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The Cells of the Vasculature: Advances in the Regulation of Vascular Tone in the Brain and Periphery

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ABSTRACT

The vasculature is a complex tissue in which multiple cell types coordinate the regulation of tissue perfusion in response to hemodynamic and biochemical signals. Advances in this field are continuing to deepen our understanding of the relative importance of these cell types through the body. In the peripheral vasculature, tone is generated primarily by smooth muscle cells and regulated by endothelial cells, and neurons. In the brain parenchyma, unique cell types including pericytes, perivascular astrocytes and microglia, also contribute to the regulation of arterial and capillary tone. Here, we provide a cell-by-cell review of the regulation of vascular tone and highlight recent advances in the regulation of vascular tone in both the periphery and cerebral vasculature.

1 | Introduction

Permeating every organ, the blood vasculature performs a diverse array of vital functions throughout the body. Perhaps the most fundamental of these is the delivery of oxygen and nutrients as well as the removal of carbon dioxide and waste from the tissue. This requires precise control of vascular tone to ensure each tissue is adequately perfused to meet its metabolic demand.

Vascular tone comprised both passive and active components. Passive tone, also referred to as arterial stiffness or elasticity, is dependent on extracellular matrix composition and is a critical component of dampening pulse waves generated by the heart to provide steady blood flow to the tissue. Changes in passive tone require remodelling of the extracellular matrix and therefore cannot be rapidly adjusted to meet the demands of the tissue. Active tone, however, is dictated by the degree of mural cell constriction and undergoes acute changes to regulate vascular resistance and tissue perfusion.

To meet the specific demands of each tissue, the pathways and signalling molecules that regulate active tone vary from organ to organ. Despite this, the cell types that regulate tone are largely the same in each tissue (e.g. smooth muscle cells [SMCs], endothelial cells [ECs] and neurons) with the primary exception being the cerebral vasculature. Here, recent work has demonstrated a fundamental role for astrocytes and microglia in the maintenance of physiologic tone in arteries and capillaries, respectively (Figure 1). In this review, we will discuss the cell types and their interactions that regulate vascular tone in the peripheral and cerebral vasculature as well as highlight recent advances in each of these fields. For a more comprehensive review of the biochemical pathways in these cell types and how they differ throughout the body, we suggest the following reviews [1–4].

Summary

- The vasculature regulates blood flow through the regulation of vascular tone, and advances in this field have deepened our understanding of how the regulation of vascular tone is coordinated between different cell types.
- Throughout the body, vascular tone is generated primarily by smooth muscle cells and is regulated by endothelial cells, and neurons.
- In the brain, unique cell types including mural pericytes, perivascular astrocytes and microglia, also regulate blood flow.
- Here, we provide a cell-by-cell review of the mechanisms that regulate vascular tone and highlight recent advances in the regulation of vascular tone in both the periphery and cerebral vasculature.

2 | Mural Cells

While there are instances of pericytes regulating blood flow in the periphery [5], it is primarily the SMCs in arteries and arterioles that regulate vascular tone and tissue perfusion. However, in the brain parenchyma, penetrating arterioles, precapillary arterioles and higher-order capillaries have all been implicated in the modulation of resting basal tone and vascular resistance.

Relative to pericytes, SMCs are capable of generating fast-acting, high-amplitude contractions and therefore contribute substantially to the regulation of vascular tone. Importantly, in resistance arteries (< 200 mm in diameter), SMCs directly sense increases in intravascular pressure and contract against it to maintain proper perfusion and protect the downstream capillary bed from damaging pressures [2, 6]. This myogenic response also provides a degree of basal tone allowing for resistance to be modulated by both vasoconstricting and vasodilating signalling pathways.

Several mechanisms have been proposed to mediate the sensing of intraluminal pressure including mechanosensitive ion channels, mechanosensitive G protein–coupled receptors (GPCRs), integrins and matrix metalloproteases [2]. The exact mechanisms and channel isoforms vary from tissue to tissue, but ultimately these pathways lead to SMC depolarisation and calcium influx via the activation of transient receptor potential (TRP) channels, L-type calcium channels, calcium-activated chloride channels and calcium-dependent potassium channels [2, 6]. Of particular importance is the opening of voltage-gated calcium channels—such as $Ca_{V1.2}$ that allows for large increases in intracellular calcium. Subsequently, calmodulin binds to and activates myosin light-chain kinase (MLCK) initiating actin/myosin interaction, cross-bridge cycling and cell shortening [2, 7]. Both large-conductance calcium-activated potassium channels (BK_{Ca}) and voltage-gated potassium channels act as negative feedback mechanisms to limit the myogenic response. Importantly, increased calcium sensitivity occurs over pressures ranging from 60 to 140 mmHg allowing for changes in SMC contractility despite moderate increases in calcium [6].

While SMCs are almost exclusively responsible for the regulation of tone in the periphery, pericytes contribute substantially to the regulation of tone in in the brain allowing the redirecting of blood flow to areas of high demand without increasing total tissue blood flow that would increase intracranial pressure [5]. The relative contributions of SMCs and pericytes in the brain continue to be thoroughly debated and is partially obfuscated by the terminology. By fluorescently labelling cells using an NG2-Cre, Hill et al. [8] was the first to provide a thorough characterisation of morphologically distinct mural cells along the cortical vascular tree (Figure 2). Pial and penetrating arterioles with diameters ranging from 15 to 40 μm possessed mural cells that enwrapped the entire circumference of the vessel wall with narrow bands, thus having a classical SMC morphology. Spatially isolated cells with clear soma yet similar circumferential band-like morphology were additionally observed on distal vessels with diameters ranging from 3 to 15 μm. These cells were found to express α-smooth muscle actin (αSMA) from the first branch-order up to the third branch order. Similar observations were made in human cortex. Cells beyond the third branch order on

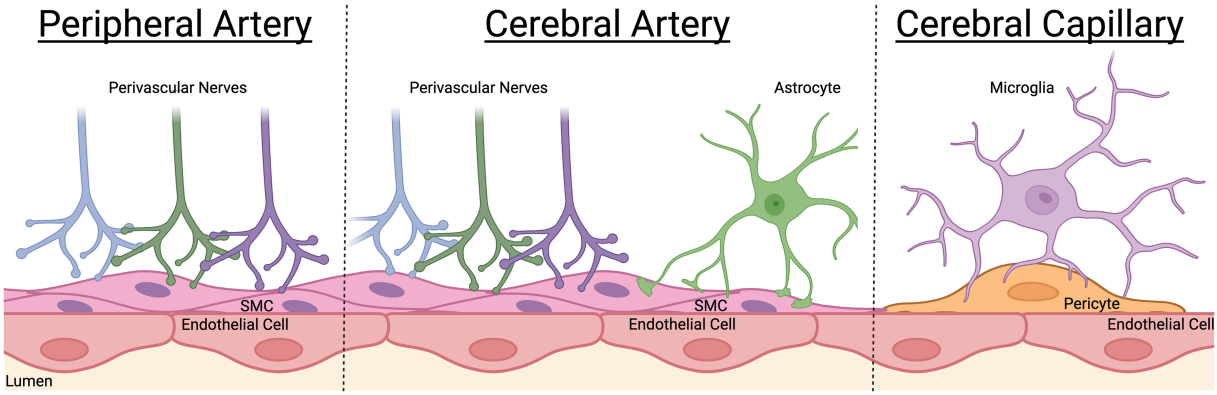

FIGURE 1 | Cell types involved in the regulation of vascular tone. In the peripheral vasculature, vascular tone is primarily generated by smooth muscle cells (SMC), which receives vasodilating and vasoconstricting signals from endothelial cells (ECs) and perivascular nerves (sympathetic, parasympathetic and sensory). In the brain, vascular tone is regulated by SMCs in arteries and pericytes in capillaries. Vascular tone in arteries is regulated by ECs, perivascular nerves and astrocytes. In the brain, tone is also tightly regulated at the capillary level which is generated by pericytes and regulated by ECs and microglia.

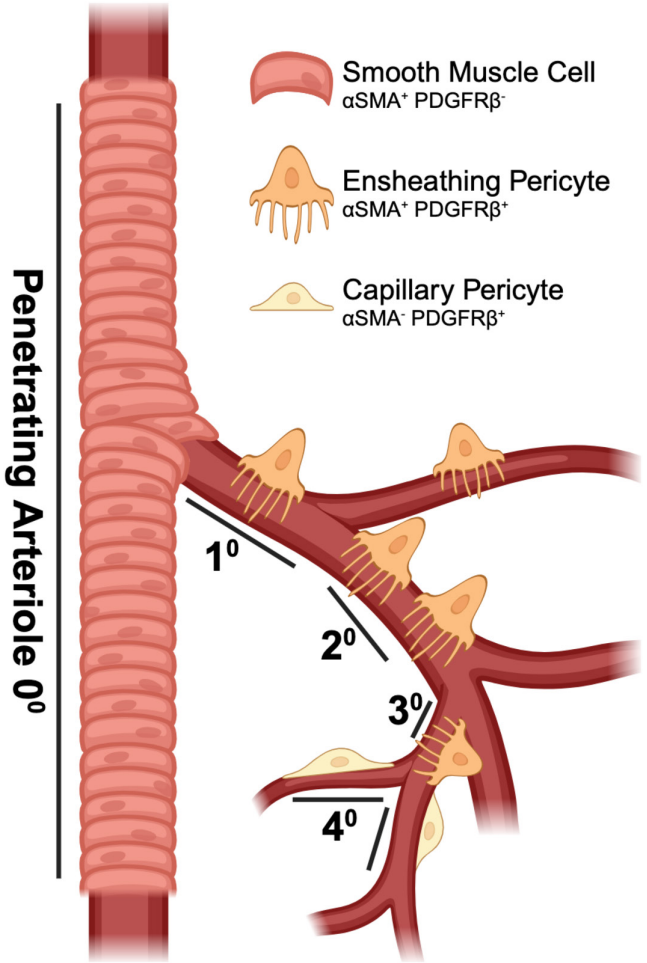

FIGURE 2 | Distribution of mural cells in the cerebral vasculature. Smooth muscle cells, which are αSMA$^+$ and PDGFRβ$^-$, ensheathe the penetrating arteriole. At the first branch (1° capillary; precapillary arteriole), the mural cells become spaced and present with a bump on a log morphology while still ensheathing the vessel. These ensheathing pericytes are both αSMA$^+$ and PDGFRβ$^+$ and continue through the 3° capillary. Further down the vascular tree, the pericytes do not fully encircle the vessel. These capillary pericytes are αSMA$^-$ and PDGFRβ$^+$ and do not contribute to vascular tone.

vessels with diameters ranging from 3 to 9 μm were identified as capillary pericytes, as they possessed processes extending parallel to the vessel for hundreds of microns but never fully enwrapping the vessel circumferentially.

When examining the spontaneous contractile properties of these mural cells in awake, head-fixed mice, Hill et al. observed a high frequency of spontaneous contractions and dilations at vessels 10–50 μm in diameter. Notably, they found a significant anticorrelation between intracellular calcium and vessel dilation in SMCs but not pericytes. They further demonstrated single-cell optogenetic activation and neural-activity-induced vasomotion only occur at these SMC-covered vessels, but not vessels with capillary pericytes. Hill et al. classification of mural cells as either SMCs or pericytes was based on morphological appearance, contractile dynamics and αSMA expression, but this classification at smaller precapillary arterioles drew criticism and a response from Attwell et al. [9] who argued the term pericyte be used for all varying morphologies (including transitions to SMCs) of spatially isolated cells with a bump on log morphology in agreement with the original 1923 Zimmerman definition. In light of this, Attwell et al. suggested that the term pre-capillary arteriole be removed from use and all vessels with continuous layer of adjacent SMCs be identified as arterioles. All other vessels with a spatially isolated contractile cells possess a pericyte and therefore cannot be an arteriole. Thus, by this definition, classical SMCs and pericytes with a SMC-like morphology contribute to vasomotion at penetrating and small arterioles in the cortex.

This pericyte with SMC-like morphology and αSMA expression has become known as an ensheathing pericyte in more recent literature. Hartmann et al. [10] was able to utilise PDGFRβ-Cre tdTomato mice combined with αSMA immunolabelling and replicate the mural cell morphological characterisation performed by Hill et al. Interestingly, these αSMA expressing ensheathing pericytes were PDGFRβ positive, which Hill et al. observed as well. Because PDGFRβ is a classical marker for pericytes, this further suggests that these contractile mural cells should be classified as pericytes.

Notably, Hartmann et al. was able detect optogenetic induced capillary constriction by increasing the time of imaging. Single-capillary pericyte optogenetic stimulation not only resulted in significantly reduced capillary diameter but also red blood cell flux and velocity demonstrating that higher branch-order cortical capillary pericytes exert a substantial influence on blood flow, albeit at slower kinetics relative to upstream SMCs and ensheathing pericytes. As these changes were induced with single-cell activation, they can be interpreted as occurring independent of changes in upstream arteriolar flow. Hartmann et al. went on to show that capillaries are slower to contract back to baseline values following hypercapnic-induced vasodilation and that capillaries dilate over minutes when recovering from single-cell optogenetic induced vasoconstriction kinetics much slower than that observed at upstream vasculature. Similarly, focal ablation of individual capillary pericytes resulted in significantly increased capillary diameter and red blood cell flux until neighbouring capillary pericytes can innervate that vacant stretch of capillary restoring the diameter to baseline values [11].

Interestingly, triple pericyte ablation studies [12] by this group revealed that local dilations are sufficient to perturb capillary flow in neighbouring capillary segments. When analysing 3 days post ablation and prior to regaining pericyte coverage, 72% of observed capillaries revealed significantly increased flux and the remaining 28% of observations significantly decreased flux. These instances of decreased flux occurred at divergent bifurcations where upon one daughter branch losing pericyte coverage, dilating and having increased flux the other daughter branch remained under perfused relative to baseline. Similarly, in silico modelling of 13–14 contiguous capillary segments revealed both increases and decreases in blood flow in hundreds of surrounding capillaries. This heterogenous division of blood flow, as also seen in the prior findings from triple pericyte ablation studies, reflects the Zweifach–Fung effect whereby increased flow rate in a dilated capillary rends a larger percentage of RBCs into a dilated branch segment. Hence, even local

manipulations to capillary pericytes can have ramifications for flow in the broader vascular network.

Taken together, classical SMCs in cortex contribute to highly rapid and dynamic vasomotion spontaneously and in response to neural activity. Spatially isolated contractile ensheathing pericytes exhibit similar physiology, while capillary pericytes at higher order upstream capillaries impact capillary flow at slower kinetics. With the recent advent of the pericyte-specific cre, Atp13a5creERT2 [13], the field is now able to perform cell-specific genetic ablation experiments so that mechanistic insight into how pericytes control basal tone can be ascertained, which can ultimately inform potential therapeutic targets for disease states where aberrant flow is observed.

The exact mechanisms that facilitate contraction of these cells remain to be further elucidated. Much of our understanding of vascular contractility has been elucidated through experiments in SMCs, and many of these same GPCRs, ion channels and kinases are found in pericytes [14]. Therefore, many of the same mechanisms that mediate SMC constriction and dilation likely mediate pericyte contractility. For example, the above study by Hartmann et al. demonstrated a role for Rho kinase–dependent rearrangement of the cytoskeleton [10]. In both the periphery and brain, mural cells maintain vascular tone and are strategically placed in proximity to or direct contact with neighbouring cells such as ECs, neurons, myeloid cells and astrocytes—all of which respond to local stimuli and tissue demands to regulate vascular tone.

3 | ECs

ECs form a monolayer along the vessel lumen and are therefore effectively positioned to modulate vascular tone in response to changes in blood flow and blood composition in both the brain and periphery. There are two primary vasodilatory pathways on which ECs rely (Figure 3): nitric oxide (NO) and endothelial derived hyperpolarisation (EDH). Both are active in the brain and periphery. Endothelial NO is produced primarily by endothelial NO synthase (eNOS) and diffuses into the neighbouring SMC acting on its receptor soluble guanylyl cyclase (sGC) catalysing the production of cGMP [1]. cGMP mediates SMC relaxation through protein kinase G (PKG) resulting in disinhibition of sarcoplasmic/endoplasmic reticulum ATPase (SERCA) and activation of potassium channels ultimately resulting in decreased cytosolic calcium and decreased membrane potential [1]. In large conduit arteries where the thick inner elastic lamina (IEL) limits direct contact between the endothelium and SMCs, endothelial control of vascular tone is dependent upon diffusible vasoactive molecules such as NOs. However, NO signalling is limited in resistance arteries by the presence of α-globin (Hbα), a potent NO scavenger [15]. We recently found endothelial Hbα also acts as a nitrite (NO_2^-) reductase, reducing NO_2^- to NO in hypoxic conditions [16]. Hbα therefore controls vascular tone by acting as a NO buffer, inhibiting NO-dependent vasodilation in sufficiently perfused tissues and mediating NO-dependent vasodilation in hypoperfused tissues.

The expression of Hbα in ECs is dependent on the direct contact of SMCs and ECs termed myoendothelial junctions (MEJs)

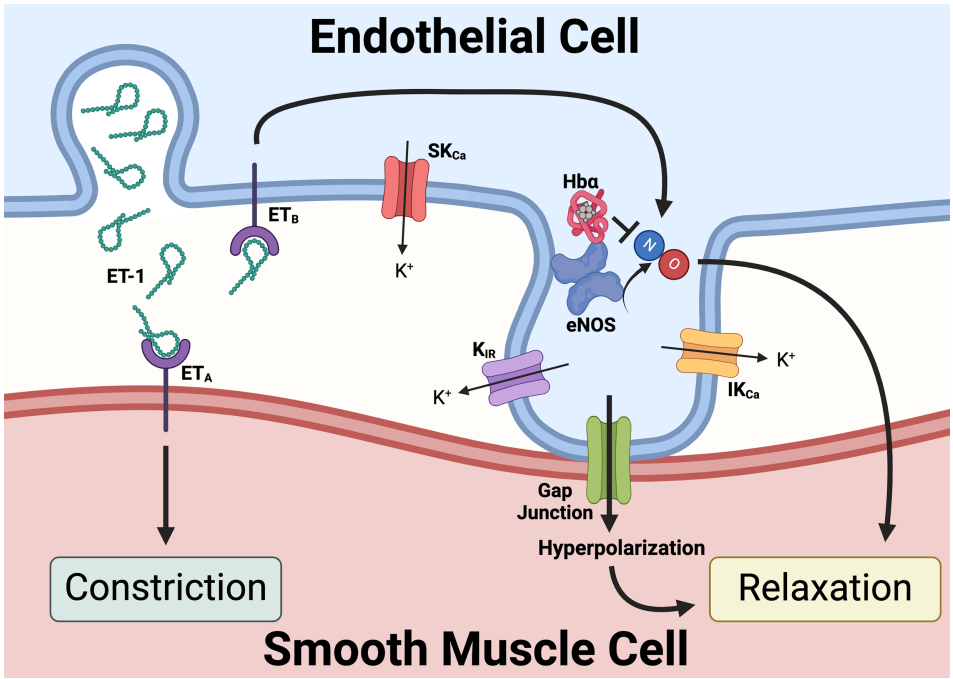

FIGURE 3 | Major pathways by which endothelial cells regulate vascular tone. There are two major pathways by which endothelial cells vasodilate. Endothelial derived hyperpolarisation is initiated by calcium sensitive K^+ channels (IK_{Ca} and SK_{Ca}) resulting in activation of inward rectifying K channels (K_{IR}). This decreased membrane potential is transmitted to SMCs through gap junctions and through accumulation of intercellular K^+. Alternatively, nitric oxide (NO) generated by endothelial NO synthase (eNOS) diffuses across the cell membranes to initiate vasorelaxation. This pathway is primarily utilised in conduit arteries as α-globin (Hbα) is expressed in the resistance artery endothelium and scavenges NO. Endothelial cells are also the primary source of endothelin 1 (ET-1), which acts on the smooth muscle ET_A receptor to promote vasoconstriction and ats on the endothelial ET_B receptor to promote dilation primarily through NO.

[15, 17]. These junctions form through holes in the IEL that are predominantly in smaller arteries of both the brain and periphery [17, 18], although there are reports of holes in the IEL in large arteries [19]. Similar junctions have been described between pericytes and ECs suggesting these junctions also play a critical role in endothelial-pericyte communication and therefore capillary tone [14]. In capillaries, these junctions allow pericytes to hyperpolarise the EC, which then can transfer the signal to the upstream arteriole and hyperpolarising the arterial SMC.

In resistance arteries, EDH is the primary mechanism by which ECs promote vasodilation. EDH is initiated by the activation of the calcium sensitive K^+ channels, IK_{Ca} and SK_{Ca}, which facilitate potassium efflux. This hyperpolarisation is then transmitted to the neighbouring SMC through gap junctions at MEJs and through the accumulation of endothelial-derived K^+ in the intercellular space [1, 20]. Increased extracellular potassium shifts the current–voltage relationship of inward rectifying K^+ channels (K_{IR}) on both ECs and SMCs such that they are outward rectifying at resting membrane potential further polarising the cell [21]. These two means of transmission, gap junctions and potassium channels, are spatially organised. Work in rat resistance arteries has found IK_{Ca} and Cx37 colocalise within the same MEJs [22], while recent work from our group in mouse resistance arteries demonstrated Kir2.1 and Cx40 localise to separate MEJs [23]. Furthermore, phosphatidylserine a lipid regulator of Kir2.1 co-localises with Kir2.1 suggesting a complex arrangement of gap junctions, ion channels and specific lipids at MEJs coordinating the regulation of vascular tone.

The most potent known vasoconstrictor, endothelin-1 (ET-1), is a peptide synthesised by the endothelium, which acts either in a paracrine or autocrine manner on the SMC endothelin A (ET_A) receptor to rapidly induce vasoconstriction (Figure 3). This vasoconstriction is buffered slightly through the ET_B receptor on ECs, which promotes vasodilation [24]. Notably, the tight blood brain barrier limits the endocrine activity of ET-1 on the brain vasculature. A recent study in human subjects demonstrated infusion of ET-1 had no impact on cerebral blood flow and caused only minor constriction of the middle cerebral artery [25]. The authors conclude this is not likely due to a lack of cerebral vasoreactivity to ET-1 as abluminal ET-1 reliably elicits vasoconstriction in the cerebral vessels of animal models. Excitingly after decades of research, aprocitentan, an dual ET_A and ET_B receptor antagonist, was approved by the US Food and Drug Administration in March 2024 for the treatment of resistant hypertension marking it as the first approval of an ET-1 receptor antagonist for systemic hypertension [26].

As our understanding of endothelial signalling continues to grow, it has become increasingly apparent that the endothelium is a heterogenous population of cells. Indeed, the ECs within a single vessel segment exhibit heterogenous receptor expression and sensitivity to vasoactive substances, which may allow the endothelium to respond to complex or counteracting signals [27]. This nuanced view of endothelial biology will only continue to expand with the use of single-cell technologies, which have unveiled a detailed view of EC heterogeneity between and within tissues. For example, single-cell RNA sequencing (scRNA-seq) of the mouse lung endothelium revealed a distinct population of lung capillary ECs, which facilitate gas exchange [28]. Interestingly, these populations are distinct from capillary ECs, which express genes involved in the synthesis of ET-1 and NO suggesting a distinct subpopulation of capillary ECs, which regulate vascular tone in the lung. Ultimately, functional studies are needed to verify the physiological relevance of these gene signatures, but these technologies offer an exciting new perspective on endothelial biology.

4 | Neurons

In both the brain and periphery, arteries are innervated by sensory, parasympathetic and sympathetic neurons allowing neuronal control of vascular tone. Parasympathetic tone is most often vasodilatory. However, it is not clear that parasympathetic signalling contributes meaningfully to vascular tone in the periphery. Acetylcholine, which is found in perivascular parasympathetic neurons, is perhaps the most well-characterised endothelial-dependent vasodilators. Acetylcholine receptors are expressed on most ECs, and acetylcholine is frequently used to test endothelial function ex vivo [29]. However, work in the rat mesentery has demonstrated that the presence of cholinergic neurons does not necessitate their importance for the regulation of vascular tone [30]. Similar in the brain, studies that have disrupted parasympathetic signalling by severing the facial nerve found no changes to the hypoxic or hypercapnic response in rats, cats, dogs, rabbits or baboons even though stimulation of the facial nerve does increase cerebral blood flow in both humans and animal models [31].

Sensory neurons are another source of vasodilatory signals in both the peripheral and cerebral vasculature primarily relying on calcitonin-gene-related peptide (CGRP) and substance P. The contribution of these neurons to arterial tone has been directly shown in mouse mesenteric arteries where they were demonstrated to blunt sympathetic vasoconstriction in response to electric field stimulation (EFS) primarily via CGRP [32]. CGRP directly hyperpolarises SMCs predominantly through activation of K_{ATP} channels resulting in endothelial independent vasodilation, while also depolarising ECs through K_{Ca} channels [33]. Substance P, however, has a much smaller impact on SMC hyperpolarisation, which it induces via endothelial NO [33]. Interestingly, in human pial arteries, the vasodilatory mechanism of substance P is heterogenous where approximately half the subjects relied entirely on NO-mediated vasodilation, whereas the other subjects relied on both NO and EDH [34]. These two neurotransmitters are highly intertwined as aberrant substance P signalling in a mouse model of inflammatory bowel disease was shown to disrupt CGRP mediated vasodilation [32].

Notably, the vasodilatory response of sensory neurons to EFS is only unmasked after sympathetic blockade, suggesting sympathetic signalling is the predominant neuronal signal dictating vascular tone [32]. In the vasculature, these neurons are primarily adrenergic and purinergic. Norepinephrine acts on SMC α_1-adrenergic receptors (α_1-ARs), which are GPRCs predominantly coupled to G_q proteins. α_1-AR stimulation increases intracellular calcium thereby promoting SMC contraction [1, 7]. Our lab has also demonstrated α_1-AR stimulation activates pannexin 1 (Panx1) channels to release ATP acting in an autocrine manor to

facilitate vasoconstriction at least partially through P2Y receptors [35]. This is in contrast to ATP released from sympathetic synaptic vesicles, which acts primarily on SMC P2X1 receptors to induce vasoconstriction [36].

There still remains some debate as to the exact contribution of SMC derived ATP compared with neuronal derived ATP in sympathetic vasoconstriction. We recently demonstrated inducible knockout of Panx1 in SMCs significantly blunts vasoconstriction to EFS in pressurised mouse mesenteric arteries, while overexpression of human Panx1 potentiates the response to EFS [37]. Notably, degradation of ATP by apyrase blunts vasocontraction in WT mice but has no impact on vasoconstriction in SMC Panx1 knockout mice, suggesting purinergic vasoconstriction in these vessels is dependent on SMC Panx1.

The importance of neuronal derived ATP to sympathetic vasoconstriction has been thoroughly debated in the literature. Disagreements between the relative contribution of ATP and norepinephrine may come down to the species studied (e.g. mouse vs rat), technical preparation (e.g. wire vs pressure myography) and method of inhibition (genetic deletion vs pharmacologic). A recent carefully designed study by Mittal et al. [38] demonstrates the point well. They investigated the relative contribution of adrenergic and purinergic vasoconstriction in response to EFS in mouse and rat mesenteric arteries prepared on a wire myograph. While both adrenergic signalling was the major mediator in both species, only mice had a component which could be blocked by a P2X1 inhibitor. This study clearly demonstrates species differences in this signalling pathway and suggests a role for neuronal derived ATP in mouse mesenteric sympathetic vasoconstriction. Finally, when considering the relative importance of these neurotransmitters, it is worth considering potential crosstalk between these pathways that are not ruled out by ours or others' experiments. For example, P2X1 signalling may have an undiscovered dependency on Panx1. It will be important to continue to elucidate and clarify the relative contributions of these neurotransmitters to better understand the components which regulate blood flow and blood pressure.

5 | Astrocytes

Astrocytes are star-shaped glial cells capable of regulating cerebral arterial tone and have been recently implicated in cerebral autoregulation. Within-subject studies have revealed that cerebral autoregulation can only maintain cerebral blood flow within a limited range of arterial pressure changes and that the cerebral vasculature possesses a low buffering capacity against reductions in perfusion pressure [39]. This has provoked investigation into what mechanisms in brain parenchyma might exist to sense changes in arterial perfusion pressure, thereby protecting the brain from hypoperfusion. While arterial baroreceptors in the carotid bifurcation are strategically positioned to monitor systemic blood pressure, they are not able to detect pressure within the brain parenchyma. It has, therefore, been hypothesised an intracranial baroreceptor exists to monitor changes in brain perfusion [40]. Specifically, astrocytes have been proposed to fulfil this role by regulating arteriole tone in both cortical and ventrolateral brainstem (Figure 4a–c); astrocytes respond to increasing intracranial pressure and reduced cerebral blood flow with robust intracellular calcium responses in all subcellular compartments, including soma, processes and perivascular endfeet. Additionally, blocking vesicular release from astrocytes in ventrolateral brainstem abolishes both cardiovascular and sympathetic responses to increased intracranial pressure and reduced cerebral blood flow suggesting astrocytes participate in the sensing of intracranial pressure [40].

The rise in astrocyte intracellular calcium has been shown ex vivo to be necessary for both maintenance of basal tone as well as sustained responses to hemodynamic forces [41]. The TRP cation channel subfamily V member 4 (TRPV4) is necessary for astrocyte calcium responses and hence regulation of penetrating arteriole tone. These channels are localised to astrocyte endfeet, thus positioning them to sense changes in arterial diameter. While some have proposed TRPV4 as the mechanosensor, others have shown TRPV4 channels do not respond directly to changes in membrane tension suggesting they act downstream of other mechanosensing pathways [42]. Both this study and the aforementioned study in ventrolateral brainstem went on to identify purinergic signalling as a key modality in mediating basal tone [40, 41].

Others have since replicated the finding that increases in arteriole tone increase astrocyte calcium via engagement with TRPV4 channels and that this subsequently engages cyclooxygenase 1 (COX1), which limits the extent of vasoconstriction (Figure 4b). They also further confirmed that clamping astrocyte calcium in vivo decreases cerebral vasomotion [43]. However, it is unclear which product of COX1 is responsible for these effects. One such metabolite, 20-HETE, has been excluded as a contributor of penetrating arterial basal tone [41].

Taken together, recent work points to a role for astrocyte TRPV4 channels responding to changes in arteriole tone by increasing astrocyte intracellular calcium. This then leads to both COX1 metabolites and purinergic signalling mediating sustained responses in resting arterial tone. Interestingly, in our recent study [44], we utilised the 2Phatal focal ablation approach and demonstrated no change in capillary diameter 10 days following astrocyte ablation (Figure 4c). This suggests that astrocyte regulation of tone might exist only at arterioles but not at capillaries. This should be clarified in future studies.

6 | Myeloid Cells

Myeloid cells are highly abundant immune cells critical to the innate and adaptive immune response [45]. In the periphery, the impact of these cells on vascular tone has been primarily considered in the pathogenesis of inflammatory conditions. For example, intermittent hypoxia induces iNOS expression in pulmonary macrophages, which subsequently blunts hypoxia induced vasoconstriction presumably through canonical NO signalling [46]. More recently, adventitial macrophages were shown to impair sympathetic and sensory nerve control of vascular tone in inflammatory bowel disease [47]. The exact mechanism remains to be elucidated, but the authors hypothesise these cells may be binding CGRP and substance P released from sensory neurons

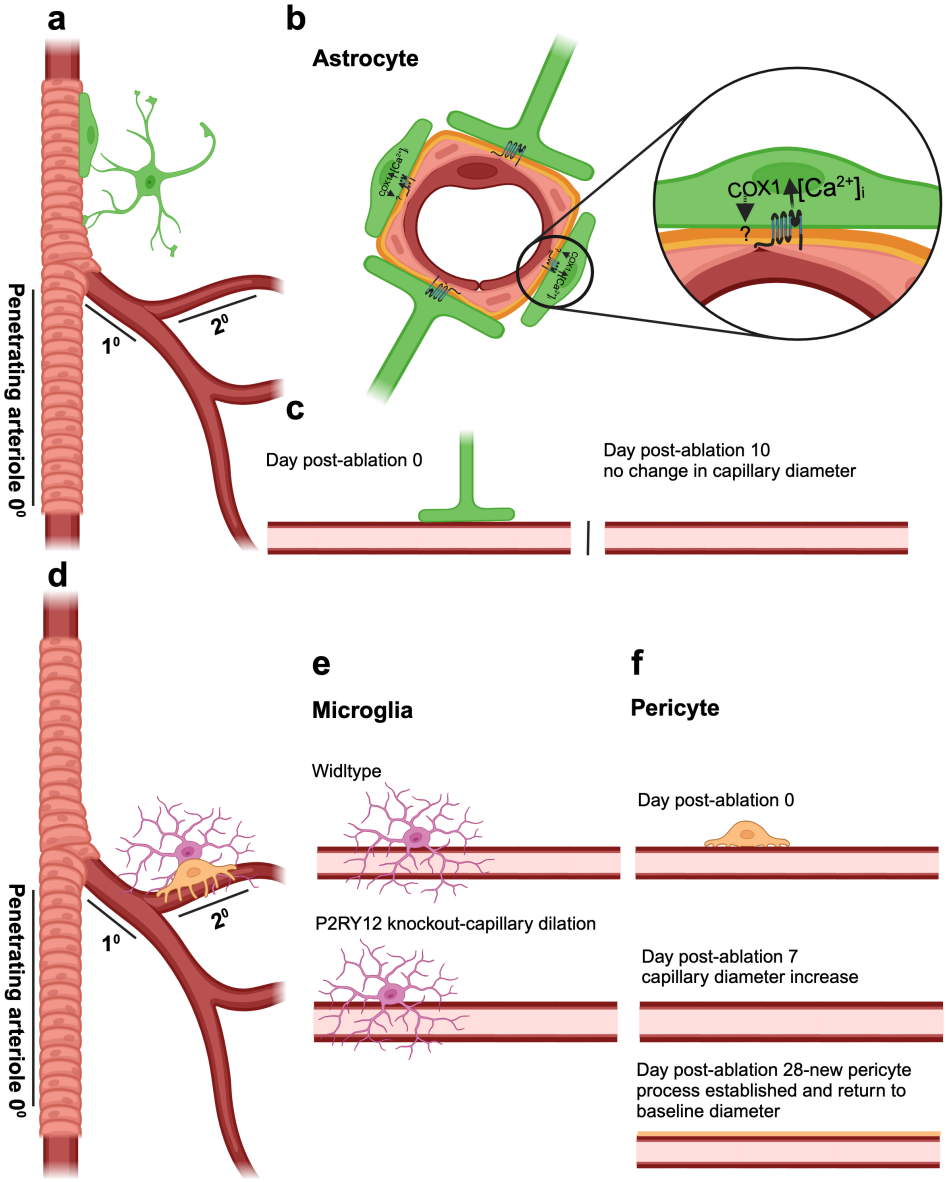

FIGURE 4 | Control of vascular tone by astrocytes and microglia. Astrocytes primarily regulate tone in arteries and arterioles (a), where they respond to changes in intravascular pressure with increased calcium and COX1-mediated signalling (b). Importantly, ablation of astrocytes shows no impact on capillary diameter (c). A subpopulation of microglia associates with capillaries (d). Knockout of P2RY12 from capillaries increases dilation demonstrating these cells respond to purinergic signals to maintain capillary tone (e). A similar phenotype is observed by ablating pericytes demonstrating the importance of pericytes in the regulation of cerebral capillary tone (f).

resulting inflammatory signalling rather than vasodilation [47]. These and other studies investigating myeloid control of peripheral vascular function have primarily focused on macrophages, but a recent study demonstrated that mast cells in the skin associate with arterioles and are in contact with arterial SMCs [48]. The full functional implications of this spatial organisation remain to be investigated further, but it is interesting to consider how this would contribute to localised vasodilation or constriction during an allergic response.

In the brain, myeloid cells are important for the physiologic regulation of vascular tone. We recently characterised a subset of microglia whose somata reside on capillaries, termed capillary-associated microglia (Figure 4d), and found the global ablation of microglia results in capillary dilation [49]. This was phenocopied in P2RY12 receptor–deficient mice and Panx1-deficient mice suggesting purines released through Panx1 channels may act on microglial P2RY12 receptors to regulate capillary tone (Figure 4e).

These findings mimic findings described above where focal laser ablation of pericytes resulted in increased capillary diameter and red blood cell flux, with both restored to baseline values following reinnervation of the vascular vacancy by a new pericyte (Figure 4f) [11]. Interestingly, focal loss of pericytes in the aged brain results in a higher magnitude of capillary dilation relative to younger mice [12]. Because both pericyte and myeloid cell loss resulted in changes to capillary diameter and red blood cell flux, more studies are needed to confirm how these cell types communicate and what changes in local capillary tone

mean for blood perfusion within larger brain regions. To that end, in silico modelling studies have demonstrated that flow perturbations of hundreds of capillaries resulted just from dilation of a few capillaries [12]. Thus, it seems that perturbation to just a few capillaries can have a large-scale effect, although imaging modalities such as fMRI should be utilised to examine global cerebral blood flow following these focal experimental ablations.

7 | Perspectives and Conclusions

Comprised of only a few cell types, the vasculature is clearly a complex tissue responding to hemodynamic and biochemical signals to coordinate the regulation of tissue perfusion, and the detailed mechanisms by which these cells regulate vascular tone are still being understood. Excitingly, recent technological and methodological advances are offering new insights into cellular heterogeneity of the vasculature. The increasing utilisation of scRNA-seq has no doubt driven the field forward by discovering new cell subsets and providing accurate markers of cell identity to aid in precise experimental design. Our understanding of vascular and perivascular cell organisation and heterogeneity will be furthered even more so by advancements in spatial transcriptomics. Until recently, this technology has been difficult to implement in the study of vascular biology due to poor resolution. In fact, many early spatial transcriptomic datasets fail to detect ECs all together; however, recent bioinformatic approaches have increased the practical resolution of these technologies and offer an exciting new avenue of research to understand cellular heterogeneity and cellular communication in vascular biology [50]. This technology will be able to help the field understand how spatial organisation and proximity to other cell types impact vascular cell identity. This technology may provide insight into why astrocytes and microglia differentially control arterial and capillary tone even though they occupy perivascular regions of both arterioles and capillaries and uncover distinct transcriptional signatures between parenchymal and capillary-associated microglia.

Similarly, understanding subcellular spatial heterogeneity and the organisation of signalling hubs continues to offer an exciting avenue of future research. One such example of this is the work by our lab and others demonstrating MEJ heterogeneity [22, 23]. The functional implications of the segregation of Kir2.1 and Cx40 and the co-localisation of IK_{Ca} and Cx37 remain to be fully understood. Thoroughly understanding points of intercellular contact throughout the vasculature will be important to understanding the regulation of vascular tone.

Continuing to investigate how vascular tone is coordinated by various cell types will no doubt offer new insights into therapeutics for cardiovascular and cerebrovascular diseases. The recent approval of aprocitentan by the US FDA marks an exciting milestone after decades of work investigating endothelin biology. Perhaps an area of even greater need is the development of therapeutics to prevent and treat diseases characterised by cerebral blood flow deficits, such as Alzheimer's disease. Understanding the complex regulation of cerebral blood flow will be critical in the development of new therapies and improving patient outcomes.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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