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Contributions of Aging to Cerebral Small Vessel Disease

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Abstract

Cerebral small vessel disease (SVD) is characterized by changes in the pial and parenchymal microcirculations. SVD produces reductions in cerebral blood flow and impaired blood-brain barrier function, which are leading contributors to age-related reductions in brain health. End-organ effects are diverse, resulting in both cognitive and non-cognitive deficits. Underlying phenotypes and mechanisms are multi-factorial, with no specific treatments at this time. Despite consequences that are already considerable, the impact of SVD is predicted to increase substantially with the growing aging population. In the face of this health challenge, the basic biology, pathogenesis, and determinants of SVD are poorly defined. This review summarizes recent progress and concepts in this area, highlighting key findings and some major unanswered questions. We focus on phenotypes and mechanisms that underlie microvascular aging, the greatest risk factor for cerebrovascular disease and its subsequent effects.

Keywords

Microcirculation; endothelium; blood-brain barrier; cerebral blood flow; nitric oxide

Introduction

Aging is the greatest risk factor for cerebral vascular disease and subsequent consequences which include loss of brain health due to strokes, mild cognitive impairment, dementias, and non-cognitive disorders such as abnormalities of gait and balance (Figure 1) (1–5). While already a major contributor to global disease burden, the size of the aged population is increasing with time. In 2015, almost 50 million people were living with dementia worldwide, a number expected to triple by 2050 (4). The personal, familial, societal, and financial costs of dementia alone are enormous (4, 6). Even with such impact, the majority of preclinical studies have not incorporated aging into common experimental models of cerebrovascular disease, including stroke. This lack of incorporating aging into the design of

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preclinical studies is despite the fact that mechanistic determinants of vascular disease including genetics, inflammation, and oxidative stress often change with age. Notwithstanding these limitations, our understanding of the biology of aging in the cerebral circulation is growing, albeit slowly.

The goal of this review is to summarize recent progress and concepts in this area, while highlighting some key findings and unanswered questions. We focus on the biology and aging of the microcirculation and select end-organ effects that are a consequence of what is often referred to as cerebral microvascular or small vessel disease (SVD). SVD is characterized by changes in the pial (leptomeningeal) and parenchymal circulations (small arteries, arterioles, capillaries, and venules). In this review, we concentrate predominantly on normal or 'healthy' aging, along with features of SVD that would commonly be linked with vascular risk factors (5, 7). Because there have been very useful reviews on related subjects such as cerebrovascular changes during Alzheimer's disease (8, 9), we do not emphasize that subject nor studies based on surgically- or pharmacologically-induced reductions in cerebral blood flow (CBF) to rapidly produce hypoperfusion, a common feature of aging.

Loss of Brain Health

Key consequences or manifestations of SVD in relation to brain health can be classified as cognitive decline or non-cognitive disorders (Figure 1). These deficits occur predominately in aged individuals (1, 3, 4, 10). There are several categories of specific changes (or clinical indexes) in this regard which include lacunes (small cavities resulting from previous infarcts), microinfarcts (small lesions of ischemic origin), microbleeds, enlarged perivascular spaces, white matter injury with changes in connectivity, and brain atrophy (Figure 1) (5, 8, 11). As an example, increased numbers of microbleeds are associated with a greater risk for cognitive decline and dementias (eg, executive function and memory) (12). While all of these changes can result from vascular dysfunction or vascular pathology, mixed and non-vascular mechanisms are thought to contribute as well.

Multiple mechanisms likely contribute to a decline in brain function with aging. Cognitive performance can be reduced differentially and in multiple domains, including executive function and forms of memory (4, 13). Aging can be associated with impaired ability to maintain cognition at normal levels, as well as loss of cognitive reserve (Figure 1) (14). In general, some degree of vascular and cognitive dysfunction occurs with normal aging, but the rate of change can be augmented or inhibited by additional factors including genetics, diet, behavior, the environment, and the presence or absence of varied diseases. Thus, the chronological and biological (or physiological) age of vessels may differ. Cognitive decline may be viewed as a biomarker for otherwise silent SVD (7, 12). Even when SVD is present as a consequence of causes other than aging [eg, a genetic mutation in the Notch3 receptor responsible for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)], the disease still has an aging component (15, 16).

A vascular contribution to mild cognitive impairment, Alzheimer's disease, or other dementias is increasingly recognized. Most humans with dementias exhibit features of mixed dementia (vascular disease and neurodegeneration), a combination of ß-amyloid-,

tau-, and vascular-dependent mechanisms (5, 7, 11, 13, 17). Mild cognitive impairment affects more people than dementias, with estimates of up to 20% of individuals age 65 and older (4). In relation to mixed dementia, up to 75% of patients with dementia have vascular pathology at autopsy (18). Alzheimer's disease has a high prevalence of coexisting age-dependent vascular disease, higher than in other forms of neurodegeneration (19). In preclinical models, it is possible to study effects of aging per se, but in humans, vascular aging often does not occur in isolation. Rather it is frequently seen with other vascular risk factors including hypertension, metabolic diseases (eg, hyperhomocysteinemia and diabetes), obesity, or genetic variations. Common vascular risk factors such as hypertension (particular during mid-life) accelerate the rate of cognitive decline (7, 20), so dementias occur at a younger age in persons with chronic hypertension (4). Augmented progression of SVD is considered a likely contributing mechanism (7, 20). Better cardiovascular health metrics are associated with less decline in cognition and less dementia in the aged (21).

Brain Microcirculation

The cerebral circulation can be grouped into distinct domains or zones. Anatomically, there are three major segments - cerebral arteries, the pial circulation, and the parenchymal circulation (Figure 2) (22, 23). Each segment has unique features but also characteristics that are common to all (22–24). Within an individual segment, such as the parenchymal circulation, considerable heterogeneity exists (22–26). Importantly, all segments are integrated to some extent (see discussion below).

Conceptualizing the vasculature as part of a neurovascular unit (NVU) is a common and useful approach, one that has been reviewed in detail recently (23). In the literature as a whole, the focus on the NVU is often at the capillary level, sometimes excluding major cell types such as vascular muscle. Considering changes in organization with progression along the vascular tree (22–25), it is appropriate to recognize that the cellular makeup and function of NVUs vary as well (Figure 2). In addition to structural and functional diversity due to size, brain region, and so forth, the transcriptome in each of the major vascular cell types in the microcirculation can vary substantially under normal conditions, depending on the specific gene (26).

Contribution of Small Vessels to Regulation of CBF

The distribution of vascular resistance in brain is unique relative to other vascular beds. As discussed in greater detail recently, a large fraction of vascular resistance normally resides in cerebral arteries and the pial circulation (23, 27). Thus, there is a substantial drop in local intravascular pressure before pial arterioles dive into the brain parenchyma (27). Direct measurements of CBF and microvascular pressure reveal that resistance across the parenchymal portion of the circulation accounts for approximately half of total cerebrovascular resistance (23, 24, 27). In contrast, a recent modeling study concluded that the capillary bed is the site of greatest vascular resistance in the cerebral cortex (28). The results of this approach are based in part on the assumption that intravascular pressure in the pial circulation is essentially equal to blood pressure in the aorta (28), an assumption not supported by direct measurements of arteriolar pressure in many laboratories (23, 27). What

can be concluded at this point is that while vascular resistance of the parenchymal circulation is nearly half of total cerebrovascular resistance (23, 24, 27), the precise relative contribution of parenchymal arterioles, capillaries, and venules is not known at present for adults, or during aging (Figure 2).

Hemodynamic Changes With Aging

There is considerable evidence that CBF declines progressively during normal aging. Such a decline has been described in rats and mice (29–33) and in many studies of humans where progressive reductions in CBF have been commonly described, independent of changes in cerebral O_2 consumption (34–39). Similarly, increases in cerebrovascular resistance and reductions in CBF were associated with indexes of cognitive decline over a two year follow-up in subjects with Alzheimer's disease (38). Local vascular and brain PO_2 levels also decrease (40), implicating age-dependent reductions in arteriolar and venular oxygenation and O_2 transport (Figure 1).

Several lines of evidence, including temporal relationships and genetic data, suggest levels of CBF are a biomarker for brain health. In addition to the observation of reductions in CBF with age, baseline levels of CBF are predictive of future cognitive function, particularly changes in the frontal lobe (37, 41). Lower baseline CBF is predictive of cognitive decline at follow-up, notably executive function and memory (42). This association was stronger in subjects with hypertension and higher degrees of white matter abnormalities (42). Thus, changes in CBF precede and can be predictive of future cognitive loss. In subjects with gene mutations that cause frontotemporal dementia, CBF begins to decline more than a decade before behavioral or neurological symptoms (41). Such work supports the possibility that interventions that maintain CBF at normal levels in presymptomatic carriers may delay or prevent the onset of cognitive or other neurological symptoms (41). Whether age-related changes in brain function due to other causes, including normal aging, can be reversed by chronically increasing CBF is an unanswered question.

Regarding a mechanistic basis for reductions in CBF, aging is associated with changes in vascular structure and mechanics, impairment of adaptive mechanisms that regulate global and local CBF, and emergence of pathological pathways that promote increases in vascular resistance, such as production of endothelin-1 by endothelial cells (Figure 1) (24, 43). Along with perfusion pressure, CBF is determined by an integration of these changes and their net impact on cerebrovascular resistance. At this point, it is difficult to assign fractional importance to individual or specific changes.

If CBF is a predictor of future brain health, defining mechanisms that underlie vascular aging, including unique features of cerebrovascular aging should be a priority. Unfortunately, relatively few studies have focused on the fundamental biology and mechanisms of vascular aging in brain. The view that studies of the extracranial circulation will generally be predictive of cerebrovascular aging and its underlying mechanisms seems unrealistic considering the numerous unique features of the brains circulation. A comparison of cerebral arteries with non-cerebral vessels suggest the cerebral circulation is particularly sensitive to features of vascular aging, including oxidative stress and endothelial

abnormalities (44–47). Mechanisms that are responsible for these fundamental regional differences are not clear at this time.

Endothelium-Dependent Mechanisms

One of the most prominent effects that endothelial cells exert within the vessel wall related to regulation of vascular tone in cerebral arteries and microvessels (Figure 3) (46, 48–52). Importantly, the state of endothelium-dependent regulation of vascular tone is predictive of clinical events including stroke (24, 53, 54). Endothelial cells normally influence the diameter of arteries and arterioles at rest while also mediating vasodilation to mechanical forces, receptor mediated agonists, ions, neurotransmitters, and therapeutic agents (24, 54–56). Impairment of this endothelium-dependent mechanism occurs with aging throughout the vasculature, from cerebral arteries to the smallest parenchymal arterioles (44–46, 48, 50–52, 57). This concept is based on direct visualization of vessels in vivo, studies of isolated vessels, and measurements of CBF.

Endothelium-dependent vasodilation occurs via several mechanisms, which can vary with brain region and hierarchy within the vascular tree. For example, nitric oxide (NO) produced by endothelial NO synthase (eNOS) is a major mediator of this response in cerebral arteries, pial and parenchymal arterioles, in multiple species including humans (44, 46, 48, 58–60). The primary molecular target of eNOS-derived NO is guanylyl cyclase (Figure 4) producing effects through cGMP formation and its subsequent targets (24, 55, 60, 61). Another example of the impact of this pathway is seen in humans expressing gain of function variants in eNOS and NO-mediated cGMP formation, that are associated with reductions in vascular events and stroke, independent of differences in arterial pressure (53). In contrast, vasodilation in response to activation of endothelial intermediate (IK) and small conductance (SK) Ca^{2+} activated K^+ channels, with spread of hyperpolarization to vascular muscle, is prominent in small arterioles (48, 62). Some literature continues to promote the view that vasoactive stimuli activate only eNOS in conduit arteries and only endothelial IK/SK channels in resistance vessels. Numerous studies demonstrate that this latter view is too simplistic for the cerebral circulation.

With regard to signaling mechanisms, the NO component of vasodilation is greatly affected by aging (33, 44, 46, 48, 50), while the response elicited by IK/SK channel activation remains relatively intact (48). What activates this form of endothelium-dependent hyperpolarization under physiological conditions is not clear. Whether this pathway could be a therapeutic target to offset or reverse age-induced reductions in CBF and their consequences remains to be tested.

Several pathways have been implicated in mechanisms that promote endothelial dysfunction during aging. Key contributors include the angiotensin II AT1 receptor, the adaptor protein $p66^{Shc}$, oxidative stress, and poly(ADP-ribose) polymerase, but not cyclooxygenase (Figure 4) (44–46, 51, 52, 63). Several of these contributors are interrelated. First, while there are multiple potential causes of oxidative stress (24), available data suggest the Nox2-containing NADPH oxidase is key based on findings from Nox2-deficient mice, effects of an inhibitory peptide targeting the oxidase, changes in expression of NADPH oxidase components, levels

of superoxide and isoprostanes, and effects of scavengers of superoxide of vascular function (44, 45, 50–52). Second, angiotensin II is a well documented activator of NADPH oxidase (54, 64, 65), and genetic deficiency in AT1 receptors protects against angiotensin II-induced vasoconstriction (66) and age-induced endothelial dysfunction (44). Third, $p66^{Shc}$ is a signaling protein that among other effects, activates NADPH oxidase (45). Fourth, Rho kinase is activated by reactive oxygen species (ROS), with additional evidence that age-induced reductions in eNOS-mediated vasodilation are dependent on activity of Rho kinase (Figure 4) (48). Lastly, levels of asymmetrical dimethylarginine (ADMA), an endogenous inhibitor of eNOS (Figure 4) (67), are elevated with aging and have been linked with SVD (5, 17). On a broader scale, changes in the small vessel endothelial cell vasculome are greater with aging in mice than in models of hypertension (BPH) or diabetes (*db/db*) (68). While the sensitivity of the endothelial transcriptome to aging is interesting, the impact of such changes in the cells expression profile remains to be defined.

The influence of endothelium on other cell types extend beyond vascular tone and include effects on vascular structure, maintenance of collateral vessels, neurotransmission, neurogenesis, and metabolism of ß-amyloid (see below)(Figure 3) (54, 55). Recently, effects of endothelium-derived semaphorin 3G have emerged. This secreted signaling molecule (part of the endothelial communicome) normally promotes neural function, synaptic organization, and memory in the hippocampus (69). Secretion of semaphorin 3G, which is expressed at higher levels in arteriolar endothelium (26), promotes hippocampal function and memory. Thus, a decline in endothelial cell health (loss of endothelial function) can promote cognitive dysfunction via vascular and non-vascular effects, including effects on CBF as well as synaptic organization (Figure 2). When the effects described above are combined with its role within the BBB (see below), the overall impact of cerebral endothelial cells for brain function cannot be underestimated.

Few studies have worked to define mechanisms in models that combine risk factors for vascular disease with aging. Some exceptions do exist however, including recent work using aged mice that are also partly genetically deficient in eNOS. The phenotype seen in these mice are relevant to SVD and its consequences, and include increased levels of vascular ß-amyloid, microthrombi, degeneration of vascular muscle, microbleeds, and reductions in cognition (Figures 1 and 3) (70, 71). Such work provides examples of approaches that may have particular relevance as preclinical models of vascular aging.

Neurovascular Coupling

Individual brain regions are perfused at levels that are proportional to their basal levels of energy consumption (eg, gray versus white matter) (23). Additional increases in local neural activity rapidly alter local CBF above baseline levels, a response referred to as neurovascular coupling (NVC) and used commonly as the basis for functional brain imaging (23). Considerable effort has been invested in defining the cellular and molecular mediators of the NVC response. While essential roles for neurons (and glutamate receptors), vascular muscle, and endothelial cells are established, the contribution of other cell types - particularly astrocytes and pericytes – have been more difficult to define and remain controversial due to conflicting findings and interpretation (23). There has been some agreement regarding

molecular mediators of NVC which include NO, adenosine, extracellular K^+, and prostanoids (23). Having identified specific roles for these cells and molecules (or ions), it is important to recognize that the contribution of each cell type and which mechanisms mediate NVC vary with brain region and segment of the vasculature (23, 24, 56, 72, 73). Such diversity in the local regulation of NVC hemodynamics support a multi-NVU concept.

An area in which much less is known involves flow-mediated and propagated vasodilation, vascular responses that are also dependent on endothelial cells (24, 56, 74). Activation of brain regions or neural subtypes deep within the parenchyma generate hemodynamic changes that occur locally, but are also transmitted upstream to reach arterioles and arteries on the surface (23, 74). Relatively little has been known regarding details of how signals originating within the brain elicit vasodilation of resistance vessels on the brain surface. Recent work implicates capillaries as sensors of K^+ and therefore neural activity. The local concentration of extracellular K^+, a potent vasodilator (56, 75), increases with each action potential (56). With neural activation, a local upward spread of hyperpolarization from endothelial cell to adjacent endothelial cell (via endothelial inward rectifier K^+ channel 2.1) occurs, along with simultaneous spread to vascular muscle via myoendothelial gap junctions (56). The result is vasodilation of arterioles within the parenchyma and on the pial surface. Importantly, this vascular communication occurs in the absence of changes in capillary diameter (56). Considering the distribution of vascular resistance in brain (23, 27), this form of integrated microcirculatory response is important in that without dilation of upstream arterioles and arteries, vasodilation that originates in distal portions of the vascular network would reduce local arteriolar pressure, resulting in a decline in local perfusion pressure (27, 76).

With aging, NVC is impaired in preclinical models and in humans (50, 52, 77–79). Reductions in NVC appear to be due to vascular defects, not changes in neural activity (78). Long term reductions in NVC are assumed to contribute to loss of brain health including cognitive decline (8, 80), although cognitive loss is also associated with reductions in baseline CBF in the absence of changes in NVC (81).

Regarding possible mechanisms that underlie NVC dysfunction with aging, oxidative stress seems to play a key role (23, 52, 79). In that sense, ROS and NADPH oxidase may be components of a common underlying mechanism that produces dysfunction of both NVC and endothelium-dependent vasodilation with aging (Figure 4) (44, 46, 51, 52). Because endothelial cells are important contributors to the NVC response (23, 56, 74), other mechanisms implicated in loss of endothelial function (discussed above) may also contribute to decreased NVC. For example, to what extent reduced propagated vasodilation contributes to loss of NVC or other adaptive responses with age is unclear.

Chemoregulation

Additional determinants of cerebral vascular resistance include chemical stimuli (eg, increases in CO_2 or hydrogen ion or reductions in O_2). Both hypercapnia, an elevation in arterial $PaCO_2$ (via reductions in extracellular pH) and hypoxia, a reduction in PaO_2 (or arterial O_2 content), are powerful vasodilators (49), with the greatest responses occurring in

the smallest arterioles (82). These are additional vascular responses that are altered by aging (49). The normally steep relationship between CBF and $PaCO_2$ is impaired with aging or in subjects with CADASIL (35, 83). Reduced vasodilation to hypercapnia is predictive of cognitive decline (84). In the Framingham study, a reduced response to hypercapnia increased the risk of dementia (84). In these subjects, the impairment was likely not due to atherosclerosis and thus reflects functional, not structural, vascular abnormalities (84).

Endothelial dysfunction is one of the early vascular phenotypes seen with aging (Figure 3). Consistent with vascular responses to hypercapnia being independent of endothelial cells (85), it is not uncommon for models of vascular disease to exhibit endothelial dysfunction or impaired NVC before changes in vascular effects of increased CO_2 become apparent (52). CBF responses to hypercapnia can become impaired with more advanced age however (49, 52).

Vascular Structure and Mechanics

It is well established that aorta and large conduit vessels such as the carotid artery become stiffer (less distensible or compliant) with aging (86). These changes in vascular mechanics are considered key events in human disease, changes that contribute to increased pulse pressure and hypertension, among other effects (7, 86). Increased arterial stiffness has been linked with cognitive decline in numerous studies (7, 86), and links between large and SVD have been described previously (27, 86).

Hypertension (particular during mid-life) accelerates the rate of cognitive decline (7), with increased stiffness of large arteries and augmented progression of SVD considered as a likely contributing mechanism (7). Recently, Chiesa et al linked changes in carotid artery hemodynamics (forward-traveling compression wave intensity) in mid- to late-life to cognitive decline at follow-up more than 10 years later (20). In a relatively large and well characterized population, reductions in cognition – particularly executive function and phonemic fluency – occurred independent of other risk factors, and were presumably due to effects of increased pulse pressure and transmission of elevated pressure to distal cerebral microvessels (20). In individuals with the greatest hemodynamic change, cognitive reductions were equivalent to approximately two years of additional aging (20).

Within the cerebral circulation, large arteries and pial arterioles become stiffer with aging, apparently due to reductions, fragmentation, and reorganization of elastin fibers along with deposition of collagens (Figure 2) (87–90). Indirect indexes suggest small lenticulostriate arteries also stiffen with age (91). In the pial circulation, reductions in arteriolar cross-sectional area (atrophy) have also been observed (89), a change that may promote formation of microaneurysms and eventual microbleeds (Figure 2). Similar to effects of aging, small cerebral arteries become stiffer with a reduced ability to vasodilate in a genetic model of CADASIL (92). Work on structural and mechanical changes in arterioles, capillaries, or venules in the parenchyma is very limited. In recent studies, aging was not associated with changes in distensibility or lumen diameter in parenchymal arterioles (48, 87).

The reduction in distensibility of large arteries correlates with, and is assumed to cause pathophysiological changes in small vessels downstream. The relative importance of loss of endothelial function, impaired communication between vascular segments and loss of blood-brain barrier (BBB) integrity are not known. Interestingly, vasoconstrictor responses to angiotensin II are selectively increased in cerebral arteries in a model of increased large artery stiffness due to genetic deficiency in elastin (93). This latter finding provides another example of selective susceptibility in the cerebral circulation to age-related vascular changes.

Normal aging is associated with progressive loss or rarefaction of pial collateral arterioles (reductions in arteriolar number and diameter), resulting in a marked increase in vascular resistance in the pial collateral circulation (Figure 2) (94, 95). Similarly, increased age was associated with poor pial collateral status in patients presenting with ischemic stroke (96). Like inward vascular remodeling, these microvascular changes can contribute to increased vascular resistance, hypoperfusion, reductions in vasodilator responses, and a predisposition to increased injury if challenged with local ischemia. Similar to effects on pial arterioles, reductions in cerebral capillary density with aging are also common (Figure 2) (30, 40, 47, 87).

Many previous studies have reported changes in vessel diameter as evidence of vascular remodeling under a variety of conditions including aging. With few exceptions, simply measuring vessel diameter does not distinguish whether alterations seen are due to changes in vascular tone, vascular mechanics, or encroachment of the vessel wall on the lumen (due to true vascular remodeling or increases in cross-sectional area of the vessel wall). In general, insight into mechanisms that underlie changes in vascular structure and mechanics in the aging brain are limited. Because normal function of eNOS is known to be required for maintenance of pial collaterals, loss of this protective effect appears to underlie rarefaction of pial arteriolar collaterals during progressive aging (94, 97).

Blood-Brain Barrier and Blood-CSF Barrier

Along with the impact of age on CBF, changes in BBB integrity and function is a second major category of age-induced alterations that affects the cerebral circulation and as a consequence, brain health (Figures 1 and 2). The ‘cornerstone’ of the BBB are endothelial cells, with adjacent cells anchored to each other by junctional complexes that include tight junctions (eg, occludin and claudins) and adherens junctions (eg, cadherins, junctional adhesion molecules) (54, 98–100). Both the level of expression and phosphorylation state of these molecules are key factors in function of these complexes (25, 101). Critical support for BBB function is normally provided by astrocytes and pericytes, with additional contributions by the basement membrane and the glycocalyx (25, 98–100). The basement membrane consists of a network of extracellular matrix proteins including type IV collagen, laminins, and proteoglycans (102).

The normal BBB exhibits some key features (25, 100, 101, 103, 104). First, the movement of molecules or cells from circulating blood into the cerebrospinal fluid or brain parenchyma is limited by the presence of endothelial cell tight junctions and is highly regulated. Second,

levels of transcytosis (trans-endothelial vesicular transport) are low in these endothelial cells. This key feature is due, at least in part, to active suppression by the transmembrane protein Mfsd2a (105), which is expressed in various microvessels, but at the highest levels in capillaries (26). Increases in BBB permeability can involve increased transcytosis, changes in expression or post-translational phosphorylation or degradation of junctional proteins, or combinations of the above (101). Third, the BBB expresses transporters which selectively move key molecules out of blood and into brain (eg, the glucose transporter GLUT1) as well as drug efflux pumps (that move molecules from brain into blood) and metabolizing enzymes (25, 106). While features of the BBB can vary in different segments of the vasculature (25, 107), the BBB is present in the pial and parenchymal circulations (both arterioles and venules) as well as in capillaries (22, 25, 54, 108). Capillaries are the site of greatest surface area for exchange and transport however.

Dysfunction of the BBB occurs with aging in both mice and humans, changes that are progressive, often region-dependent, and include white matter – a key event in relation to subsequent cognitive dysfunction (Figures 1 and 2) (47, 109–112). In humans, age-dependent but selective increases in permeability of the BBB have been described in the hippocampus (111, 113). Such changes can occur early in the progression of SVD, and include white matter, but be present before detectable alterations in cognition (111). Changes in the BBB were also independent of changes in ß-amyloid or tau biomarkers (113). Mice deficient in expression of platelet-derived growth factor receptor-ß have age-dependent reductions in pericyte coverage on capillaries, and exhibit similar changes in permeability of the BBB in the hippocampus (114). In addition, there are changes in expression of tight junction proteins (101, 104, 112), alterations in the thickness and composition of extracellular matrix proteins (collagen type IV, laminins, and proteoglycans) as well as lipid accumulation in the basement membrane (102, 104, 109), and loss or degeneration of pericytes (Figure 2) (104, 109, 115).

Because the brain relies almost entirely on glucose as an energy source, relatively large quantities of glucose are constantly being transported from blood to brain (25, 106). While a large percentage of all proteins in cerebral endothelial cells are transporter molecules, GLUT1 is the major facilitator for glucose transport (25, 106). GLUT1 is localized on both the luminal and abluminal endothelial cell membranes, with normal activity of GLUT1 being essential for brain health (25, 106). In addition to the constant basal delivery, acute changes in glucose transport can occur. Studies using glucose biosensors have shown that local concentrations of glucose increase within seconds in response to sensory stimulation or glutamate receptor activation (116). Reductions in GLUT1 expression or activity result in reduced BBB integrity and varied neurological symptoms (25, 106, 117). With aging, GLUT1 expression and brain glucose uptake is reduced in humans and rodents, changes that are associated with increased BBB permeability and cognitive decline (Figures 1 and 2) (25, 106).

Recent studies have highlighted other potential mechanisms that impact alterations in BBB function with aging. The transcriptional regulator sirtuin 1 has been broadly implicated in oxidative stress and longevity. In aged mice, increases in permeability of the BBB and reduced expression and fragmentation of the tight junction protein claudin 5 are linked to

reduced expression of sirtuin 1 in microvessels (112). Reducing expression of sirtuin 1 in endothelial cells in adult mice increased permeability of the BBB (112). Conversely, claudin 5 and the BBB are protected in aged transgenic mice with increased expression of sirtuin 1 (112). Together, these results suggest that sirtuin 1 normally plays a protective role and may be a therapeutic target to prevent loss of BBB integrity during aging.

Acid sphingomyelinase (ASM, a sphingolipid metabolizing enzyme) may also play a key role. In both humans and mice, plasma concentrations of ASM increased with aging (47). Levels of ASM in brain are increased in aged mice, with microvessels and specifically endothelial cells as the primary source. Increases in ASM levels in brain were greater than in other tissues and were associated with increased BBB permeability, increased brain H_2O content, capillary rarefaction, and memory loss (47). Partial genetic inhibition or endothelial-specific deficiency in the gene that produces ASM (*Smpd1*) protected mice against loss of BBB integrity, increases in brain H_2O content, loss of capillaries, and memory deficits (47). In contrast, genetically altered mice that overexpress ASM in endothelium had greater impairment of the BBB and loss of memory (47). Of note, changes in permeability of the BBB with age were due to increased transcytosis, with no detectable change in tight junction proteins (47). Thus, endothelium-derived ASM has emerged as an additional regulator of age-dependent BBB function and consequently memory.

The concept that endothelial health is a key determinant of brain health is important, but not new. Beyond the links between endothelial health and atherosclerosis (54), there are other examples. Cell-specific deficiency in endothelial NF-κB essential modulator causes impaired endothelium-dependent vasodilation, reduced NVC, hypoperfusion, loss of BBB integrity, and behavioral changes (118). The endothelial cell adhesion molecule VCAM1 may be a key contributor to loss of hippocampal-dependent function with age (119).

In addition to the BBB, other barriers are present within the brain (106, 120). Within each brain ventricle is a highly vascular choroid plexus, structures that are perfused at very high levels and have unique microvascular features. While capillaries of the choroid plexus are fenestrated, the nearby adjacent epithelial cells are joined by tight junctions so it is these cells (not endothelial cells) that form a blood-CSF barrier (120, 121). Functions of the choroid plexus include being the primary site of CSF formation (120) as well as immune surveillance and interface for trafficking of immune cells (106, 121). The choroid plexus is also a site of molecular transport for nutrients and vitamins and a source of many bioactive molecules, extracellular vesicles, and exosomes (122). With aging, numerous changes occur at the choroid plexus, including loss of barrier integrity, reduced CSF formation, local morphological, molecular, and transcriptome alterations, low grade inflammation, cytokine formation and potential immune cell trafficking (106, 121, 122).

Sex-Dependent Effects

There are now many examples of sex-dependent effects related to stroke (123). Sex-dependent differences in the presence of mild cognitive impairment and Alzheimer's disease have been described, but can be complex, including varying with age (123). Much less is known regarding to what extent vascular aging and the biology of SVD per se are sex-

dependent. In the current context, a few examples can be noted. Regarding reductions in CBF with age, both sex-dependent and sex-independent differences have been described (34, 35). Both the number and diameter of pial collateral arterioles in adult male and female mice and the progressive loss of pial collaterals with aging are not sex-dependent (124). In contrast, there is evidence in cerebral arteries that endothelial dysfunction occurs earlier in males than in females as they age, although both sexes eventually reach similar levels of maximal impairment (44).

Genetic Background

Most preclinical studies are currently performed using rodents. Due to differences in genetics, the strain of animal used can sometimes have a dramatic impact on baseline phenotypes and effects of interventions. In relation to atherosclerosis for example, C57BL6 mice are much more susceptible than FVB/N mice (48). Despite some prominent differences and stain dependent effects, most investigations are performed using a single strain of animal, very often C57BL6 mice in studies related to stroke and the cerebral circulation. While the study of more than one strain requires greater effort and resources, it can provide insight into genetic determinants of vascular disease, including disease susceptibility or resistance. One example are the features and impact of pial arteriolar collaterals on focal ischemic-induced injury (125). A second example are effects of aging on endothelial function. In a recent study of cerebral arteries, endothelium-dependent vasodilation was similar in adult C57BL/6 and FVB/N mice (48). In contrast, while endothelial dysfunction occurred with aging in both strains, the effects were greater in C57BL/6 mice. Despite the quantitative difference in the impairment, the underlying mechanism involved Rho kinase in both strains of mice (48).

Caloric Restriction

Caloric restriction (CR), a reduction in caloric intake without malnutrition, is known to improve lifespan (or healthspan) in multiple organisms (126). Effects of CR on the cerebral circulation have been examined in a few studies. Reductions in CBF and memory with aging in mice are attenuated by CR (32). In relation to function of endothelial cells, CR increased eNOS expression, reduced levels of ROS, and improved endothelium-dependent vasodilation (the NO-component of the response) in models of aging (32, 46).

Because it is difficult to maintain a sustained CR diet in humans, interest in identifying and testing effects of potential CR mimetics has become a priority (126), with the hope of discovering agents to slow loss of health with aging, or reverse already established phenotypes. For example, the plant-derived polyphenol resveratrol is accepted as a CR mimetic (126). Short-term treatment with resveratrol in aged mice improved endothelial function and NVC by augmenting the NO component of both responses, effects that may be due to reductions in activity of NADPH oxidase and levels of ROS (50).

In relation to therapeutic CR alternatives, a molecule with strong CR mimetic activity is the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ) (127). Within the vasculature, PPARγ normally exerts prominent effects in both endothelium and vascular

muscle, particularly in the cerebral circulation where it affects NO-dependent signaling, myogenic tone, vasoconstriction to endothelin-1, microvascular oxidative stress, arteriolar cross-sectional area, and lumen diameter (128–132). The hypertrophy and inward remodeling that was evident in small cerebral arterioles following genetic interference with PPARγ was sex-independent (131).

In humans 60 years of age and older, long-term use of the PPARγ ligand pioglitazone reduced the risk of developing dementia by approximately one-half (133). In mechanistic experiments, genetic interference with endothelial PPARγ accelerated age-related endothelial dysfunction in carotid arteries via mechanisms involving oxidative stress and Rho kinase (134). These effects were sex-independent (134). Vascular expression of CDKN2A (a marker of senescence) was also significantly increased compared to age-matched controls. Collectively, these results implicate an essential role for endothelial PPARγ to protect against key features of vascular aging (134). Related to these findings and other aspects of vascular aging, it is noteworthy that semaphorin 3G has emerged as a downstream target of endothelial PPARγ (135).

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Abbreviations:

ADMA	asymmetrical dimethylarginine
ASM	acid sphingomyelinase
BBB	blood-brain barrier
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CBF	cerebral blood flow
CR	caloric restriction
eNOS	endothelial nitric oxide synthase
IK	intermediate conductance Ca^{2+} activated potassium channel
NO	nitric oxide
NVC	neurovascular coupling
NVU	neurovascular unit
PO_2	partial pressure of oxygen
PPARγ	peroxisome proliferator-activated receptor-γ
ROS	reactive O_2 species

SK	small conductance Ca^{2+} activated potassium channel
SVD	small vessel disease

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Summary Points

- SVD contributes to a range of neurological abnormalities and loss of brain health. This impact includes cognitive and non-cognitive aspects of brain function.
- In relation to vascular cells, endothelial cells and vascular muscle play a major role in SVD. For endothelial cells, the impact is broad and includes effects on vascular structure, CBF, and BBB, but also higher aspects of brain function - cognition, memory, and behavior.
- CBF is a biomarker for brain health and predictor of future cognitive function.
- Several common underlying mechanisms and signaling pathways have been described. New contributors and modulators continue to emerge as a focus on vascular aging in brain grows.
- Although aging impacts large and small vessel structure, mechanics and function – the precise consequences and relative importance of each change is poorly defined.

Future Issues

- Current concepts regarding vascular disease and brain health are often based on temporal associations. Most interventional or therapeutic approaches that have been attempted lacked vascular specific manipulations.
- Targeted experimental and therapeutic strategies are needed to directly define the impact of specific vascular cells on end-organ effects and function. Testing the importance of lesser studied cell types (eg, perivascular macrophages).
- When cell specific approaches have been used, effects of aging have rarely been included in the design.
- Studies that provide greater mechanistic depth are needed. For SVD, this includes details of the endothelial transcriptome and communicome in models of disease and aging, the impact of hematopoietic cells, non-coding RNAs, and the various microbiome's (oral cavity, gastrointestical tract, skin, lung, placenta, vagina and uterus), each with potential interactions with brain.
- In addition to pial and parenchymal vessels, greater understanding of the biology of other microcirculations are needed including meninges, choroid plexus, and circumventricular organs.
- Vascular aging very often does not occur in isolation. Relevant models and studies are needed that combine aging with chronic major risk factors for SVD, including hypertension and diabetes.

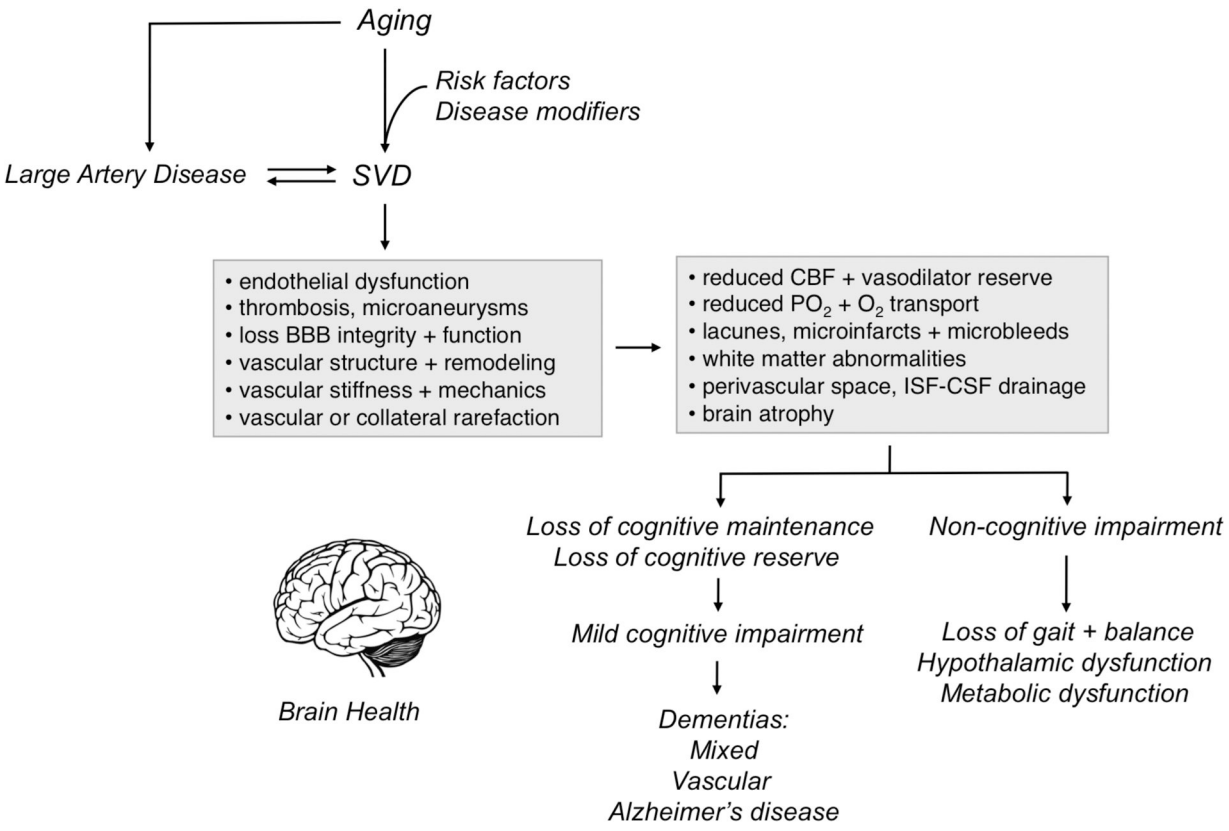

Figure 1.

Schematic summarizing effects of aging, vascular risk factors, and disease modifiers for SVD (small vessel disease), followed by changes in the vasculature and consequences of these changes for brain health.

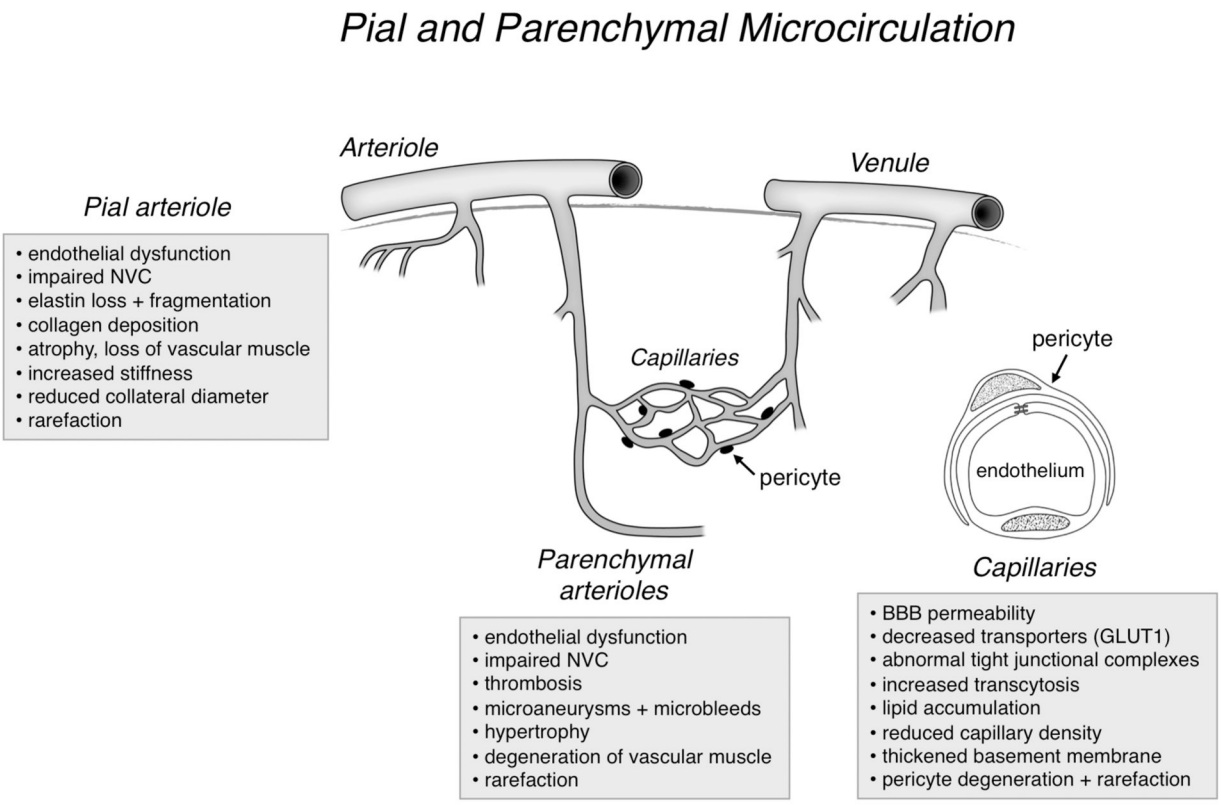

Figure 2.

Schematic illustrating segments of the pial and parenchymal microcirculations, with major changes in each segment during SVD. The illustration is based on features of the human cortical microcirculation (136), with specific characteristics based on the following references (30, 40, 47, 48, 50–52, 63, 71, 87, 89, 94, 102, 104, 109, 115, 137). See text for discussion and additional details.

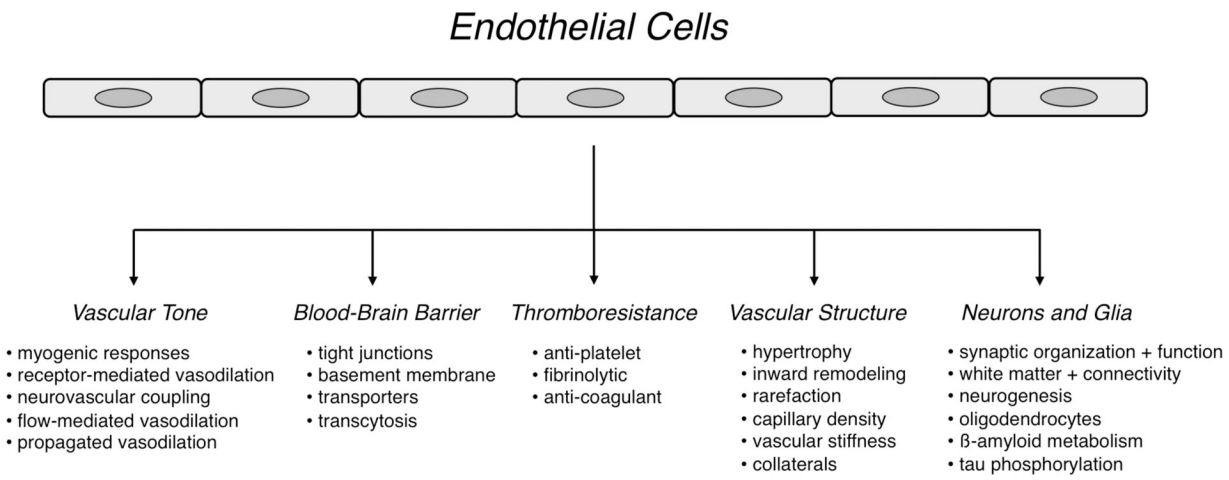

Figure 3.

Summary of diverse effects exerted by endothelial cells in relation to vascular tone, the blood-brain barrier (BBB), thrombosis, vascular structure, neurons, and glia.

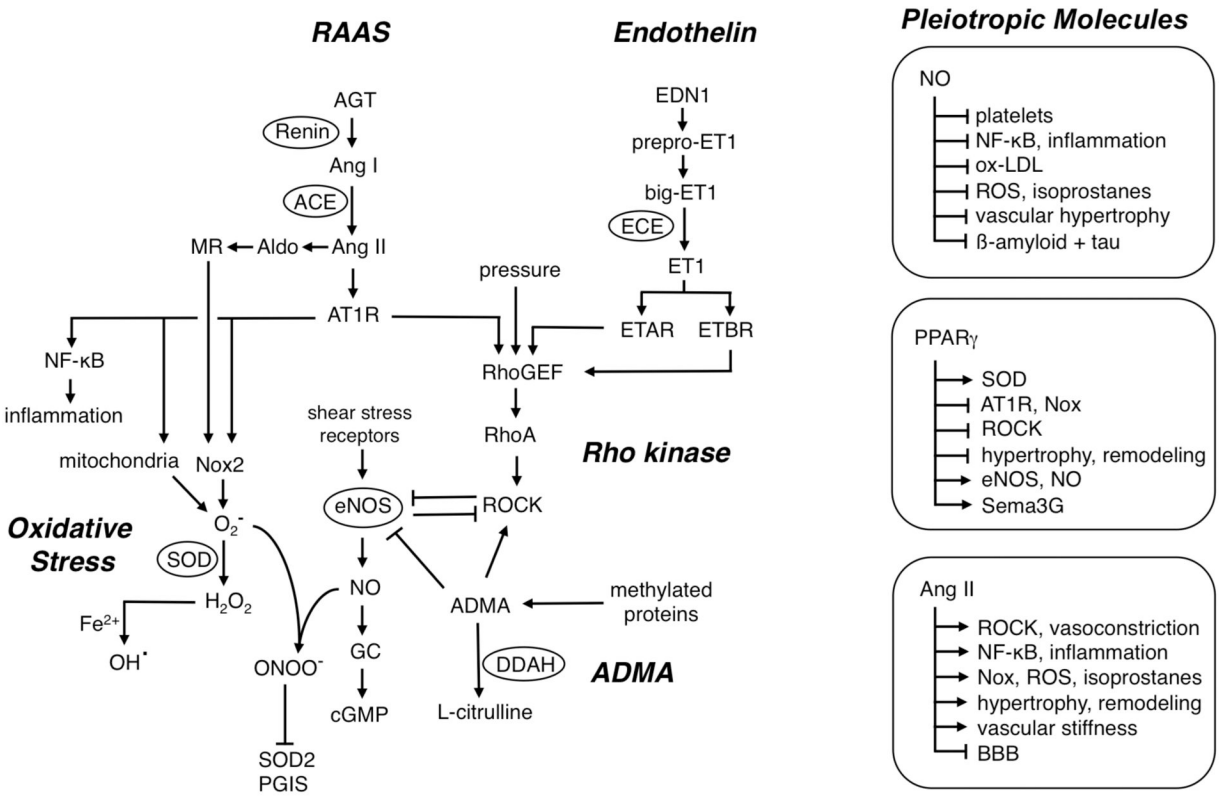

Figure 4.

Several major mechanisms that have been implicated in vascular aging. The renin-angiotensin-aldosterone system (RAAS) produces angiotensin II (Ang II) from angiotensinogen (AGT) via enzymatic actions of renin and angiotensin-converting enzyme (ACE). In addition to activating the Ang II type 1 receptor (AT1R), Ang II can stimulate production of aldosterone (Aldo), which acts via the mineralocorticoid receptor (MR). Both Aldo and Ang II promote oxidative stress by activating a Nox2 containing NADPH oxidase which produces superoxide anion. Superoxide reacts readily with NO to form peroxynitrite ($ONOO^-$), with inhibitory effects on mitochondrial SOD (SOD2) and prostaglandin I_2 synthase (PGIS) due to effects on tyrosine residues. Superoxide can also be converted to H_2O_2 by SOD, and H_2O_2 can be converted to hydroxyl radical (OH·) in the presence of Fe^{2+}. Via the AT1R, Ang II can activate NF-κB promoting inflammatory signaling, stimulate mitochondria to produce superoxide, or increase activity of Rho kinase (ROCK) via effects on RhoGEF. In addition to increasing vascular tone directly (not shown), ROCK inhibits production of NO by eNOS. ROCK is also activated by the endothelin-1 (ET1) pathway, where prepro-ET1 is produced from the ET1 gene (EDH1) and formed by endothelin-converting enzyme (ECE) from big-ET1. ET1 can then activate ET1 receptors (ETAR or ETBR, the latter of which can activate RhoGEF in vascular muscle). Activity of eNOS can be inhibited by ADMA (asymmetric dimethylarginine) or ADMA can be degraded by dimethylarginine dimethylaminohydrolase (DDAH). For clarity, the boxes on the right summarize related pleiotropic effects of three key molecules in this context: NO, PPARγ, and Ang II.