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Pericyte Control of Blood Flow Across Microvascular Zones in the Central Nervous System

David A. Hartmann,[1] Vanessa Coelho-Santos,[2,3] and Andy Y. Shih[2,3,4]

[1]Department of Neurology and Neurological Sciences, Stanford University, Stanford, California, USA

[2]Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, Washington, USA; email: Andy.Shih@SeattleChildrens.org

[3]Department of Pediatrics, University of Washington, Seattle, Washington, USA

[4]Department of Bioengineering, University of Washington, Seattle, Washington, USA

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Abstract

The vast majority of the brain's vascular length is composed of capillaries, where our understanding of blood flow control remains incomplete. This review synthesizes current knowledge on the control of blood flow across microvascular zones by addressing issues with nomenclature and drawing on new developments from in vivo optical imaging and single-cell transcriptomics. Recent studies have highlighted important distinctions in mural cell morphology, gene expression, and contractile dynamics, which can explain observed differences in response to vasoactive mediators between arteriole, transitional, and capillary zones. Smooth muscle cells of arterioles and ensheathing pericytes of the arteriole-capillary transitional zone control large-scale, rapid changes in blood flow. In contrast, capillary pericytes downstream of the transitional zone act on slower and smaller scales and are involved in establishing resting capillary tone and flow heterogeneity. Many unresolved issues remain, including the vasoactive mediators that activate the different pericyte types in vivo, the role of pericyte-endothelial communication in conducting signals from capillaries to arterioles, and how neurological disease affects these mechanisms.

BRAIN MICROVASCULATURE

The brain is the most metabolically demanding organ, consuming 20% of the total body's oxygen while weighing only 2% of its mass. Because it lacks a local energy reserve, the human brain relies on a continuous supply of blood delivered by an astounding total vascular length of 400 miles (1). While arteriole and venous networks are visible to the naked eye, these larger caliber vessels are dwarfed in number by dense microscopic capillaries that serve as the blood's vast distribution network.

Clinical imaging cannot yet resolve individual capillaries in vivo. However, capillary perfusion can be appreciated from digital subtraction angiography, where a contrast agent transiting the microvasculature creates a ubiquitous blush across the hemisphere (**Figure 1*a***). This blush predominantly represents flow through capillaries. It is here that the blood fulfills its role to supply oxygen and remove waste from the brain. Strikingly, the contrast agent perfuses the collective

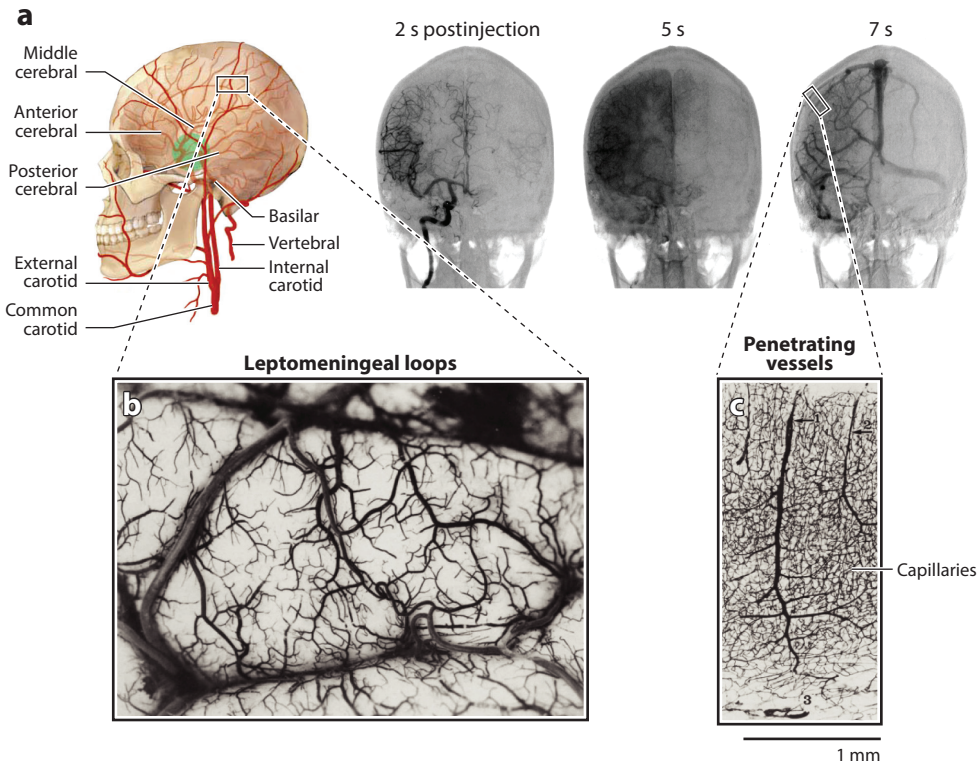


Figure 1

Capillary perfusion and structure in the human brain. (*a*) Schematic of major extracranial and intracranial arteries perfusing the brain are shown on the left. Digital subtraction angiography images on the right show movement of the contrast agent as it enters the cerebral arteries (2 s postinjection in the right internal carotid artery), transits capillaries (5 s), and then exits through the venules and veins (7 s). The image at 5 s represents capillary blush as the contrast agent is visible, but the individual microvessels perfused are below the resolution limit of the imaging technique. (*b*) Image of pial vasculature on the surface of the human cerebral cortex. (*c*) Micrograph of a tissue slice from human cortex, showing a cortical penetrating vessel (arteriolar or venular origin unclear) surrounded by the capillary bed. Panels *b* and *c* adapted with permission from Reference 4; copyright 1981, Elsevier.

400 miles of vasculature in less than 5 s, highlighting the efficiency of this microcirculatory process. In various diseases that affect the cerebrovasculature, the transit of blood cells through capillaries becomes impaired, contributing to brain dysfunction and neurodegeneration (2, 3).

Elegant anatomical studies by Duvernoy and colleagues used India ink injections to reveal the elaborate vascular networks of the human brain (4). They imaged and depicted the arterioles and venules of the brain surface (**Figure 1*b***) and the underlying capillary networks (**Figure 1*c***), which were densely packed because each capillary measured only about 5 μm in diameter, i.e., one-tenth the diameter of a human hair. These studies made it clear that capillaries represent the great majority of the brain's vascular length and that their function was essential to understand.

Early histological studies led many to hypothesize that capillaries were involved in cerebral blood flow dynamics such as functional hyperemia, a phenomenon in which neural activity leads to increased local blood flow. Functional hyperemia is achieved by communication between neurons and vessels in a process called neurovascular coupling (5). At the level of brain arterioles, neurons communicate directly or indirectly through astrocytes to control the tone of arteriolar smooth muscle cells (SMCs). Similarly, the current theory is that neurons or astrocytes also communicate with mural cells of vessels downstream of arterioles, called pericytes, to control blood on a finer scale (6). There is logic behind this concept, as autonomous control of capillary diameter by pericytes could lead to more precise delivery of blood, making the redistribution of blood cells and delivery of oxygen more efficient.

Pericytes are the ideal candidate for the regulation of capillary diameter. They line the walls of all microvessels that span between arterioles and venules and have similar abluminal positions as SMCs, which are known to exert a powerful influence over arteriole diameter and flow. Pericytes are embedded within the basement membrane of the capillary wall and form communicatory junctions (gap and adherens junctions) with endothelial cells at sites where the basement membrane thins (7). Through communication with the endothelium, pericytes serve diverse functions in brain vascular health. These functions have been surveyed by many recent reviews, which detail classic pericyte roles in blood–brain barrier establishment, blood flow control, and angiogenesis (8–11). Novel roles in regulation of brain immune responses are also emerging (12).

We focus this review on the data surrounding the hypothesis that pericytes control cerebral blood flow in vivo. This hypothesis has proven challenging to address for over a century owing to many factors, including (*a*) a dearth of tools to unequivocally identify and genetically target pericytes on capillaries, (*b*) inconsistent nomenclature of pericyte subtypes, (*c*) the correlative nature of observational in vivo studies, and (*d*) a lack of spatial resolution to resolve small changes in capillary diameter. However, recent advances have overcome some of these challenges, yielding novel data from ex vivo and in vivo preparations. Our goal is to synthesize these findings and navigate past controversies to distill key concepts and next steps.

HISTORICAL ASPECTS OF PERICYTES IN BLOOD FLOW CONTROL

Pericytes have been proposed to modulate capillary blood flow since their discovery in the frog by Eberth in 1871 (13) and Rouget in 1873 (14). This potential role of pericytes was later rejuvenated by research showing that capillary blood flow is dynamic. August Krogh discovered that capillaries in skeletal muscle become perfused in response to muscle activity, a discovery that culminated in his Nobel Prize; see Poole et al. (15) for a review of Krogh's work. An excerpt from Krogh's speech accepting the Nobel Prize for "the capillary motor regulating mechanism" aptly describes the field in 1920, and the field now, over 100 years later:

> There must be a special capillary-motor system by which the bore of the capillaries can be regulated, but stating that fact raises a whole series of new questions: is variation in diameter of capillaries independent of the arteries, or does it follow from them? In what way can the capillaries be excited—chemical, electrical or mechanical? Are they under nervous control, and, if so, by which nerve? (16)

Based on Krogh's work, there was consensus that capillary blood flow was heterogeneous and dynamic, but questions remained as to why and how. Pericytes emerged as answers to these questions, launching several epochs of pericyte research, each characterized by new methodological advancements and fueled by controversy.

One initial controversy asked whether endothelial cells or pericytes were responsible for the observed narrowing of capillaries in frog nictitating membrane or rodent mesentery. Many groups observed focal changes in capillary diameter, but there was disagreement as to whether these changes were correlated with the location of pericytes, identified by their abluminal position on the capillary wall (17). In moving from observation to manipulation, the next wave of experiments activated mesenteric pericytes by touching them or electrically stimulating them with glass micropipettes (18). Some groups observed localized contraction when stimulating pericytes, while others did not.

With the advent of transmission electron microscopy, several groups noted electron-dense bands of filaments in pericytes that abutted endothelial cells in the brain and retina, which in some cases stained for actin, nonmuscle myosin, and tropomyosin (17). Scanning electron microscopy confirmed and elaborated on earlier drawings of mural cells, showing that mural cell morphologies in the brain and retina exist in a continuum, which serves as the basis of ongoing disagreements about how to refer to different vascular segments and their associated mural cells. As Sims (17, p. 161) said in 1986, "a continuum of cell morphologies will occur in these regions, so that discrepancies of cell names will inevitably arise."

Cell culture studies next suggested that pericytes, not endothelial cells, were contractile and responsive to known vasoactive agents (19, 20). However, the morphology of pericytes, and expression of some contractile proteins (21), changes drastically under culture conditions, making it difficult to translate these results into the in vivo functions of pericytes.

Thereafter, ex vivo studies in the retina confirmed that pericytes are excitable cells that can modulate vessel diameter over the course of minutes in response to vasoactive molecules such as angiotensin II, endothelin, and adenosine triphosphate (ATP) (22, 23). The modern era of pericyte and blood flow research was ushered in by landmark ex vivo studies showing that direct pericyte stimulation, neurotransmitters, and ischemia modulate vessel diameter at pericyte locations in the brain and retina (24). With this work, pericytes became implicated in functional hyperemia and in states of pathologically reduced blood flow such as stroke, dementia, and diabetic retinopathy. Many studies then began to investigate pericyte function and dysfunction in new ways, with new technologies including in vivo two-photon microscopy.

Despite the diverse experimental approaches and accumulating evidence, it remained unknown if pericytes were an answer to August Krogh's question: Do capillaries autonomously change their blood flow to meet metabolic demands of the tissue? However, the proliferation of new tools such as two-photon imaging, mouse models for targeting pericytes, and optogenetics permitted detailed in vivo investigations of the role of pericytes, adding new insight into a centuries-old question.

VASCULAR AND CELLULAR STRUCTURE

While there continues to be inconsistent nomenclature in the field (see 11, 25 for reviews of nomenclature variations), there is sufficient information at present to distinguish mural cell types

in different vascular zones. One goal of this review is to clarify these mural cell types as seen in rodent cerebral cortex and to propose a nomenclature that suitably distinguishes each cell type and the vascular zone it occupies. These distinctions are key because, if carefully considered and reported, they will enable different laboratories to compare and contrast their results.

The preponderance of data from physiological studies indicates there are at least four types of mural cells, which define four major microvascular zones (discussed in further detail below): SMCs on pial and penetrating arterioles, ensheathing pericytes on the arteriole-capillary transition (ACT), capillary pericytes (displaying mesh and thin-strand processes) on capillaries, and venular SMCs on ascending and pial venules (**Figure 2*a***). We also depict mesh pericytes on post-capillary venules, although it is currently unclear whether these cells represent a meaningfully distinct population. Our suggested nomenclature describes mural cell and vessel types based on their morphology and location, not their function. This is because ascribed functions may change as we learn more. For example, the terms contractile versus noncontractile pericytes become a source of confusion, because new data suggest that all pericyte types are contractile but function on different timescales (26–28).

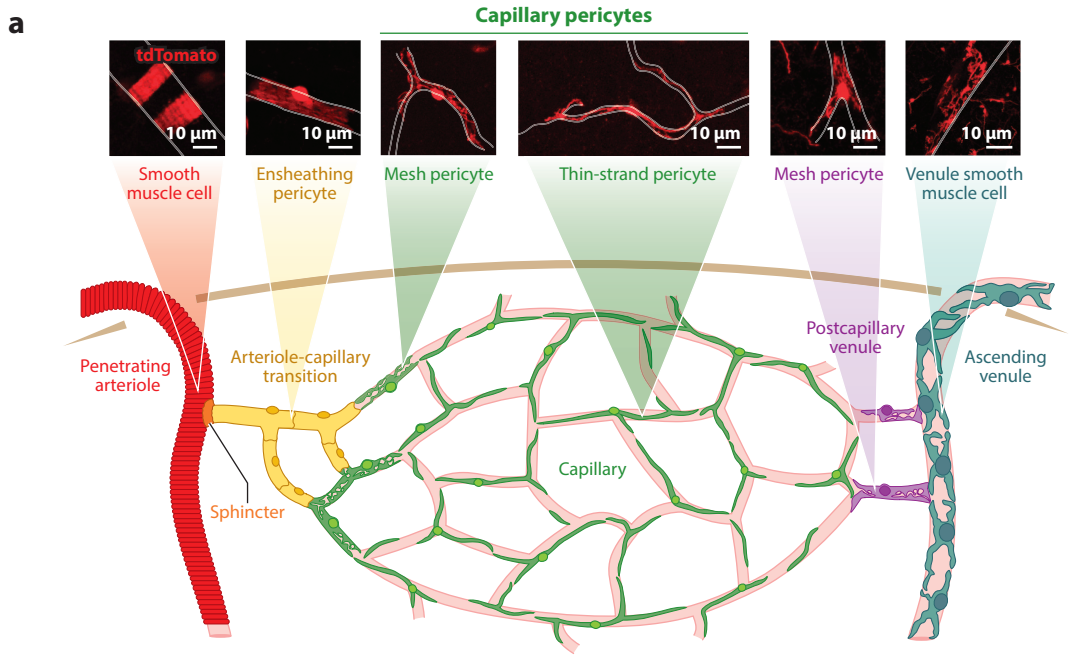
Naturally, the vascular zones described above can vary across different tissue beds. For example, the cortex has vascular structure and physiology that are distinct from those of the hippocampus (29). However, recent in vivo studies of the brain have focused on the upper layers of cerebral cortex (mostly sensory cortex), providing a common framework for discussion. In this vascular network, branching order from the cortical penetrating arteriole (denoted zero order) has been the primary means for communicating the location of microvessels within the network (**Figure 2*a*,*b***). Penetrating arterioles send small offshoots that form the entryway into the capillary bed; these offshoots are denoted first order. Thereafter, branching order increases by one at each bifurcation. When assigning a branch order to all capillaries, the most numerous are branch orders 3 to 6 (30, 31), but branch orders up to the ninth can be reliably traced during in vivo imaging (26).

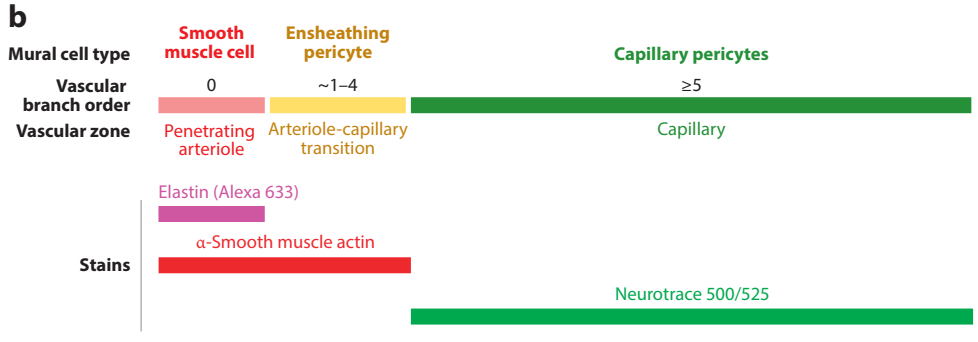
Mural cells exhibit diverse morphologies as cortical penetrating arterioles transition to capillaries (32). Penetrating arterioles are covered in concentric ring-like SMCs that are rich in α-smooth muscle actin (α-SMA; encoded by the *acta2* gene), a component of the actomyosin contractile machinery (33) (**Figure 2*a*,*b***). The penetrating arterioles also have a layer of elastin that is labeled by Alexa 633 hydrazide (34) (**Figure 2*b***). These attributes facilitate rapid and robust contraction and relaxation of the penetrating arteriole during blood flow regulation.

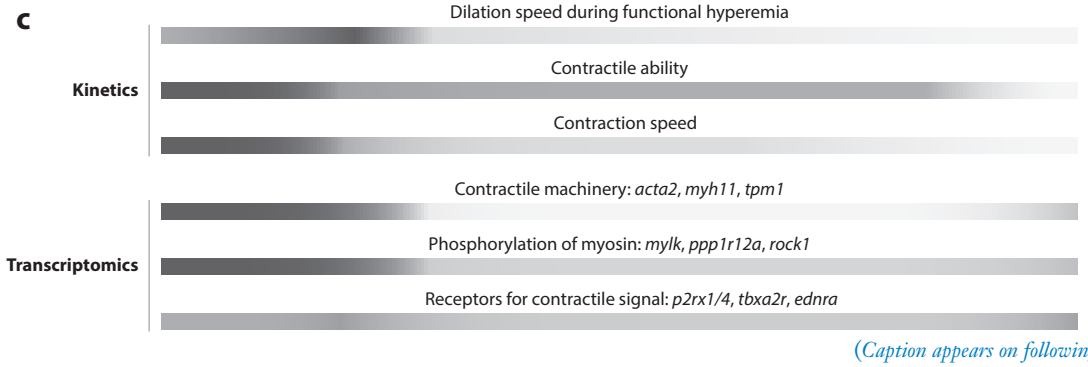
Offshoots from the penetrating arteriole and loss of the elastin layer mark the junction between the penetrating arteriole and ACT zones (**Figure 2*a*,*b***). In 20–50% of the cases, annular α-SMA-positive sphincter cells are positioned at the mouth of the offshoot (35). These sphincters maintain a localized region of constriction to buffer the capillary bed from pressure fluctuations occurring upstream. They also dilate during functional hyperemia to promote blood flow to the capillary bed. Sphincter cells have also been reported in the microvasculature of the retina (36).

Just downstream of the sphincter, the vessel is covered by ensheathing pericytes. Ensheathing pericytes express α-SMA like SMCs but possess the hybrid morphology of both SMCs and classic pericytes of the capillary bed. Similar to SMCs, their processes enwrap the entire endothelial tube and interdigitate as they run circumferential to the vessel lumen. Yet, they are elongated and exhibit protruding cell bodies like classic pericytes (33) (see the sidebar titled Nomenclature Clash).

As microvessels branch further, α-SMA expression drops abruptly to low or undetectable levels (26, 33, 37, 38). This marks the junction between the ACT and the true capillary zone and the shift from coverage by ensheathing pericyte to capillary pericytes with classic pericyte morphology. The ACT-capillary junction can occur anywhere between the first and fourth branch orders,







(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Mural cell types across the microvascular network and their contractile abilities. (*a*) Schematic showing different zones of the brain microvasculature, including the pial arteriole, penetrating arteriole, arteriole-capillary transition (ACT), capillary, postcapillary venule, ascending venule, and pial venule. The precapillary sphincter is occasionally seen at the start of the ACT zone. The appearance and names of mural cells occupying these distinct zones, as seen from sparsely labeled (NG2-tdTomato or Myh11-tdTomato) mice, are shown. (*b*) Mural cell type and corresponding vascular branch order, with small-molecule/immunohistochemical stains that aid in distinguishing the zones. (*c*) Dilatory and contractile features of mural cells differ across microvascular zones. Smooth muscle cells and ensheathing pericytes dynamically control vessel diameter. Capillary pericytes are contractile but function on a slower timescale. This may be the result of differential expression in genes that encode proteins for contractile machinery or control the phosphorylation of myosins. Key receptors for agonists that engage mural cell contraction via ATP, thromboxanes and endothelin-1, are broadly expressed in mural cells across vascular zones. The gradients are qualitative only and derived from observations of single-cell transcriptomic data reported by Vanlandewijck et al. (42). To match transcriptomic and physiological data, we make the assumption that aSMCs, aaSMCs, and PCs, as defined by Vanlandewijck et al., are SMCs of pial/penetrating arterioles, ensheathing pericytes of the ACT, and mesh and thin-strand pericytes of the capillary zone, respectively.

with larger-diameter offshoots at the first-order brain exhibiting α-SMA expression out to higher branch orders (33).

Capillary pericytes are unlike ensheathing pericytes, in part owing to a stark difference in α-SMA content. Morphologically, capillary pericytes extend thin, longer processes that incompletely cover the endothelium (thin-strand processes). Their processes can reach out to contact hundreds of micrometers of capillary length, unlike the short-range coverage of ensheathing pericytes (25, 33). As such, capillary pericyte processes usually span multiple capillary segments, and they bifurcate at capillary junctions. Closer to the ACT and in the postcapillary venule zone, capillary pericyte processes take on a mesh-like appearance, though still incompletely covering the endothelium. However, in the mid-capillary regions that are the majority of the capillary length, pericytes exhibit thin-strand processes. Mesh and thin-strand pericytes are difficult to objectively distinguish and represent a true continuum in morphologies (33).

The precise boundary between the ACT and capillary zones remains difficult to pinpoint in vivo unless labels for pericyte morphology, α-SMA, or capillary pericyte–specific dye (Neurotrace 500/525) are used (37, 39) (**Figure 2*b***). Basal vessel diameter alone is not a reliable means to delineate the vascular zones in vivo, as there is overlap in the diameters of ACT vessels and capillaries (33). In perfusion-fixed tissues, a cut-off of 7 μm in diameter has been used to categorize capillaries in mouse brain connectome data (31), which is reasonable based on studies that have considered diameter alongside α-SMA and pericyte labeling (33). However, there is the caveat that contraction of ensheathing pericytes in vivo can make ACT vessels smaller in diameter than capillaries

NOMENCLATURE CLASH

Ensheathing pericytes have been referred to by a variety of names in the literature, as has the vascular zone they occupy. They have been called precapillary SMCs, which creates the view that they are identical to concentric, ring-like SMCs of larger arterioles, or called simply pericytes, which conjures images of thin, elongated pericytes of capillaries. This is the core of the controversy in recent literature on pericytes (107). Yet, ensheathing pericytes are distinct from both SMCs and capillary pericytes in their morphology, topological position, dynamics, and gene expression, warranting an independent name. Adding more confusion, the nomenclature for the zone occupied by ensheathing pericytes has also varied (precapillary arteriole or simply capillary). Here, we support using the term arteriole-capillary transition because pericytes, by definition, cannot be located on arterioles, as this is where SMCs reside. Further, the all-encompassing term capillary for vessels of the first order and beyond obscures functional differences between the transitional and true capillary zones.

(37). Using only branch order in upper layers of cortex, one can ensure they are within the true capillary bed (>fifth order), based on studies that have carefully charted the probability of α-SMA expression as a function of branch order in mouse cortex (26, 33, 37, 38).

Ensheathing and capillary pericyte somata also vary in their locations, with somata at capillary junctions (junctional) or on intervening portions of the capillary (en passant) (32, 40). Junctional capillary pericytes are more prevalent. This may be a consequence of capillary network construction during early postnatal development, where the sprouting of new capillary branches occurs preferentially at pericyte somata (41).

In summary, we recommend the following nomenclature based on current data and use it in further discussions of mural cell physiology in vivo: SMCs on pial and penetrating arterioles, ensheathing pericytes on the ACT, capillary pericytes on capillaries, and venular SMCs on venules. Critically, this system avoids confusion between the two functionally distinct pericyte types (ensheathing and capillary) that exist downstream of the penetrating arteriole.

These mural cell types need to be reconciled with data and nomenclature from single-cell transcriptome studies, which have identified two brain mural cell classes, each containing cells within a continuum of transcriptional states (42). One class is α-SMA/*acta2* rich and is subdivided into arterial smooth muscle cells (aSMCs) and arteriole smooth muscle cells (aaSMCs). The second class is α-SMA/*acta2* low to negative and is subdivided into pericytes (PCs) and venous smooth muscle cells (vSMCs). The location of these transcriptionally identified subtypes has not been extensively mapped onto the three-dimensional vascular architecture. However, based on α-SMA/*acta2* expression, we assume that aSMCs are SMCs of pial/penetrating arterioles, aaSMCs are ensheathing pericytes of the ACT, and PCs are capillary pericytes on capillaries. This assumption allows us to begin linking mural cell physiology to cell-specific gene expression data.

DYNAMICS OF VASCULAR SEGMENTS

Vascular Smooth Muscle Cells

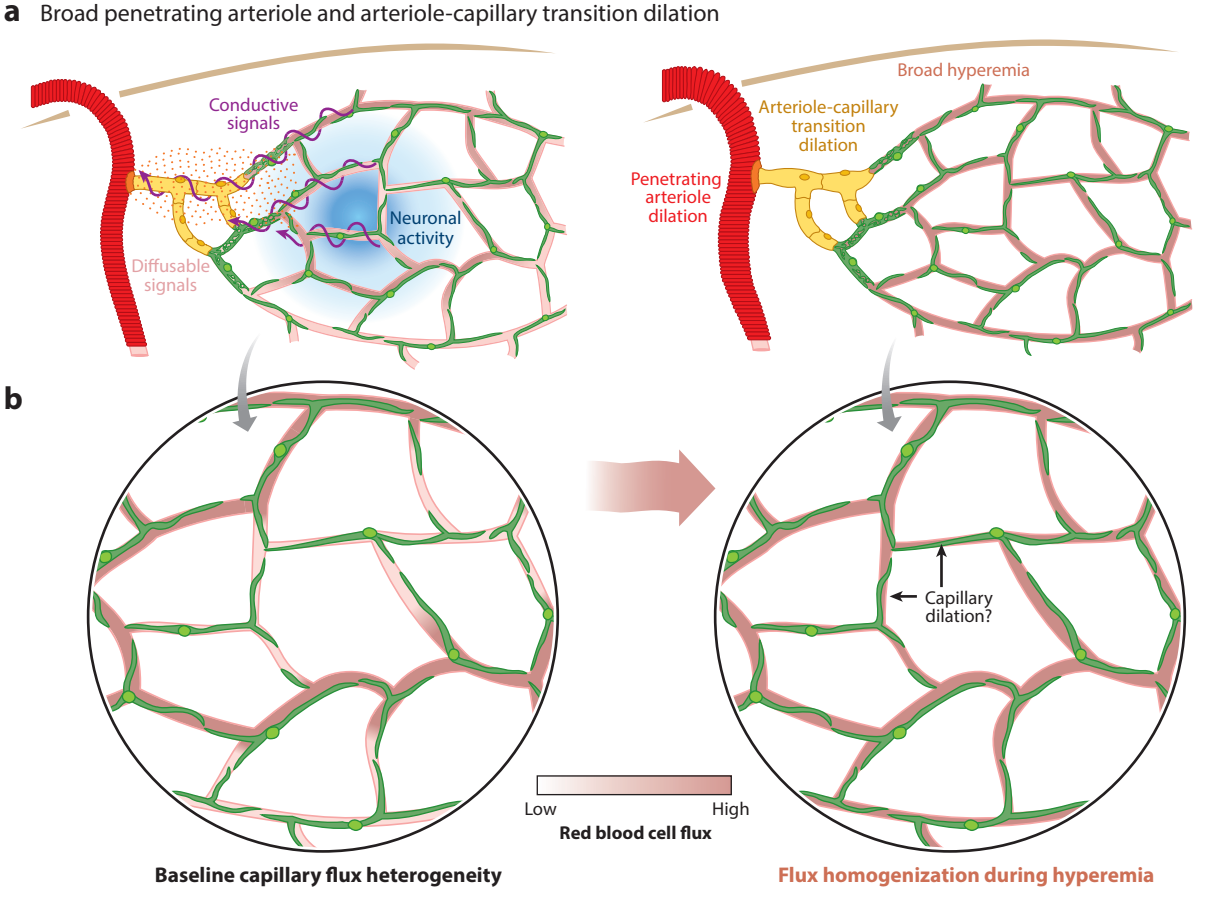
SMCs surround brain arterioles, of which there are two primary types that serve cerebral cortex: pial arterioles and penetrating arterioles. Pial arterioles sit between the arachnoid and pia mater and are distant from cells of the parenchyma. Penetrating arterioles are within the brain parenchyma and thus intimately in contact with astrocytic endfeet and the metabolites they release (43).

At baseline in awake rodents, the diameter of pial arterioles ranges between 8 and 80 μm (44, 45). Penetrating arterioles range from 6 to 40 μm, with an average ~10 μm (35, 46, 47). There are many factors governing basal diameter, such as proximity to the sourcing arteriole, blood gases, blood pressure, and vasoactive mediators released through local brain activity, such as nitric oxide from interneurons (48) and astrocyte-derived signals (49). Pial and penetrating arterioles exhibit vasomotion, where lumen diameter oscillates by about 10% at low frequency (~0.1 Hz) (37, 50). This is partly driven by slow fluctuations in γ-band power from neural activity (50) and partly by intrinsic rhythmicity of SMCs (51).

Hypercapnia is a strong vasodilatory stimulus. From their baseline diameter, pial arterioles (52) and penetrating arterioles (53) have been reported to increase in diameter by ~20% and 15%, respectively, in response to several minutes of hypercapnia. During sensory stimulation in awake mice (vibrissal stimulation), pial arterioles can dilate up to a peak of ~20% in a few seconds (average of ~10%) (44). Similarly, penetrating arterioles can increase in diameter by ~10–15% within a few seconds, in response to sensory stimuli (**Figures 2*c*** and **3*a***) (54). Thus, SMCs provide robust and rapid control of pial and penetrating arteriole diameter in vivo.

Ensheathing Pericytes

Ensheathing pericytes have emerged as important cells because they are very dynamic at rest and dilate rapidly during sensory stimulation (**Figures 2*c*** and **3*a***) (37, 55–57). The ACT zone on which they reside generally has a baseline lumen diameter range from 2 to 10 μm, with an average of



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Effect of vessel diameter change across microvascular zones. (*a*) A large region of neuronal activation leads to the production of diffusible vasodilatory signals and conductive hyperpolarization from the capillary bed. This leads to dilation of the arteriole-capillary transition (ACT), precapillary sphincter, penetrating arteriole, and pial arterioles, resulting in a relatively broad region of hyperemia. (*b*) Capillary pericytes provide tone and contribute to heterogeneity in capillary diameter and blood cell flux. During functional hyperemia, flux among capillaries homogenizes by increasing flow through initially low-flux capillaries. This process improves extraction of oxygen by redistributing blood within the capillary network. (*c*) Ensheathing pericytes positioned at vascular junctions in the ACT exhibit preferential dilation of specific daughter branches based on the direction of the arriving conductive signal. This allows for finer-tuned control of hyperemia within the territory of the penetrating arteriole.

~5 μm in vivo (26). Similar to SMCs on arterioles, the ACT exhibits vasomotor oscillations, with diameter changes on the order of 10% in the awake mouse brain (37, 39).

From their baseline state, vessels in the ACT zone have the capacity to dilate by ~25% in response to hypercapnia in anesthetized mice (26, 58). During sensory stimulation of alpha-chloralose-anesthetized mice, Hall et al. (56) showed the first-order branch within the ACT dilated by ~5%, taking ~15 s to reach peak diameter. Critically, this dilation was slightly faster than that of the neighboring penetrating arteriole, revealing that ensheathing pericytes are first to respond during the regional blood flow increase during neurovascular coupling (**Figures 2*c*** and **3*a***). Follow-up studies corroborated these findings and added that the precapillary sphincter was also highly responsive to whisker stimulation, both of which dilate slightly earlier than neighboring penetrating arterioles (35, 57). Similar observations were made in the olfactory bulb in response to a brief odor presentation (55).

Capillary Pericytes

Having firmly established that ensheathing pericytes of the ACT rapidly control blood flow during neurovascular coupling, we now direct our attention to data on capillary pericytes, which are pericytes downstream of ensheathing pericytes, with low to undetectable levels of α-SMA expression. Baseline diameters among capillaries are very heterogeneous, ~1.5–8 μm, but their individual diameters are quite stable over time (26, 59). At baseline, there is no discernible vasomotion in capillaries, in contrast to pial/penetrating arterioles and the ACT (37). The heterogeneity in capillary diameter contributes to variance in blood cell flux among capillaries within a network under basal conditions (discussed further below) (26).

With the strong stimulus of hypercapnia, capillaries can dilate by ~5–10% over the course of several minutes (26, 52, 58, 60). This dilation may not solely be a passive effect of upstream pressure, which would affect all downstream capillaries similarly. Hypercapnia produces homogenization of flow among capillaries, such that smaller capillaries show disproportionately greater dilation and increases in blood cell flux than capillaries with large baseline diameters (52, 60). Further, capillaries constrict back to baseline after hypercapnia at a slower rate than upstream vessels, suggesting independent action by capillary pericytes (26).

During sensory stimulation, studies consistently show that capillaries exhibit small and slow dilations on the order of 1–2% from baseline (37, 38, 55, 61, 62). These diameter changes are very small, which calls into question whether they are truly resolvable with in vivo two-photon microscopy. However, some recent evidence suggests that the dilations may be physiologically meaningful. Capillary pericytes (thin-strand morphology) decrease their frequency of calcium transients in response to neural activity simultaneous with small dilations, suggesting potential relaxation of a calcium-dependent contractile machinery (55, 63). Further, a small dilation of capillaries was necessary to achieve a full functional hyperemic response, according to modeling studies (55). In silico studies also suggest that small capillary dilations produce hemodynamic responses that are much more tightly localized near neural activity than scenarios where capillaries are rigid (64).

PERICYTES AND AUTOREGULATION

There is some evidence for autoregulation or compensatory dilation at the capillary level. Stenosis of upstream vessels in atherosclerosis (108) and mechanical carotid artery occlusion (109) models produces dilation of capillaries. These may correlate with improved collateral flow that has been observed over time in humans with stenosis of intracranial arteries (110).

Thus, capillaries exhibit small and delayed dilations during neurovascular coupling, and this change may be permissive for blood cell passage during neurovascular coupling (**Figure 3*b***). However, the existing experimental data fall short of confirming an active role of capillary pericytes in local blood flow control during functional hyperemia. This is an important open question, as small dilations could exert a large effect when multiplied across many capillaries, particularly if they occur at high resistance points within the capillary network (see the sidebar titled Pericytes and Autoregulation).

POTENTIAL CONTRACTILE MACHINERY IN CAPILLARY PERICYTES

Single-cell transcriptomic studies indicate that capillary pericytes express receptors for vasoactive mediators (discussed further below), but they also express some genes involved in SMC actomyosin contraction, such as myosin (*myh11*, *myl9*, *myh9*), regulators of the myosin phosphorylation state (*mylk* and *ppp1r12a*, *rock1*), and L-type voltage-gated calcium channels (*cacna1c*) (42) (**Figure 2*c***). However, smooth muscle actin transcripts (acta1, acta2, actg2, actc1) expected to bind myosin proteins are low to undetectable in expression. Conceivably, low α-SMA content may still be sufficient for contractility via canonical actomyosin cross-bridging and may explain the slower kinetics of capillary pericytes. Recent studies in the retina showed that immunohistological detection of low α-SMA may be enhanced by special fixation procedures, and blockade of α-SMA expression limits the contraction of retinal capillary pericytes (65, 66). It is also possible that weak actomyosin contraction is coupled to F-actin polymerization, known to promote constriction in SMCs through RhoA-Rho kinase signaling.

CONDUCTIVE RESPONSES

Active neurons in the brain parenchyma receive increased blood flow from arterioles that may be located hundreds of micrometers away. Recent studies demonstrate that capillary endothelial cells detect neural activity and communicate the need for increased blood flow to arterioles upstream (**Figure 3*a***). Longden et al. (67) reported that extracellular K^+, released as a by-product of neural activity, leads to endothelial hyperpolarization through the activation of inward rectifier potassium family, subtype 2 channels (Kir2). Endothelial cells conduct this hyperpolarization upstream, from capillaries to the ACT zone, where electrical transfer through myoendothelial junctions leads to rapid vasodilation (**Figures 3*a*,*c*** and **4*b***).

In parallel, neurons also activate endothelial transient receptor potential ankyrin 1 (TRPA1) channels, a pathway that conducts hyperpolarization to upstream arterioles more slowly than Kir2-mediated signals (68). The slower kinetics are likely due to intermediate steps that involve TRPA1 activation of endothelial pannexin (Panx1) proteins, which release ATP that then activates calcium-permeable P2X receptors on neighboring endothelial cells. In these capillary-to-arteriole signaling mechanisms, the capillary bed functions as an extensive sensory web to detect and transmit neuronal signals during neurovascular coupling (**Figure 4*b***).

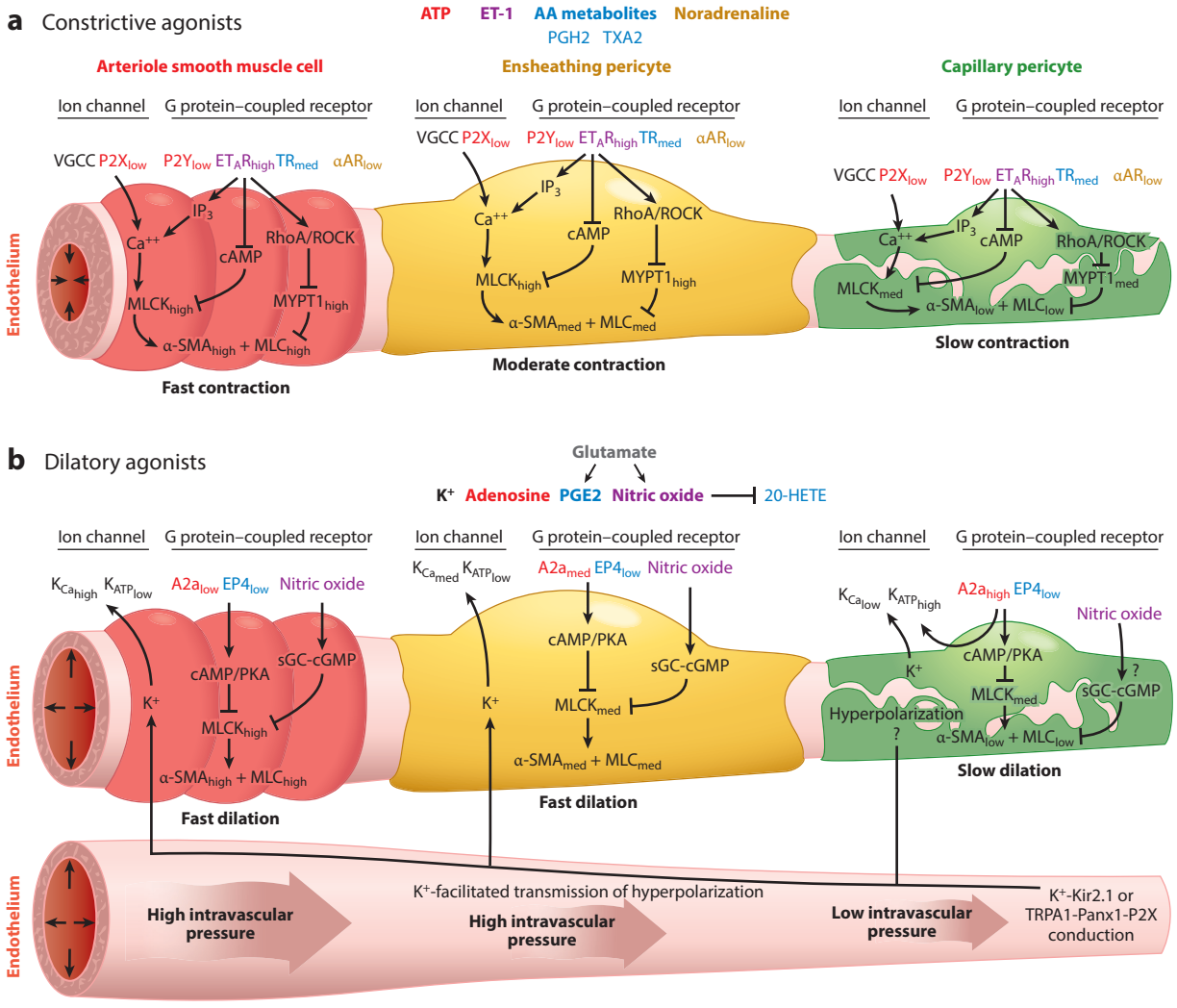


Figure 4

Signaling and contractile machinery leading to different dynamics across vascular zones. (*a*) Schematic depicting how vasoconstrictive agonists act on mural cells across microvascular zones, simplified to discuss arteriole (SMC), ACT (ensheathing pericyte), and capillary zones (capillary pericyte). The ion channel or G protein–coupled receptors for the agonists are expressed at varying levels in these mural cells (based on single-cell transcriptomic data), and the qualifiers low, med, and high are appended as subscripts to each protein to show general expression level. Intracellularly, the expression levels of actomyosin contractile machinery components also vary across mural cell types. The contractile capacity of each mural cell type is based on observations from pharmacological studies and in vivo cell-specific optogenetic manipulations (26). (*b*) Dilatory agonists and their receptors with likely pathways leading to relaxation of the mural cell. Endothelial conductive hyperpolarization from capillary-to-arteriole promotes relaxation of upstream mural cells via transmission of hyperpolarization through myo-endothelial junctions. The speed of dilation is, in part, driven by intravascular pressure at each vascular zone. Abbreviations: A2a, A2a purinergic receptor; AA, arachidonic acid; ACT, arteriole-capillary transition; α-AR, α adrenoreceptor; α-SMA, α-smooth muscle actin; ATP, adenosine triphosphate; cAMP/PKA, cyclic AMP-protein kinase A; EP4, prostaglandin E_2 receptor 4; ET-1, endothelin-1; ET_AR, endothelin A receptor; IP_3, inositol 1,4,5-trisphosphate; K_{ATP}, ATP-sensitive K^+ channel; K_{Ca}, calcium-activated K^+ channel; Kir2.1, inward rectifying K^+ channel 2.1; MLC, myosin light chain; MLCK, MLC kinase; MYPT1, myosin light chain phosphatase 1; Panx1, endothelial pannexin; P2X, P2X purinoreceptor; P2Y, P2Y receptor; PGE2, prostaglandin E_2; PGH2, prostaglandin H_2; RhoA/ROCK, RhoA-Rho kinase; sGC-cGMP, soluble guanylyl cyclase–cyclic guanosine monophosphate; TR, thromboxane receptor; TRPA1, transient receptor potential ankyrin 1; TXA2, thromboxane A2; VGCC, voltage-gated calcium channel.

Pericytes are known to express gap junction proteins (Cx37 and Cx45), and new evidence in the retina suggests that pericytes utilize them to communicate with endothelial cells, astrocytes, and other pericytes (69–71). There is also a clear anatomic basis for pericyte-endothelial contact, as pericytes and endothelial cells form interdigitating peg-and-socket junctions that have recently been demonstrated with three-dimensional electron microscopy techniques (72). Interestingly, there is a directional bias in gap junction–mediated dye transfer from capillary pericytes toward upstream arterioles, further suggesting a role of pericytes in communicating dilatory signals to upstream arterioles (73).

A recent study showed that ensheathing pericytes may preferentially gate flow down specific ACT branches, based on the direction of the incoming hyperpolarizing signal (40). By dilating just one branch at a time, ensheathing pericytes provide a more economical means of regulating blood flow when compared to the dilation of the whole penetrating arteriole (**Figure 3*c***).

CAUSE-AND-EFFECT STUDIES OF BLOOD FLOW CONTROL BY CAPILLARY PERICYTES

To clearly dissect the role of capillary pericytes, new approaches were needed for selective pericyte manipulation in vivo independent of the ACT and arteriole zones. In recent years, three cause-and-effect approaches have been implemented to study capillary pericytes: single-cell optogenetics, single-cell optical ablation, and genetic ablation using diphtheria toxin.

Hill and colleagues (37) were the first to apply optogenetics to stimulate mural cells in vivo, focusing on the question of which vascular zones were contractile and could contribute to functional hyperemia. These studies expressed the H134R variant of channelrhodopsin-2 (ChR2) in all mural cells and used two-photon ChR2 excitation to stimulate mural cells of specific zones in a spatially restricted manner. They discovered that arteriolar SMCs contracted readily when stimulated. However, capillary pericytes produced no change in capillary diameter with similar stimulation over tens of seconds, leading to the conclusion that they were incapable of regulating local blood flow in capillaries at the timescale of SMCs and ensheathing pericytes (called precapillary SMCs in their study).

Hartmann et al. (26) reassessed the contractile ability of capillary pericytes using the same approach. They found that capillary pericytes were indeed contractile but required longer and more intense stimulation owing to their slower dynamics. Their contraction led to reductions in blood cell velocity and flux. Further, the slow dynamics of capillary pericytes were inhibited by the vasodilator fasudil, suggesting that capillary pericytes use a form of contractile machinery similar to SMCs. Similar probing of upstream α-SMA-positive ensheathing pericytes and SMCs led to rapid contractions, consistent with the findings of Hill et al. (37). Additionally, a separate study by Nelson et al. (27) expressed ChR2 in capillary pericytes with a cell-specific Cre driver (pericyte-CreER) and also found a slow contraction of pericytes in response to ChR2 activation.

Demonstrations that capillary pericytes could contract in vivo were complemented by studies showing that optical ablation of capillary pericytes led to vasodilation. Berthiaume et al. (25) optically ablated capillary pericytes in the cortex of adult mice, finding that it led to the consistent dilation of regions lacking pericyte contact. This dilation resulted in aberrantly high levels of blood cell flux in the uncovered capillary (26). Capillary tone was regained once pericyte contact was reestablished by the growth of neighboring pericytes. This effect was not restricted to laser-induced pericyte loss, as capillary dilation was seen in response to pericyte loss in a model of epileptic seizure (74). In this model, capillary tone was regained with gradual pericyte remodeling and endothelial contact, and also after accelerated remodeling through exogenous administration of platelet-derived growth factor BB (PDGF-BB). A recent study in the retina showed that the

halorhodopsin-induced hyperpolarization of capillary pericytes specifically could induce blood cell flux increase, though zone-specific dynamics were not compared (36). Collectively, these data provide strong evidence that capillary pericytes are necessary and sufficient for basal capillary tone in vivo. This contractile ability also suggests a role in slow modulation of capillary diameter, though their specific contributions to blood flow changes during physiological stimuli still remain unclear.

Using a genetic strategy, Nikolakopoulou et al. (75) examined cerebral blood flow changes in response to the global ablation of capillary pericytes. Here, capillary pericytes were ablated en masse using pericyte-CreER mice crossed with diphtheria toxin receptor–expressing mice. Extensive pericyte loss was observed and accompanied by a marked decrease in cerebral blood flow, most likely due to cerebral edema and tissue swelling. However, whether contractile tone of capillary pericytes changed in vivo was difficult to ascertain with the larger scale of injury. Global pericyte ablations and optical single pericyte ablations lie at two ends of the spectrum and provide insight into how different scales of pericyte loss can affect cerebral hemodynamics.

CAPILLARY HETEROGENEITY

As briefly discussed above, capillary flow is heterogeneous at rest, which provides reserve space for the redistribution of blood in capillary networks during functional hyperemia (76). As blood flow increases, the flux among capillaries homogenize (**Figure 3*b***). This mechanism has been strongly supported by in vivo imaging studies, which have revealed that flux among capillaries homogenizes with sensory-evoked functional hyperemia (61, 77–80). In particular, low baseline flux capillaries rapidly increase in flux, while high baseline flux capillaries tend to decrease slightly in flux. This homogenization of flow is expected to improve the efficiency of oxygenation extraction from the blood, and this concept has been described theoretically (81) and shown empirically (82).

The homogenization of capillary blood flow implies an active mechanism whereby pericytes covering small diameter capillaries act differently than those on large diameter capillaries. This mechanism may work in concert with increased compliance of red blood cells that carry more oxygen (38), with the end goal of promoting blood cell passage through the smallest diameter capillaries of the network during functional hyperemia.

Related to these findings from sensory cortex, recent in vivo imaging studies in the retina show that some pericytes link neighboring capillaries together with tunneling nanotubes (71). These so-called bridging pericytes selectively increase flow in one capillary while decreasing flow in others. The capillary diameter changes associated with these flow changes in the retina are also small, averaging 1–2% from baseline, similar to those described in cortex.

VASOACTIVE MEDIATORS KNOWN TO ACT ON PERICYTES

Although cause-and-effect studies define the capabilities of pericytes in vivo, they do not inform us about the physiological drivers that engage pericytes. An excellent review from Hariharan et al. (83) explored the battery of ion channels and G protein–coupled receptors expressed by pericytes using single-cell transcriptomic data from mouse brain. This revealed numerous signaling mechanisms through which pericytes might interact with their environment. While many pathways remain to be clarified by rigorous experimental studies, some of the findings reinforce more than a decade of research identifying vasomediators of pericyte contraction and dilation, as reviewed in detail by Hamilton et al. (6). We begin by discussing vasoactive signals that predominately lead to vasoconstriction of pericytes (**Figure 4*a***).

ATP

ATP is released by many cell types and has well-established effects on the tone of arteries and arterioles, both constrictive and dilatory. Application of ATP to isolated whole retina induces the contraction of pericyte somata around capillaries (24, 84). Direct injection of ATP into the brain parenchyma leads also to potent vasoconstriction in the ACT zone, with slower and more prolonged responses occurring with higher branch orders (57). ATP injection in vivo also led to delayed vasodilation, possibly because of metabolism of ATP to vasodilator adenosine (57). ATP may act directly on purinergic receptors expressed by pericytes, and transcriptomic data confirm the expression of P2X1 (*p2rx1*) and P2X4 (*p2rx4*) by some but not all sampled ensheathing and capillary pericytes (42). The activation of these receptors would cause flux of Na^+ and Ca^{++} into the cell, initiating cell depolarization and engagement of contractile machinery. Recent studies showed robust calcium increases in capillary pericytes, consistent with direct action on these cells (63).

The most immediate source of ATP to brain and retinal pericytes would be the adjacent astrocytes, and activity in retinal astrocytes is known to cause ATP release (85). Inhibition of P2X1 in vivo blocks sensory-evoked dilation consistent with ATP involvement in neurovascular coupling (86). Application of the P2Y receptor agonist, UTP, can also cause contraction of pericytes (24). UTP reacts with glucose to produce UTP-glucose, which is an agonist for the P2Y14 receptor (*p2ry14*) that is highly expressed by brain capillary pericytes. Activation of P2Y14 may lead to vasoconstriction through a decrease in cAMP production via G protein signaling. As discussed above, TRPA1 activation in endothelial cells also causes ATP release, which may act on nearby pericytes and initiate local contraction or conductive responses (68).

Noradrenaline

The superfusion of noradrenaline on brain slices leads to robust contraction of pericytes (24). In vivo, noradrenaline levels in the forebrain are modulated by the locus coeruleus, and its vasoconstrictive effect is important for coordination of blood distribution during neurovascular coupling (87). Locus coeruleus projections form varicosities around brain capillaries (in addition to arterioles) but do not appear to directly innervate pericytes (88). Further, adrenoceptor gene expression (*i.e.*, *adra1a*) is very low in capillary pericytes based on single-cell transcriptomic data (42, 83). This suggests that noradrenaline may act on other cell types such as astrocytes that then secondarily release constrictive signals to pericytes.

Arachidonic Acid Derivatives

The thromboxane receptor A2 gene (*tbxa2r*) is strongly expressed by most capillary and ensheathing pericytes, and various arachidonic acid metabolites are endogenous agonists of the receptor. Astrocytes secrete arachidonic acid that can be metabolized by cyclooxygenase 1 to produce prostaglandin H2 (PGH2), which can be further processed into thromboxane A2, a potent thromboxane receptor agonist that can cause pericyte contraction. The stable synthetic analog of the endoperoxide prostaglandin PGH2, U46619, effectively contracts pericytes and is commonly used to create basal capillary tone in brain slice studies (86). In vivo superfusion of U46619 onto the cerebral cortex causes slow contraction of capillary pericytes (28) and an increase in intracellular calcium (63). In the retina, application of U46619 leads to marked contraction of ensheathing pericytes but slow contraction of capillary pericytes. This slow contraction was blocked with inhibitors of actin polymerization (cytochalasin D and latrunculin B), again indicating a slow-acting contractile mechanism in capillary pericytes (40). Arachidonic acid can also be processed by the

cytochrome P450 ω-hydroxylase pathway to produce 20-HETE, which is also a thromboxane receptor agonist that may modulate capillary pericyte tone. Thus, broad signaling pathways initiated by arachidonic acid metabolism can potently contract capillary pericytes.

Endothelin-1

Both ensheathing pericytes and capillary pericytes express high levels of endothelin receptor A (*ednra*) and contract robustly in response to treatment with endothelin-1 (ET-1), a potent vasoconstrictive peptide expressed by endothelial cells and other cell types (22). A recent study showed that the precapillary sphincter and ensheathing pericytes (first- to third-order branches) were most responsive to intraparenchymal delivery of ET-1 (89). Endothelin receptor signaling results in the opening of intracellular Ca^{++} stores and Ca^{++} influx through membrane channels, which activates the Ca^{++}-dependent contractile machinery in capillary pericytes (63). Studies also suggest that ET-1 inhibits ATP-sensitive K^+ (K_{ATP}) channels, which would prevent their ability to hyperpolarize the cell and promote dilation (84). Prolonged exposure to ET-1 leads to a potential pathological condition that diminishes gap junction connectivity between pericytes (84).

Pathways to Vasodilation

Vasoactive signals leading to pericyte dilation have also been identified (**Figure 4*b***). The major excitatory neurotransmitter glutamate causes dilation of capillaries when superfused onto brain slices after capillaries had been partially constricted by noradrenaline. A series of elegant pharmacological studies by Attwell and colleagues (56, 86) concluded that glutamate likely activates arachidonic acid metabolism in astrocytes or neurons to produce prostaglandin E_2 that then activates prostaglandin E_2 receptor 4 (EP4) receptors on pericytes to promote vasodilation. Single-cell transcriptomic data suggest moderate and heterogeneous expression of EP4 receptor mRNA (*ptger4*) among capillary pericytes. Intracellularly, EP4 signaling may promote vasodilation by increasing cAMP production and protein kinase A activation, which in turn inhibits myosin light chain kinase activity. Further, inhibiting 14,15-EET signaling, which also acts via EP4 receptors, causes vasoconstriction and blunts functional hyperemia in vivo (90). Alternatively, glutamate-induced nitric oxide production inhibits 20-HETE production to promote dilation by removing a constrictive signal (86).

Adenosine may also cause pericyte relaxation by acting through A2a purine receptors to promote ATP-K_{ATP} channel flux (91). The gene encoding the A2a purine receptor (*adora2*) is expressed more in capillary and ensheathing pericytes than in SMCs (see the sidebar titled Comparing Apples and Oranges: Kinetics of Dilation and Constriction).

COMPARING APPLES AND ORANGES: KINETICS OF DILATION AND CONSTRICTION

Dilation is the passive process of losing contractile tone, and the speed of dilation is therefore also driven by intravascular pressure experienced at each vascular zone. Pressure is higher at arteriole and ACT zones, enabling fast dilation, but pressure is lower in capillaries, leading to slow dilation. Optogenetic studies that have selectively contracted different mural cells types in vivo give insight into their individual contractile kinetics (26). The ACT dilates rapidly and robustly in response to neurovascular coupling, but it contracts more weakly than upstream arterioles. Thus, speed of contraction does not infer dilation speed.

K_{ATP} Channels

A striking observation from single-cell transcriptomic data is that the ion channel repertoire expressed by capillary pericytes is dominated by proteins for K_{ATP} channels. Both subunits for the channel, i.e., inward rectifier (Kir) subunit Kir6.1 (*kcnj8*) and sulfonylurea receptor 2 (*abcc9*) protein, are highly expressed. The role of pericyte K_{ATP} channels remains to be clarified, but their contribution to membrane hyperpolarization might lead to local pericyte dilation. Consistent with this, recent work by Zambach et al. (89) showed that the K_{ATP} channel opener, pinacidil, evoked vasodilation, with strongest responses at the precapillary sphincter and ACT. In contrast, blockade of K_{ATP} channels with PNU37883 had no effect on basal vessel diameter, but it did blunt vasodilation evoked by sensory stimulation. Although these drugs exert their greatest effects at the ACT, they may also act at the capillary zone and influence conduction of hyperpolarizing signals. Endogenously, K_{ATP} channel conductance is dependent on intracellular ATP levels, with lower levels leading to channel opening and potassium efflux. This raises the intriguing hypothesis that the reduction in pericyte ATP content during brain activity increases K_{ATP} channel conductance, leading to pericyte relaxation (83).

PERICYTE PATHOLOGY AND CEREBRAL BLOOD FLOW DEFICITS IN DISEASE

Stroke

Pericyte loss and dysfunction are mechanisms of blood flow impairment in the acute stages of stroke. Pericytes contract in rigor during exposure to ischemia (24) and clamp down onto the capillary lumen, limiting blood cell passage. This can initiate during ischemia and persist even after successful recanalization of the artery (92). Pericytes die as a result of excitotoxic injury (56) and also injury caused by oxidative/nitrosative stress (92). Sustained contraction is also observed at the level of ACT, where ensheathing pericytes contract just prior to entry to the capillary bed (37). Spreading depolarization alone, which can occur in the acute phase of stroke, was also shown to cause lasting constrictions in the ACT zone (93).

In the stroke penumbra, recent studies have shown increased incidence of capillary flow stalls, which contribute to impaired perfusion and oxygen delivery. While these flow stalls have been ascribed to adhesion of neutrophils, aberrant pericyte contractions may heighten capillary plugging (94, 95). Spontaneous deep intracerebral hemorrhage is a devastating subtype of stroke involving leakage of blood into deep brain regions. A recent study using a mouse model of intracerebral hemorrhage revealed specific changes at the ACT, where ensheathing pericytes proliferate and hypermuscularize the vessel (96). This leads to the buildup of upstream pressure from arterioles increasing likelihood of vascular rupture.

Dementia

In protracted diseases such as Alzheimer's disease (AD) and vascular contributions to cognitive impairment and dementia, pericyte loss has been widely reported (97–99) and is predictive of cognitive decline in *APOE4* carriers (100). Patients with AD have 25–50% fewer pericytes compared to age-matched controls (98). Pericyte pathology is also seen in CADASIL, a monogenic form of vascular dementia primarily affecting arterioles and capillaries (101). A series of elegant studies from Zlokovic and colleagues on *APOE4* and MMP9-CypA signaling have linked pericyte pathology in AD to blood–brain barrier disruption (102, 103), which has been confirmed by both postmortem histology and in vivo MRI (100). The gradual pericyte loss seen in AD likely perturbs brain capillary flow, but the basis of this disease mechanism is less well understood. Recently, Nortley et al. (104) showed in rodent and human tissues that Aβ-induced reactive oxygen

species promote the release of ET-1, which binds to endothelin receptor A on pericytes, leading to aberrant sustained contraction. The death of pericytes and loss of endothelial coverage may lead to heterogeneous capillary dilation, altered capillary flow patterns, and the maldistribution of blood flow and tissue oxygenation. Increased capillary flow heterogeneity would also raise the threshold for capillary flux homogenization, further impairing supply of oxygen and nutrients at times of increased metabolic demand. Consistent with this concept, MRI studies have reported altered capillary flow patterns in individuals with AD (105).

Epilepsy

SMCs and pericytes are lost during seizure and replaced over time through mural cell remodeling. This leads to the reorganization of mural cell coverage and changes in basal vascular tone, which also alters capillary flow patterns (74). Treatment with PDGF-BB, a signal for pericyte migration and proliferation, improves mural cell coverage. In a separate study, seizure activity was shown to associate with local constriction of pericytes and tissue hypoxia in hippocampus (106).

SUMMARY POINTS

1. The gene expression profile, cellular composition, and physiological roles of distinct microvascular zones in the brain are active areas of research and of central importance to cerebral blood flow regulation under basal and active states.
2. The arteriole-capillary transition (ACT) and capillary zones are both covered by pericytes, but they have distinct physiological roles in blood flow control. Ensheathing pericytes of the ACT dynamically gate blood flow into the capillary bed, while the slow dynamics of capillary pericytes contribute to basal capillary heterogeneity and perhaps flux homogenization during functional hyperemia.
3. Cause-and-effect optogenetic manipulations were used in vivo to confirm that capillary pericytes are sufficient to reduce capillary diameter but are slow in their kinetics. Optical ablation of capillary pericytes confirmed that they are essential for the maintenance of basal capillary tone.
4. The slow kinetics of capillary pericytes may be explained by lower contractile protein expression. However, like smooth muscle cells (SMCs) and ensheathing pericytes, capillary pericytes express many of the receptors bound by vasoactive mediators, indicating that pathways to engage contractile tone in vivo are present.
5. Aberrant pericyte contraction during neurological disease can impair cerebral blood flow. Loss of pericyte coverage can also have negative effects on cerebral blood flow by increasing capillary heterogeneity, resulting in the maldistribution of blood flow and oxygen.

FUTURE ISSUES

1. Recent studies have clarified the contractile ability and kinetics of the two major pericyte subtypes. However, the in vivo signals that lead to the vasodynamic changes remain unclear. What are the molecular contributors to basal capillary tone, capillary heterogeneity, and neurovascular coupling?

2. How do pericytes communicate in vivo with other brain cells in the neurovascular unit to regulate processes such as conductive signaling and capillary flux homogenization during neurovascular coupling?
3. Inconsistent semantics continue to be a hindrance in studying mural cells and vascular zones. Here, we clarify the morphology, function, vascular location, and gene expression of mural cell subtypes as they pertain to physiology studies and suggest nomenclature to distinguish mural cells and the zones they occupy in cerebral cortex. Importantly, vascular zones can differ in tissues other than cerebral cortex, warranting separate characterization.
4. A difference in contractile kinetics is apparent between mural cell types (26), and this is reflected in differing transcriptomes between SMCs, ensheathing pericytes, and capillary pericytes (42). It is unknown which contractile proteins are important for capillary pericyte contraction, given that their expression of α-smooth muscle actin transcript (*acta2*) is low to undetectable.
5. How can we leverage our knowledge of pericytes to alleviate neurologic disease? For example, restoring pericyte coverage with exogenous PDGF-BB in a mouse model of epilepsy (74) reduced seizure burden, and preventing aberrant pericyte contractions by amyloid beta was achieved with C-type natriuretic peptide (104). These are promising strategies that may have beneficial effects in other diseases.

DISCLOSURE STATEMENT

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