular events and cerebrovascular disease (122, 202).

ANG II produces inward remodeling independent of changes in blood pressure (23, 42) (Fig. 7). Despite similar levels of hypertension, cerebral arterioles undergo hypotrophy with inward remodeling during ANG II-dependent hypertension but only hypertrophy in ANG II-independent hypertension (23). In preliminary studies, chronic treatment with a nonpress-
sor dose of ANG II caused inward remodeling of cerebral arterioles without a change in vessel cross-sectional area, while infusion of a pressor dose of ANG II produced inward remodeling with vascular hypertrophy (42). These changes did not occur in Nox2-deficient mice, indicating a critical role for NADPH oxidase (42). Thus inward remodeling is a unique response to ANG II. Inward vascular remodeling is present in a genetic model of ANG II-dependent hypertension (23) and, as would be expected, is accompanied by greater susceptibility to focal cerebral ischemia (45).

Endogenous Antioxidants

An array of antioxidant enzymes are expressed in vascular cells. These enzymes reside in different subcellular compart-
ments and include SODs and glutathione peroxidases (GPx) (92, 173, 279). One of the simplest approaches to examine the impact of oxidative stress involves testing effects of genetic deletion of individual antioxidant genes. Studies utilizing mice with genetic alterations of specific isoforms of SOD or GPx have revealed key roles for these enzymes in protecting the vasculature under normal conditions as well as in models of disease and aging (Fig. 6).

SOD1 (CuZn-SOD) is the predominant form of SOD in the vessel wall, accounting for approximately two-thirds of total
SOD activity (75, 92). SOD1 is localized in the cytosol and portions of the mitochondria and within the cell nucleus (92). Genetic deficiency in SOD1 increases vascular superoxide, augments vasoconstrictor responses, and impairs endothelium-dependent dilation in cerebral arteries and in the brain microcirculation (22, 75, 96, 198). Because they convert superoxide to H_2O_2, SODs are a major source of H_2O_2 (88, 92) (Fig. 4). Effects of arachidonic acid on vascular tone are greatly impaired in animals lacking SOD1 (198), consistent with the concept that ROS, and more specifically H_2O_2, mediates this response. Increased expression of SOD1 protects against endothelial dysfunction in response to ANG II and ceramide (a sphingolipid signaling molecule) and in models of inflammation and Alzheimer’s disease (66, 69, 70, 134), while decreased expression of SOD1 augments loss of BBB integrity following cerebral ischemia (155).

ROS have important effects on the growth of vascular cells and the structure of blood vessels. Deficiencies in SOD1 increase superoxide and produce marked hypertrophy in cerebral arterioles (22), demonstrating that a key role for SOD1 is normally to inhibit this form of vascular growth. This function of SOD1 is particularly prominent, as the magnitude of hypertrophy in cerebral arterioles in SOD1-deficient animals is greater than that seen in other models in which vascular hypertrophy occurs, including models with lifelong hypertension (20–23).

Mitochondria are a key source of ROS that affect the vasculature (282). Complete deficiency in the mitochondrial form of SOD (SOD2, manganese SOD) is lethal, so genetic studies of SOD2 have focused on phenotypes present in heterozygous deficient mice (92). Partial deficiency in SOD2 enhances vasoconstrictor responses and impairs endothelium-dependent dilation in the microcirculation under baseline conditions (96). In addition, deficiency in SOD2 predisposes to increased oxidative stress and endothelial dysfunction in response to ANG II as well as during aging and hypercholesterolemia (32, 49, 214). In addition, interactions or cross talk between mitochondria, mitochondria-derived ROS, and other sources of superoxide including NADPH oxidase have emerged (78, 147, 284).

Studies on the impact of antioxidants and eNOS highlight an important issue regarding the use of genetically altered mice (22, 47, 49, 69, 71, 96, 155, 160, 214). When defining mechanisms, an extremely valuable approach is to use animals in which both copies of a gene have been deleted through gene targeting. However, in relation to understanding disease, models in which only one gene copy is absent (heterozygotes) can also be very insightful (265). Partial reductions in the expression or activity of a protein during disease or as the result of a genetic polymorphism are more likely to occur than complete loss of function of that same protein. It is noteworthy that multiple phenotypes have been described in both SOD1 and SOD2 heterozygote mice (22, 32, 49, 69, 71, 96, 155, 214). For example, while effects on endothelial function at baseline are not detectable, partial SOD1 deficiency accelerates oxidative stress and vascular dysfunction with aging and in response to ANG II (49, 69, 71). Similarly, complete loss of GPx1 expression produces marked endothelial dysfunction (47). In contrast, a partial deficiency in GPx1 does not affect vascular function under control conditions but increases sensitivity to ANG II-induced vascular dysfunction by ~10-fold (47). Thus partial reductions in expression of GPx-1 or SOD1 or SOD2 predispose to ANG II-induced vascular dysfunction (Fig. 6). Consistent with these findings, activity of GPx is inversely related to the risk for cardiovascular events in people (28, 173), supporting the concept that H_2O_2 plays a key role in vascular disease.

In relation to translational approaches that may protect the vasculature from oxidative stress, it is possible to enhance expression of endogenous antioxidants within the vessel wall. Expression of SOD1 can be increased by virus-based gene transfer (292) and exercise (237) and with pharmacological approaches including treatment with erythropoietin or exogenous activators of peroxisome proliferator-activated receptor-γ (PPARγ) (79, 135).

**Putting the Brakes on Vascular Inflammation**

Components of the inflammatory response are activated within the vasculature during aging and in many diseases including atherosclerosis, hypertension, and diabetes (57, 72, 180, 243, 257). Nuclear factor-κB (NF-κB) is a key transcription factor that promotes inflammation (57, 180). Through activation of NF-κB and other mechanisms, ANG II and ROS increase expression of proinflammatory cytokines, adhesion molecules, chemokines, toll-like receptors, inducible NOS (iNOS), and matrix metalloproteinases (31, 57, 72, 140, 175, 180, 296), all of which are known contributors to vascular disease. ANG II-dependent hypertension increases vascular expression of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and p22phox (a component of NADPH oxidase) (73). Increases in superoxide, vascular dysfunction, and vascular hypertrophy in response to ANG II are greatly reduced in IL-6-deficient mice (249). Overall, these studies and others support the concept that after activation of AT1 receptors, inflammation-related mechanisms act in synergy with oxidative stress to promote vascular disease (Fig. 6) (175, 180, 278).

While proinflammatory mechanisms promote the progression of vascular disease, endogenous mechanisms are also present to limit the impact of vascular inflammation. The anti-inflammatory cytokine IL-10 plays a key role in this regard (36, 112–114, 176, 267). Through effects on IkB kinase activity, NF-κB degradation, and DNA-binding activity, IL-10 is a potent inhibitor of NF-κB-mediated effects (15) (Fig. 6). Many of the protective effects of IL-10 may be through this mechanism (15, 228). Endogenous IL-10 attenuates increases in superoxide, expression of iNOS, and vascular dysfunction in response to lipopolysaccharide and during diabetes (36, 112–114, 176). IL-10 also protects against thrombosis and progression of atherosclerosis (36, 176).

In relation to ANG II, treatment with exogenous IL-10 protects against ANG II-induced endothelial dysfunction (293). Genetic deletion of IL-10 greatly increases vascular sensitivity to ANG II (72). Endogenous IL-10 limits the expression of IL-6 and p22phox and increases in superoxide and protects against endothelial dysfunction in models of ANG II-induced vascular dysfunction (72) (Fig. 6). These findings provide additional support for the concept that ANG II is proinflammatory and that IL-10 is a key protective molecule in vascular disease. These findings are also consistent with the work of Harrison and others implicating a key role for the immune system and T cells in contributing to the pathogenesis...
of hypertension (120). In this regard, it is noteworthy that IL-10 strongly inhibits T-cell function (15).

Other mechanisms can suppress vascular inflammation. For example, eNOS-derived NO decreases activation of NF-κB and expression of proinflammatory genes (63). Thus reductions in the bioavailability of NO during oxidative stress promote vascular inflammation. IL-10 may contribute to this mechanism as well, as the cytokine increases eNOS expression following activation of the transcription factor signal transducer and activator of transcription-3 (41).

**Dimethylarginine Dimethylaminohydrolase: Protecting eNOS**

Oxidative stress can also produce vascular dysfunction as a result of oxidant-induced inactivation of dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that degrades asymmetric dimethylarginine (ADMA) (Figs. 3 and 4). ADMA is an endogenously produced inhibitor of eNOS, and circulating levels of ADMA increase in many disease states including hypertension (29, 273). ADMA is taken up and accumulates in endothelial cells, so intracellular concentrations may be much higher than circulating levels (40).

A major determinant of ADMA levels is the activity of DDAH, which accounts for most of the clearance of ADMA (29, 273). Thus DDAH protects the vasculature by preventing increases in local ADMA concentrations. Importantly, DDAH is an oxidant-sensitive enzyme whose activity is reduced during oxidative stress (167, 244, 245). In addition, ADMA may contribute to the uncoupling of eNOS (273). Thus reductions in the activity of DDAH as well as increased levels of ADMA may contribute to oxidative stress-induced vascular disease (Fig. 4).

Physiologically relevant concentrations of ADMA inhibit activity of NOS, increase vascular tone (via inhibition of basal production of NO), and impair endothelial function in the cerebral circulation (91). ADMA reduces cerebral perfusion in humans (149). Overexpression of DDAH in transgenic mice reduces plasma levels of ADMA, increases basal levels of vascular NO, and protects against ADMA-induced endothelial dysfunction in brain (62). Hypertrophy of cerebral arterioles during hyperhomocysteinemia is prevented in DDAH transgenic mice (233).

**Peroxisome Proliferator-Activated Receptor-γ**

PPARγ is a member of the nuclear hormone receptor superfamily that forms heterodimers with retinoid X receptors and functions as a ligand-activated transcription factor (146, 247, 252) (Fig. 8). Although it was initially thought that key roles for PPARγ were limited to adipocyte function, as well as glucose and lipid metabolism (247), it is now clear that PPARγ is expressed and is functional in many cell types including many within the vasculature. PPARγ regulates the expression of clusters of target genes through several mechanisms, including binding to PPAR-response elements and protein-protein interactions with other transcription factors including NF-κB (247, 252).

Endogenous ligands that activate PPARγ include lipids, eicosanoids, and nitro-fatty acids (247, 277) (Fig. 8). Thiazolidinediones (TZDs) (e.g., rosiglitazone and pioglitazone) are synthetic activators of PPARγ used to treat type 2 diabetes (162). Studies using vascular cells in culture or extracranial blood vessels have described numerous protective effects of TZDs (146, 239, 252) (Fig. 8). In relation to effects of ANG II on the vasculature, both antioxidant and anti-inflammatory effects of TZDs have been observed, including reductions in the expression of AT1 receptors, components of NADPH oxidase (Nox1, Nox2, and Nox4), and IL-6 (77, 128, 135, 140, 146, 259). In contrast, TZDs increase expression of SOD1 and IL-10 (135, 268) and the activity of eNOS (146) (Fig. 8). Expression of PPARγ in atherosclerotic carotid arteries highly correlates with expression of IL-10 (30). While TZDs can activate PPARγ, ANG II may inhibit PPARγ-mediated effects by increasing activity of Brc kinase, resulting in phosphorylation and reductions in PPARγ transcriptional activity (5). The consequence of reduced transcriptional activity of PPARγ is increased activation of NF-κB and local inflammation (5).

In relation to the cerebral circulation, PPARγ is expressed in endothelium in the carotid artery and brain vessels of humans and other species (182, 229). In the carotid artery and cerebral vasculature, TZDs have beneficial effects on both endothelial function and regulation of vascular tone, as well as on vascular permeability in models of Alzheimer’s disease and hypertension (52, 206, 209, 210, 232, 239). TZDs prevent inward...
vascular remodeling during hypertension (52) and produce outward remodeling of cerebral arterioles in nonpregnant animals, thus mimicking changes that occur during pregnancy (55) (Fig. 7). In contrast, treatment with an inhibitor of PPARγ in pregnant animals prevents outward vascular remodeling (55). In spontaneously hypertensive rats, pioglitazone reduced superoxide and both the expression and activity of NADPH oxidase, improved endothelial function, and reversed vascular remodeling in cerebral arteries and arterioles (206). Vascular dysfunction in the brain in aged mice overexpressing amyloid precursor protein, a model of Alzheimer’s disease, was restored toward normal by TZD treatment (209, 210). These findings suggest that pharmacological activation of PPARγ has protective effects on cerebrovascular structure and function.

An underlying assumption of TZD-based studies is that the effects observed are mediated by PPARγ. However, TZDs can have off-target or PPARγ-independent effects as well (46, 223, 235). Because systemic administration of TZDs affects cellular metabolism, it is not always clear whether phenotypes produced are due to direct vascular effects of PPARγ or indirect changes that result from other effects of whole body TZD treatment.

Patients with dominant-negative mutations (V290M or P467L) in the ligand-binding domain of PPARγ exhibit early-onset hypertension (19). These mutations inhibit transcriptional activity of wild-type PPARγ (19, 145) and exert effects on vascular gene expression that are the opposite of those produced by TZDs (19, 145). Mice that express dominant-negative forms of PPARγ provide a novel approach to interference with wild-type PPARγ and thus allow insight into the importance of endogenous PPARγ without TZD treatment. In addition to being clinically relevant mutations, these models mimic reductions in activity of PPARγ described in disease states and with other genetic polymorphisms (123). In heterozygous knockin mice with an equivalent PPARγ mutation (P465L, designated L+/+) (271), oxidative stress and endothelial dysfunction occur in cerebral arteries and arterioles, but not in the aorta or carotid artery (Ref. 26; unpublished observations). Cerebral arterioles in L+/+ mice exhibit hypertrophy with inward remodeling (26). As expected, these vascular changes are accompanied by greater susceptibility to ischemia (285). Consistent with these findings, neointimal proliferation in the carotid artery following mechanical injury is augmented in L+/+ mice (195). Thus interference with PPARγ has pronounced effects in the cerebral circulation that mimic effects of ANG II including oxidative stress, endothelial dysfunction, and inward remodeling. Collectively, these findings suggest that the balance between the activity of PPARγ and effects of ANG II may be a key determinant of the progression of cerebrovascular disease (Fig. 8).

Studies to this point had not examined cell-specific effects of PPARγ in the vasculature in vivo. Cell-specific alterations in PPARγ avoid potential metabolic effects seen with global genetic manipulation of PPARγ or whole body TZD treatment. To advance beyond this limitation, mice expressing dominant-negative mutations in PPARγ in either smooth muscle or endothelium have been developed (27, 115). In mice expressing V290M under control of the endothelium-specific vascular cadherin promoter, expression of NADPH oxidase and SOD1 were increased and decreased, respectively (27). As shown by responses to acetylcholine, endothelial function was not altered under baseline conditions in these mice (27). In contrast, feeding of a high-fat diet for 12 wk produced superoxide-mediated reductions in responses to acetylcholine in the basilar artery but not in the aorta (27). Thus interference with PPARγ in endothelium predisposes the cerebrovasculature to oxidative stress and endothelial dysfunction in a model of diet-induced obesity.

To examine the role of PPARγ in vascular muscle, transgenic mice expressing a dominant-negative form of PPARγ under the control of the smooth muscle myosin heavy chain promoter were developed (115). Cerebral arterioles in these mice underwent hypertrophy with increased distensibility and inward remodeling (115). Preliminary studies indicate that cerebrovascular function is markedly impaired after genetic interference with PPARγ in vascular muscle (97). Thus studies of several mouse models expressing dominant-negative mutations suggest that PPARγ is active under basal conditions (in the absence of TZD treatment), exerting a particularly profound influence on the cerebral circulation.

In relation to therapy, PPARγ is particularly attractive because rather than targeting a single molecule or pathway, PPARγ affects many target genes and ultimately may mediate protective effects at multiple levels (Fig. 8). Genetic variations in PPARγ genotypes are associated with carotid artery disease (6). Thus cell-based strategies to suppress the progression of cerebrovascular disease using approaches that retain beneficial but reduce detrimental or off-target effects of current pharmacological activators of PPARγ may have substantial potential.

Conclusions

Vascular endothelium plays a major role in the cerebral circulation in both health and disease. The impact of endothelium on its multiple target cells is particularly prominent and quite diverse in the brain. All major cardiovascular risk factors are associated with endothelial dysfunction. Both oxidative stress and local inflammation are key mechanisms that promote vascular disease. Activation of the RAS and AT1 receptors plays a prominent role in producing these abnormalities. In contrast, endogenous molecules, including antioxidants, IL-10, DDAH, and PPARγ, protect vascular cells by limiting these changes. A more precise understanding of mechanisms that promote or protect against cell-based vascular dysfunction may result in targeted approaches that prevent or delay the progression of cerebrovascular disease.

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DISCLOSURES

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