Astrocyte Regulation of Cerebral Blood Flow in Health and Disease

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Astrocytes play an important role in controlling microvascular diameter and regulating local cerebral blood flow (CBF) in several physiological and pathological scenarios. Neurotransmitters released from active neurons evoke Ca^{2+} increases in astrocytes, leading to the release of vasoactive metabolites of arachidonic acid (AA) from astrocyte endfeet. Synthesis of prostaglandin E_2 (PGE_2) and epoxyeicosatrienoic acids (EETs) dilate blood vessels while 20-hydroxyeicosatetraenoic acid (20-HETE) constricts vessels. The release of K^+ from astrocyte endfeet also contributes to vasodilation or constriction in a concentration-dependent manner. Whether astrocytes exert a vasodilation or vasoconstriction depends on the local microenvironment, including the metabolic status, the concentration of Ca^{2+} reached in the endfoot, and the resting vascular tone. Astrocytes also contribute to the generation of steady-state vascular tone. Tonic release of both 20-HETE and ATP from astrocytes constricts vascular smooth muscle cells, generating vessel tone, whereas tone-dependent elevations in endfoot Ca^{2+} produce tonic prostaglandin dilators to limit the degree of constriction. Under pathological conditions, including Alzheimer's disease, epilepsy, stroke, and diabetes, disruption of normal astrocyte physiology can compromise the regulation of blood flow, with negative consequences for neurological function.

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Astrocyte endfoot processes completely envelop all blood vessels in the brain.[6] Work over the past 20 years has demonstrated the importance of astrocytes in cerebral blood flow (CBF) regulation. However, several other cell types also contribute to this process, which has made untangling the specific contributions of astrocytes an enduring area of research. Indeed, neurovascular coupling (NVC) is accomplished by the coordinated activity of excitatory and inhibitory neurons, vascular endothelium, mural cells, astrocytes, and even microglia (Bisht et al. 2021;

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Howarth et al. 2021; Császár et al. 2022). Each cell type uses multiple cellular pathways and diffusible messengers, many of which are recruited in a redundant fashion to create a "fail-safe" system. This parallel processing has likely evolved because matching CBF to the metabolic demands is essential for healthy brain function. The goal of this work is to describe the mechanisms and circumstances under which astrocytes modify CBF.

The maintenance of brain homeostasis and cognitive processing requires substantial energy expenditures relative to the rest of the body. It is estimated that the brain accounts for 20% of total energy consumption although it represents only 2% of body weight (Attwell and Laughlin 2001). The greatest proportion of the brain's energy expenditure is due to excitatory synaptic transmission (Howarth et al. 2012), suggesting that glutamatergic transmission may be preferentially impacted by reduced energy supply. Brain metabolism is almost exclusively due to oxidative phosphorylation, where glucose is the primary energy substrate and O_2 is the final electron acceptor in the mitochondrial electron transport chain (Magistretti et al. 1995). Although some glycogen is stored in granules in astrocytes (Brown and Ransom 2007; Oe et al. 2016; Howarth et al. 2021; Dienel et al. 2023), the brain lacks sufficient energy reserves to maintain function over extended periods greater than a few minutes. Therefore, the moment-to-moment delivery of O_2 and glucose through the blood is fundamental to providing a consistent energy supply to adequately support brain function.

There are at least four important physiological states under which CBF is locally regulated. First, basal CBF, which is remarkably higher than the rest of the body (Magistretti et al. 1995; Raichle 2015), is regulated so that the brain receives an adequate supply of energy substrates at all times. This process, in part, helps feed intrinsic fluctuations in cortical neural activity, even in the absence of overt sensory, motor, or cognitive activities (Raichle 2015). As such, resting CBF oscillates at ~0.1 Hz, corresponding to fluctuations in high frequency neural activity (Mateo et al. 2017). Second, autoregulatory mechanisms limit CBF variability in the face of changes in systemic blood pressure. Here, the vasculature itself is intrinsically sensitive to increases in pressure, which trigger the myogenic response (vasoconstriction) to ensure relatively constant CBF (Schaeffer and Iadecola 2021). Third, changes in blood gasses result in altered CBF to maintain proper balance between energy substrates and byproducts of metabolism (Schaeffer and Iadecola 2021). Fourth, CBF is regulated in response to sudden changes in brain activity triggered by external (sensory inputs) or internal (motor command or neural processing) events. This homeostatic response, named functional hyperemia, increases delivery of glucose and O_2 at times of enhanced metabolic demand (Howarth et al. 2021). Astrocytes regulate CBF under each of these physiological states, although most work has been done on functional hyperemia.

HISTORICAL OVERVIEW OF FUNCTIONAL HYPEREMIA

Functional hyperemia was first hypothesized in the 1880s by Angelo Mosso (1880). In patients with skull defects, which allowed direct observation of the cortical surface, Mosso found that sensory stimulation increased brain volume, representing increased CBF. A decade later, Roy and Sherrington (1890) showed that stimulation of sensory nerves in dogs produced increases in cortical blood flow. They speculated that "the chemical products of cerebral metabolism … can cause variations of the caliber of the cerebral vessels: that in this reaction the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variations of functional activity" (Roy and Sherrington 1890).

Around the same time, astrocyte morphology was described by Virchow (1858), Golgi (1894), Ramón y Cajal (1995, first published in 1897), and others. They observed that astrocytes contacted both blood vessels, which are enveloped by astrocyte endfeet, and neurons (Fig. 1). Cajal wrote, "The perivascular neuroglial cells live only in the proximity of the capillaries of the gray matter, to which they send one or more thick appendages inserted in the outer side of the endothelium… . The object of such

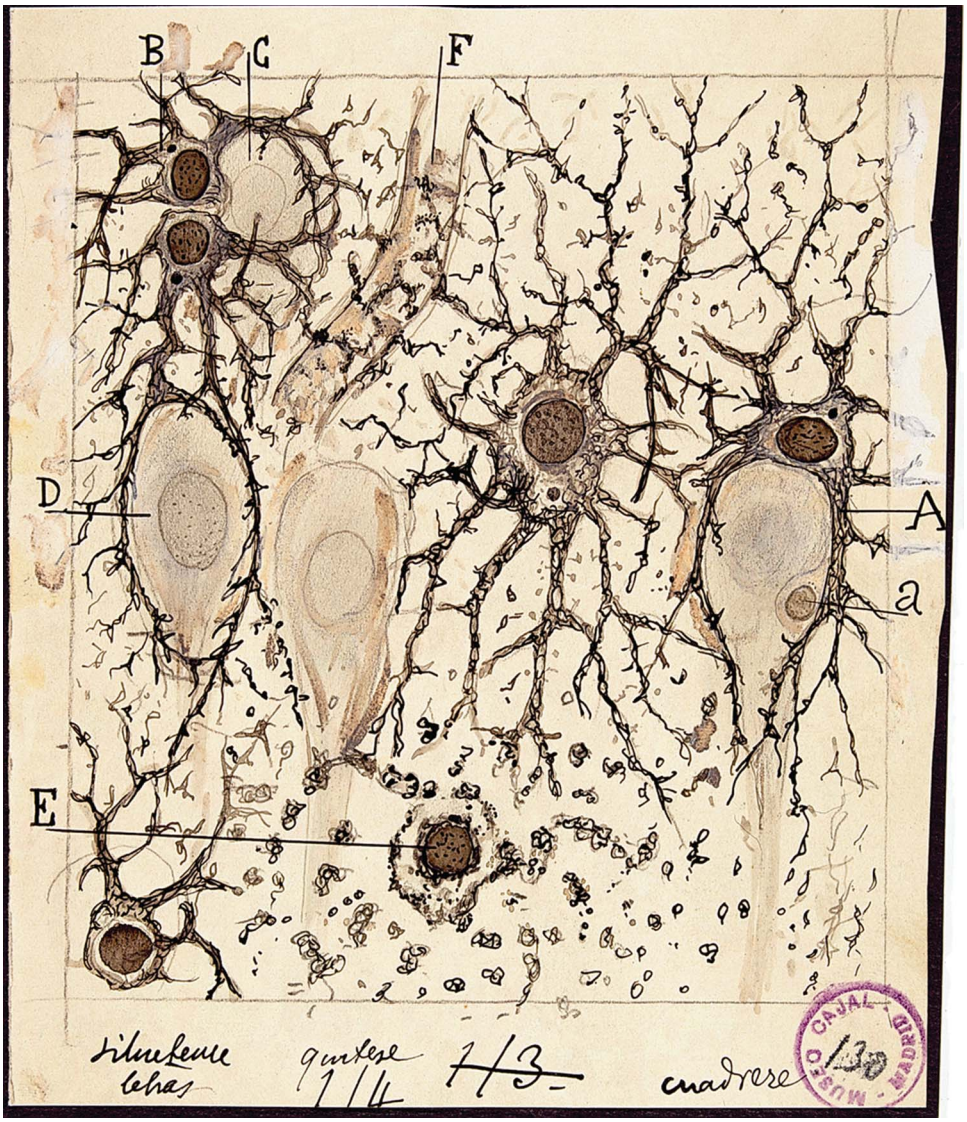

Figure 1. Drawing of brain astrocytes by Santiago Ramon y Cajal. Astrocytes, the darker cells in the drawing (*A*, *B*) contact both neurons, the lighter cells (*a*, *C*, *D*), and a blood vessel (*F*). As suggested by Cajal, astrocytes are ideally situated to mediate signaling from neurons to blood vessels and to regulate cerebral blood flow in response to neuronal activity. Drawing is reprinted with permission from Ricardo Martínez Murillo, Director of the Instituto Cajal.

elements is to evoke, by contraction of the aforementioned appendices, local dilations of the vessels" (Cajal 1895). Cajal's suggestion that astrocytes regulate blood flow, albeit by an incorrect mechanism, was prescient.

More recently, Paulson and Newman (1987) proposed that astrocytes mediate functional hyperemia by a K^+ siphoning mechanism, releasing K^+ onto blood vessels from their endfeet in response to neuronal activity. Later, Harder et al. (1998) found that epoxyeicosatrienoic acids (EETs) derived from arachidonic acid (AA) in astrocytes underlie functional hyperemia. Soon after, Zonta et al. (2003) showed that Ca^{2+}-de-

pendent synthesis of prostaglandins in astrocytes mediates NVC. Since these reports, the role of astrocytes in mediating functional hyperemia has been studied intensely, as detailed below.

NEUROVASCULAR COUPLING

The control of CBF was originally hypothesized to be mediated by negative feedback whereby the metabolites generated by active neurons (such as CO_2) were the signals that caused increased CBF (Roy and Sherrington 1890). While a recent study has brought new light to the CO_2 hypothesis (Hosford et al. 2022), work over the past two decades has shown that brain activity can directly increase CBF in a feedforward manner, independent of negative feedback. Indeed, CBF increases to such an extent that more O_2 is provided to active brain regions than is consumed (Offenhauser et al. 2005; Devor et al. 2011). This oversupply of O_2 is the basis of the blood oxygenation level dependent (BOLD) effect in functional magnetic resonance imaging (fMRI). It is well recognized that neuronal activity is causal for enhancing CBF and the BOLD signal (Lee et al. 2010), with synaptic activity (rather than spiking) being the primary driver (Logothetis et al. 2001; O'Herron et al. 2016). Indeed, direct signaling from neurons to blood vessels contributes to local CBF regulation through several well-described mechanisms (Howarth et al. 2021). Additionally, astrocytes also play an important role in regulating NVC. While astrocytes were originally thought to be the key mediator, directly transducing neural signals into vasodilation (Zonta et al. 2003), more recent discoveries suggest that they modulate, rather than mediate, CBF through several mechanisms depending on the physiological context. These include (1) augmenting CBF increase only when astrocytes are recruited during intense or sustained neural activity (Dunn et al. 2013; Gu et al. 2018; Institoris et al. 2022); (2) preferentially regulating capillaries over arterioles (Mishra et al. 2016); (3) controlling the dynamic range and polarity of the neural-evoked CBF response (Blanco et al. 2008; He et al. 2012); (4) potentially resetting microvascular diameter after functional hyperemia (Gu et al. 2018); (5) setting steady-state microvascular tone to control basal CBF (Kur and Newman 2014; Kim et al. 2015; Rosenegger et al. 2015); and, finally (6) contributing to intrinsic arteriole oscillations (Haidey et al. 2021).

MECHANISMS OF ASTROCYTE-MEDIATED NEUROVASCULAR COUPLING

Arachidonic Acid-Mediated Neurovascular Coupling

Elevation in astrocyte free Ca^{2+} bidirectionally controls vascular diameter (Fig. 2). This link was best demonstrated by uncaging Ca^{2+} in astrocytes in brain slices, retinal explants, and in vivo. These experiments showed unequivocally that Ca^{2+} transients in astrocytes can induce dilations and constrictions in adjacent vasculature. Zonta et al. (2003) showed that arterioles exhibited dilations, as opposed to the constrictions reported by Mulligan and MacVicar (2004), when, in the latter, Ca^{2+} was uncaged in adjacent astrocyte endfeet (see section on Bidirectional Control of Vessel Diameter for potential mechanisms that underlie the difference in polarity of NVC). The vasoconstriction was blocked by inhibiting phospholipase A2 (PLA2), which liberates AA from membrane lipids, and by preventing the conversion of AA to the vasoconstrictive lipid 20-HETE. Takano et al. (2006) showed that Ca^{2+} uncaging in vivo dilates cortical arterioles via a mechanism dependent on cyclooxygenase-1 (COX1), an enzyme expressed in astrocytes that synthesize PGE_2 from AA. Later, He et al. (2012) demonstrated that evoked arteriole dilations were absent or inverted to constrictions in brain slices from mice lacking IP_3 receptor type 2 (IP_3R_2), which gives rise to G_q-coupled receptor-dependent Ca^{2+} signals in astrocytes, or those lacking cytoplasmic PLA2 enzyme. These findings cemented a role for astrocyte Ca^{2+} and AA-derived substances in arteriole NVC.

Accumulating evidence shows that NVC also occurs at the capillary level (Chaigneau et al. 2003; Lacar et al. 2012; Hall et al. 2014; Kornfield and Newman 2014), where even small changes in diameter can give rise to pronounced effects on CBF as a significant fraction of total vascular resistance resides in capillaries (Blinder et al. 2013; Hall et al. 2014). Capillary contractility is

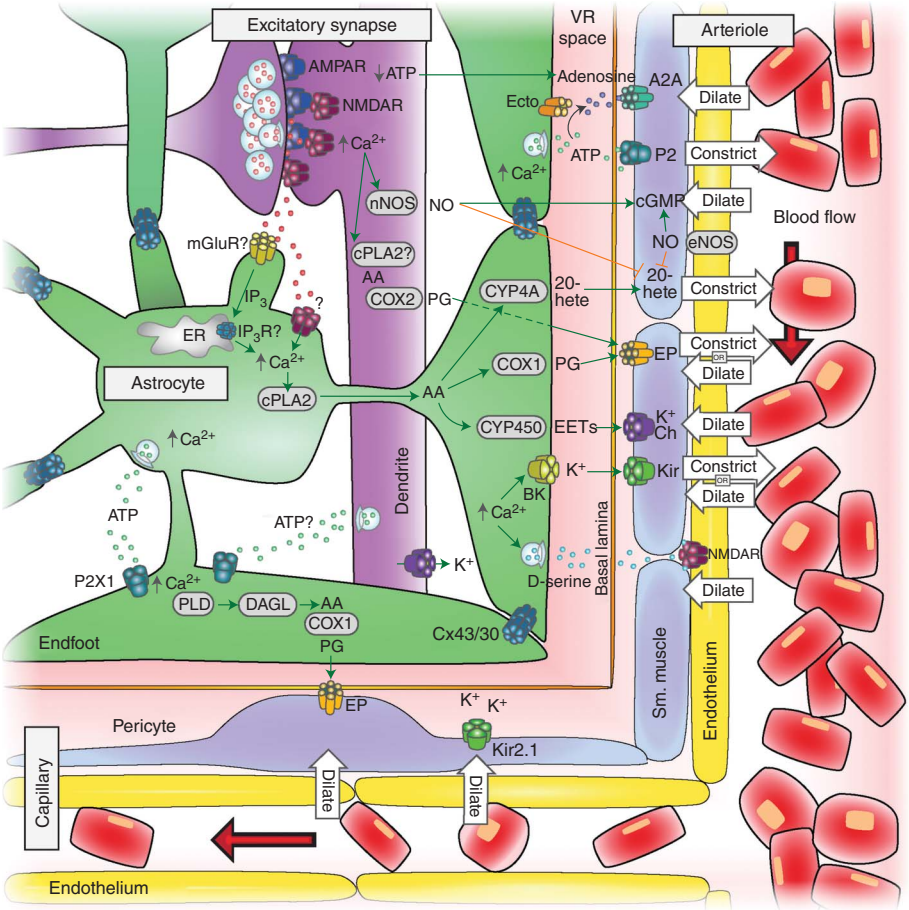

Figure 2. Summary of proposed Ca^{2+}-dependent signaling pathways in neurovascular coupling. Signaling to arterioles and capillaries by glutamatergic synaptic transmission is shown, either through direct neuronal mediators or through astrocyte pathways. For neuronal to vessel signaling subsequent to AMPAR and NMDAR activation, NO from nNOS or NOS1 causes vasodilation either through the canonical cGMP pathway or via inhibition of 20-HETE synthesis by acting on a CYP450 enzyme subtype. Prostaglandin (PG) production in neurons occurs via the enzyme COX2, acting on vascular EP4 receptors. Extracellular K^+ elevation by voltage-gated K^+ channels may trigger K^+-mediated dilation via Kir 2.1 channels on mural cells. For astrocyte to vessel signaling, glutamate release causes an elevation in free Ca^{2+} that occurs through an unknown mechanism. While IP_3R2 is a prominent Ca^{2+} source, it may not be involved in functional hyperemia. Activation of cPLA2 liberates AA from the plasma membrane and its metabolism leads to three primary pathways acting on arterioles: (1) the conversion to 20-HETE by CYP4A to cause vasoconstriction, (2) the conversion to PGs by COX1 to cause vasodilation, and (3) the conversion to EETs via a subtype of CYP450 enzyme to cause dilation. Calcium elevation in endfeet can also lead to K^+ efflux via BK channels to cause dilation, and trigger the release of D-serine, which facilitates opening of endothelial NMDARs causing dilation. At the capillary level, astrocyte endfoot Ca^{2+} triggers PLD and DAGL activity to generate AA. COX1 then converts AA to PGs to cause dilation. This mechanism involves ATP release and P2X1 receptor opening, but the source of the ATP is unclear. (A2A) Adenosine A2 receptor, (AA) arachidonic acid, (AMPAR) α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid receptor, (ATP) adenosine 5′-triphosphate, (BK) large conductance Ca^{2+} and depolarization-gated K^+ channels, (cGMP) cyclic guanosine monophosphate, (COX1) cyclooxygenase 1, (COX2) cyclooxygenase 2, (Cx43/30), connexin 43 or 30 gap junction channels, (CYP4A) cytochrome P450 4A, (CYP450) cytochrome 450 enzymes, (DAGL) diacylglycerol lipase, (Ecto) ectonucleotidase, (EETs) epoxyeicosatrienoic acid, (eNOS) endothelial nitric oxide synthase, (EP) E-type prostanoid receptor, (20-HETE) 20-hydroxyeicosatetraenoic acid, (IP3) inositol 1,4,5-trisphosphate, (IP3R2) IP3 receptor 2, (Kir) inwardly rectifying K^+ channels, (mGluR) metabotropic glutamate receptor, (NMDAR) *N*-methyl-D-aspartate receptor, (nNOS), neuronal nitric oxide synthase, (NO) nitric oxide, (P2) purinergic 2 receptors, (P2X1) purinergic 2 receptor X1, (PG) prostaglandin, (PLA2) cytosolic phospholipase A2, (PLD2) phospholipase D2, (VR) Virchow Robin space.

mediated by pericytes (Peppiatt et al. 2006; Puro 2007; Hartmann et al. 2021). Mishra et al. (2016) showed that neural activity-evoked Ca^{2+} signals in astrocytes stimulate the synthesis of PGE_2, which acts on EP4 receptors on pericytes to dilate capillaries. Preventing Ca^{2+} rises in astrocytes by introducing the Ca^{2+} chelator BAPTA into them inhibited dilation of capillaries, but not arterioles (Mishra et al. 2016). Around the same time, Biesecker et al. (2016) showed that mice lacking IP_3R2 exhibit reduced dilation of capillaries but not arterioles in the retina. These reports provided the first evidence for the necessity of astrocyte Ca^{2+} in neurovascular signaling. Astrocytes expressed PLD2, which can also generate AA, and was required for capillary dilation. Astrocyte endfeet also contained both COX1 and prostaglandin E synthase enzymes required to produce PGE_2 from AA, but lacked COX2 (Mishra et al. 2016). Evidence from two other independent groups further demonstrated that Ca^{2+} transients in astrocyte endfeet precede activity-dependent capillary dilation (Otsu et al. 2015; Lind et al. 2018). Together, these results indicate that astrocytes can dilate both arterioles and capillaries via the synthesis and release of PGE_2, and thus regulate CBF (Fig. 3).

More recent work conducted in vivo using optogenetics has further demonstrated the sufficiency of astrocyte activation in controlling CBF. Optically activating ChR2 expressed selectively in cortical astrocytes drove dilation of both penetrating and, more surprisingly, pial surface arterioles over a broad area (Hatakeyama et al. 2021), and drove increases in BOLD signals (Takata et al. 2018).

Potassium and D-Serine

NVC may also be mediated by the glial release of K^+ on to blood vessels. Raising extracellular K^+ concentration ($[K^+]_o$) from a resting level of ~3 mM up to ~15 mM dilates blood vessels. Potassium-induced vasodilation is mediated by an increase in the conductance of inwardly rectifying K^+ channels (Filosa et al. 2006; Haddy et al. 2006) and by activation of the Na^+-K^+ ATPase on the vascular smooth muscle cells (Bunger et al. 1976; Haddy 1983), both resulting in their hyperpolarization and relaxation. Larger K^+ increases, above ~15 mM, depolarize vascular smooth muscle cells, resulting in vasoconstriction (Girouard et al. 2010). However, $[K^+]_o$ in the brain normally reaches ~12 mM only during intense activity and has not been observed to exceed 15 mM except during pathological processes such as spreading depolarization or stroke (Vyskocil et al. 1972; Somjen 2001).

Active neurons release K^+ into the extracellular space, resulting in an increase in $[K^+]_o$. Light stimulation produces slow, transient $[K^+]_o$ increases of ~1 mM in the cat visual cortex (Singer and Lux 1975; Connors et al. 1979) and in the cat and frog retina (Dick and Miller 1985; Karwoski et al. 1985). Paulson and Newman (1987) proposed that NVC is mediated by a feedforward glial cell K^+ siphoning mechanism, whereby $[K^+]_o$ increase due to neuronal activity generates an influx of K^+ into astrocytes and K^+ efflux from their endfeet, which have a high density of inwardly rectifying K^+ channels (Newman 1984, 1986; Newman et al. 1984). However, the glial K^+ siphoning hypothesis, when tested in the retina, was shown not to contribute significantly to NVC (Metea et al. 2007). Later studies showed that NVC may be mediated by a second K^+-associated mechanism. Neuronal activity-evoked increases in Ca^{2+} and production of EETs in astrocytes activate Ca^{2+}-activated large conductance K^+ (BK) channels expressed in endfeet (Price et al. 2002; Gebremedhin et al. 2003; Filosa et al. 2006; Dunn and Nelson 2010), which result in K^+ efflux onto blood vessels and vessel dilation (Filosa et al. 2006; Girouard et al. 2010).

Another potential astrocyte-derived mediator for NVC is the Ca^{2+}-dependent release of D-serine. This amino acid is an important coagonist for *N*-methyl-D-aspartate receptors (NMDARs), which are critical for CBF regulation. The canonical view of NMDARs involves the generation of nitric oxide (NO) via neuronal nitric oxide synthase (NOS). However, these receptors are also expressed on vascular endothelial cells (Lu et al. 2017), and D-serine mediates vasodilation in brain slices evoked by direct astrocyte stimulation (Stobart et al. 2013). Furthermore, endothelial knockdown of NMDARs sig-

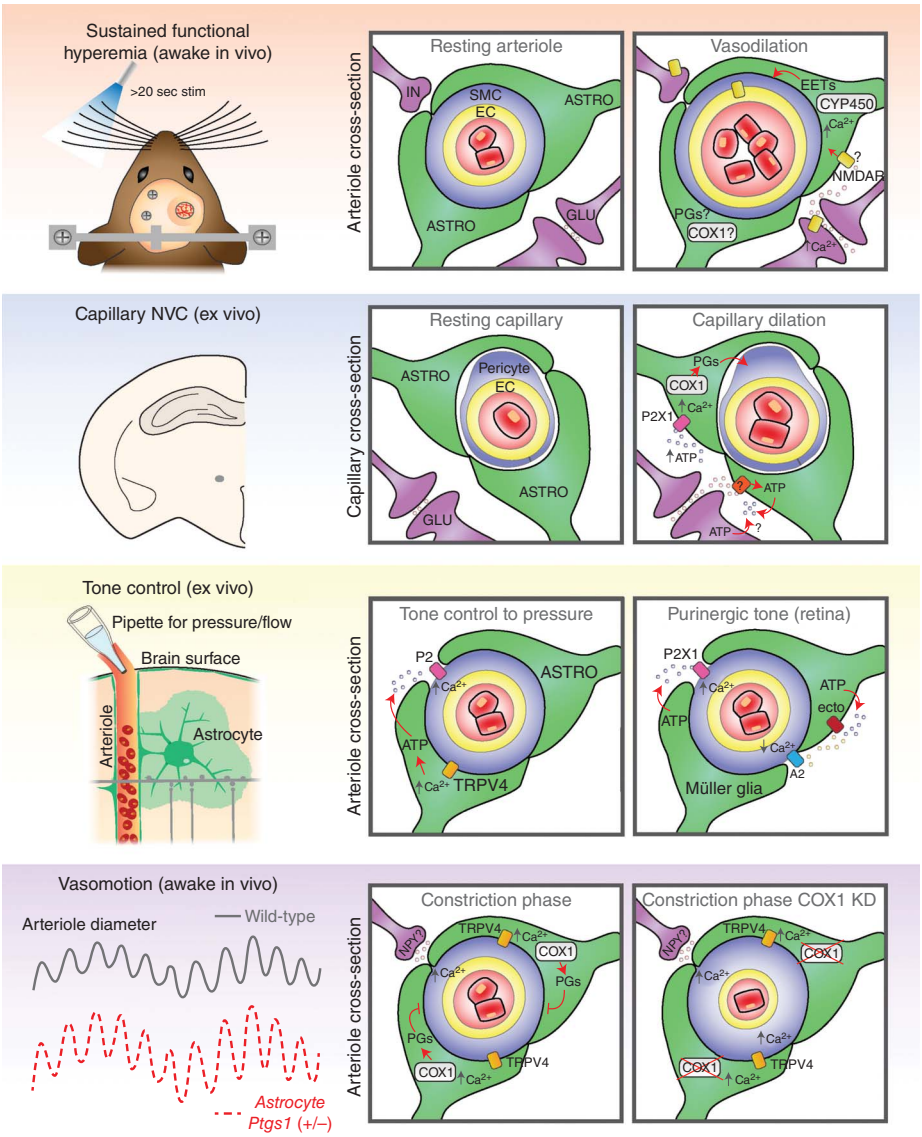

Figure 3. Diagram showing signaling pathways in astrocytes involved in four types of cerebral blood flow (CBF) regulation beyond brief functional hyperemia, which is typically studied. From *top* to *bottom*: (1) Astrocytes help amplify functional hyperemia to sustained neuronal activity through an NMDAR-dependent mechanism. The location of this receptor is unclear. NMDARs activate a CYP450 enzyme to generate EETs, boosting vasodilation that is primarily mediated by neuronal messengers. (2) Astrocytes participate in neurovascular coupling at the capillary level, but have no Ca^{2+}-dependent role at the arteriole level to brief neuronal activity. This involves an ATP- and COX1-dependent mechanism (see Fig. 2 for full pathway). (3) Astrocytes continually act to control steady-state arteriole tone in response to luminal pressure via TRPV4 in the neocortex, or through tonic purinergic signaling in the retina via P2X receptors. (4) Astrocyte endfeet sense vasoconstriction via TRPV4 and act to limit constriction by generating vasodilatory PGs from COX1 activity. Knocking down COX1 (*Ptgs1*) from astrocytes enhances vasoconstriction and oscillatory vasomotion in vivo. This mechanism likely acts more generally to constrain cerebral vasoconstriction. (A2) Adenosine A2 receptor, (ASTRO) astrocyte, (ATP) adenosine 5′-triphosphate, (COX1) cyclooxygenase 1, (CYP450) cytochrome 450 enzymes, (Ecto) ectonucleotidase, (EC) endothelial cell, (EETs) epoxyeicosatrienoic acid, (GLU) glutamatergic excitatory neuron, (IN) interneuron, (NMDAR) *N*-methyl-D-aspartate receptor, (P2) purinergic 2 receptors, (P2X1) purinergic 2 receptor X1, (PGs) prostaglandins, (Ptgs1) prostaglandin-endoperoxide synthase 1, (SMC) smooth muscle cell, (TRPV4) transient receptor potential cation channel subfamily V member 4.

nificantly reduced functional hyperemia in vivo (Hogan-Cann et al. 2019).

Bidirectional Control of Vessel Diameter

The first hints of the complexity of astrocyte regulation of CBF came from Metea and Newman (2006), where Ca^{2+} uncaging in Müller glia in retinal explants triggered both arteriole constrictions via 20-HETE or dilations via EETs. Here, NO was shown to be an important factor for switching the direction of the response: an NO donor converted light-evoked dilations to constrictions, whereas an NO scavenger switched light-evoked constrictions to dilations. A second explanation for the divergent observations that astrocyte Ca^{2+} signaling could trigger both dilations and constrictions came from Gordon et al.'s (2008) work in the hippocampus. The authors reported that the polarity of the vascular response to astrocyte Ca^{2+} transients in brain slices depended on the level of pO_2 in the superfusion solution. Arteriole constrictions evoked by Ca^{2+} uncaging in astrocytes in high O_2 (95%) solutions were reversed to arteriole dilations in more physiological O_2 (20%) solutions. Low pO_2 increased extracellular lactate concentrations, which in turn reduced the uptake and clearance of PGE_2 by the prostaglandin transporter, allowing PGE_2-mediated dilations to dominate. In high pO_2, lower lactate levels facilitated increased clearance of PGE_2 and allowed the conversion of AA to 20-HETE to dominate and cause constrictions (Fig. 3). The increased adenosine tone in low pO_2 solutions further reduced vasoconstriction (Gordon et al. 2008). A similar switch from arteriole dilation to constriction was observed in retinal explants when tissue pO_2 was altered from a low O_2 level to a high level (Mishra et al. 2011). However, this effect of O_2 was not evident in the in vivo retina when animals were made hyperoxic. This could be because hyperoxia-induced vasoconstriction (Mishra et al. 2011) maintains tissue pO_2 within a physiological range in vivo (Yu et al. 1999), allowing vasodilatory mechanisms to prevail (Mishra et al. 2011). High pO_2 has similar constrictive effects on capillaries in the cerebellum (Hall et al. 2014) and cortex, in a 20-HETE-dependent manner (Hirunpattarasilp et al. 2022). Another study found that astrocytes in vivo and in vitro respond with elevations in free Ca^{2+} when pO_2 goes below 15 mmHg, in a manner dependent on mitochondrial O_2 sensing, downstream IP_3 signaling, and ATP release (Angelova et al. 2015), although CBF effects were not explored. These results indicate that the polarity of astrocyte modulation of CBF may reflect the metabolic state of the tissue and may be modified by the levels of extracellular lactate and ATP/adenosine. Intriguingly, a link between the magnitude of CBF changes and lactate levels has also been observed in human subjects. Functional hyperemia correlates with brain lactate levels (Lin et al. 2010) and exogenously increasing the plasma lactate/pyruvate ratio results in larger CBF changes in response to physiological visual stimulation (Mintun et al. 2004).

Another variable dictating the polarity of the vessel's response may be the concentration of Ca^{2+} reached within astrocyte endfeet. Two independent groups have demonstrated this in acute brain slices. First, Girouard et al. (2010) showed that uncaging Ca^{2+} in astrocytes led to bidirectional responses of adjacent arterioles, which could be stratified by the size of the Ca^{2+} increase achieved in endfeet: large Ca^{2+} signals (>500 nM) caused vasoconstrictions while smaller Ca^{2+} signals (<500 nM) evoked vasodilation. Both constrictions and dilations depended on Ca^{2+}-sensitive BK channel opening, corresponding to high or low levels of K^+ efflux from endfeet onto vessels, respectively (Girouard et al. 2010). Separately, Haidey and Gordon (2021) used the whole-cell patch technique to directly clamp free Ca^{2+} within astrocytes to different concentrations and measure the resulting arteriole response. They reported the same general phenomenon: elevating Ca^{2+} to the moderate value of 250 nM caused dilations and elevating it highly to 750 nM caused constrictions. Here, the moderate rise in astrocyte Ca^{2+} increased dilations via PGE_2, while large astrocyte Ca^{2+} increases caused constrictions via 20-HETE. This study did not explore a role for BK channels. Haidey and Gordon's findings also support the O_2 switching hypothesis, showing that the moderate elevation to 250 nM free astrocyte Ca^{2+} during hyperoxia resulted in vasoconstriction.

Together, these findings indicate that the divergent observations made in early studies—where NVC could result in dilations in some cases while leading to constrictions in others—were likely due to differences in the extent of astrocyte Ca^{2+} increases or ambient conditions in the microenvironment (e.g., tissue concentration of O_2, NO, lactate, etc.).

RESOLVING ASTROCYTE CONTROVERSIES

While astrocyte stimulation is sufficient to induce changes in vessel diameter and CBF both ex vivo and in vivo, the question of whether they are necessary for physiological NVC is still open. It is well established that neuronal activity evokes Ca^{2+} signaling in astrocytes in vivo, but how consistent this response is and whether it precedes changes in CBF has been contentious. Many studies show that astrocyte Ca^{2+} signals develop slowly (>3 sec), and follow vasodilation (Nizar et al. 2013; Bonder and McCarthy 2014; Paukert et al. 2014; Tran et al. 2018), suggesting astrocytes do not initiate functional hyperemia. However, lack of observing fast or high-fidelity astrocyte Ca^{2+} signals could be due to limitations of the Ca^{2+} indicator tools available, ROI based analysis, and/or signal-to-noise problems. Recent studies using unbiased event-based analysis and targeted genetically encoded Ca^{2+} indicators have demonstrated rapid Ca^{2+} signaling in astrocyte processes and endfeet preceding arteriole dilation (Lind et al. 2013, 2018; Otsu et al. 2015; Stobart et al. 2018; Del Franco et al. 2022), but they did not examine a causal role for astrocyte Ca^{2+} signals in functional hyperemia. One prominent pathway to raise astrocyte cytoplasmic $[Ca^{2+}]$ is IP_3R2-dependent release of Ca^{2+} from the endoplasmic reticulum. Multiple groups reported that functional hyperemia (Takata et al. 2013; Bonder and McCarthy 2014; Del Franco et al. 2022) and BOLD fMRI signals (Jego et al. 2014) still occurred in IP_3R2 knockout animals and concluded that astrocyte Ca^{2+} signaling was not involved in vascular regulation. IP_3-dependent Ca^{2+} signaling occurs downstream of Gq-coupled receptors, and recent evidence from chemogenetic and optogenetic Gq activation studies suggest that astrocyte Gq-coupled receptor activation either do not contribute to NVC (Ozawa et al. 2023) or contribute only to the late phase following sustained neuronal activation (Institoris et al. 2022). However, there are other smaller and/or spatially restricted Ca^{2+} signals that can be detected in astrocytes lacking IP_3R2 (Srinivasan et al. 2015; Rungta et al. 2016; Stobart et al. 2018), which could still contribute to functional hyperemia. A second important consideration is the interpretation of experiments involving genetic knockout of IP_3R2. As discussed earlier, astrocyte-mediated regulation of CBF reflects only one component of several NVC systems (interneuron release of peptides, NO, PGE2 release from neurons etc. [Howarth et al. 2021]) that modify CBF and compensation from other pathways, within astrocytes or other cell types, can occur in knockout mice, even when genes are conditionally deleted in adulthood (Hösli et al. 2022). Therefore, the intact functional hyperemia observed in IP_3R2 knockouts does not definitively prove that this pathway has no role in CBF regulation. In support of astrocyte Ca^{2+}-dependent vascular control, ex vivo experiments show that buffering Ca^{2+} in astrocytes with BAPTA eliminates neuronal activity-dependent cortical capillary dilation without reducing arteriole dilation (Mishra et al. 2016). In awake mice in vivo, clamping and lowering astrocyte-free Ca^{2+} with a high-affinity plasma membrane Ca^{2+} ATPase pump called CalEx, had no effect on arteriole dilation evoked by a short 5-sec whisker stimulation, but reduced the late component of the response when stimulation was prolonged for 30 sec (Institoris et al. 2022). This suggests that astrocyte Ca^{2+} amplifies the late component of functional hyperemia. If astrocyte Ca^{2+} is also involved in the initiation of NVC, this process may only require small-amplitude, kinetically fast increases in Ca^{2+} in spatially restricted regions that astrocyte CalEx is unable to silence (Mishra et al. 2016). Identifying the endogenous trigger(s) and source(s) of the Ca^{2+} signals within astrocytes that underlie NVC remains an ongoing endeavor.

Another point of contention is the glutamate receptor involved in activating astrocytes. Astrocytic mGluR5 was proposed to mediate NVC (Zonta et al. 2003), but this work was performed

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on young tissues. mGluR5 is expressed in and can mediate astrocyte Ca^{2+} signaling in juvenile but not in adult mice (Sun et al. 2013) or rats (Duffy and Macvicar 1995). While it is now clear that mGluR5 is not responsible for functional hyperemia in adults (Calcinaghi et al. 2011; Mishra et al. 2016), which glutamate receptors, and on which cell types, are responsible for astrocyte-mediated CBF effects is still uncertain. This is important to resolve because synaptic glutamate release is the primary driver of CBF increases. NMDARs are well known to be critical for CBF regulation, and while the existence of functional astrocyte NMDARs are debated, there is some evidence for their ability to help control steady-state arteriole tone (Mehina et al. 2017). Furthermore, work in awake mice shows that NMDAR antagonism with APV dramatically reduces the late component of functional hyperemia when sensory stimulation is prolonged; the same time period in which astrocytes are implicated to enhance the CBF response (Institoris et al. 2022). Thus, selective knockout of astrocyte NMDARs as well as other glutamate receptors will be important to determine whether this is an important astrocyte-mediated pathway in NVC. Besides glutamate, many other neurotransmitters can evoke Ca^{2+} signaling in astrocytes (Schipke and Kettenmann 2004) and could also lead to CBF modulation. While we do not review all the possibilities here, a notable example is the local release of ATP, subsequent to an increase in neuronal activity. ATP is important for astrocyte-mediated NVC at the capillary level via P2X1 receptors (Mishra et al. 2016). However, it is still unclear whether ATP is generated by active neurons themselves or by astrocytes upon stimulation via another messenger from neurons (Figs. 2 and 3). It is also unclear whether P2X1 receptors on astrocytes themselves are involved, or whether the receptors are on another cell type upstream of astrocytes.

OTHER ROLES FOR ASTROCYTES IN CBF REGULATION

Regulation of basal vascular tone ensures a constant supply of O_2 and nutrients to the brain and is mediated by several mechanisms, including extrinsic autonomic innervation, intrinsic subcortical innervation, cortical interneurons, and vascular cells themselves (Cohen et al. 1996; Cauli et al. 2004; Hamel 2006; Bekar et al. 2012). Recent evidence indicates that vasoactive agents from astrocytes also contribute to vascular tone. As discussed above, astrocytes can produce the vasoconstrictor 20-HETE (Imig et al. 1996; Zou et al. 1996; Gebremedhin et al. 1998). The CYP450 enzymes that synthesize 20-HETE are inhibited by NO, a potent vasodilator (Oyekan et al. 1999; Hall et al. 2014), suggesting that a balance between 20-HETE and NO is important for setting the basal tone of cerebral blood vessels (Gordon et al. 2007; Metea et al. 2007). This conclusion is supported by observations that blocking NO synthesis constricts vessels via 20-HETE (Zonta et al. 2003; Mulligan and MacVicar 2004) and appears to be an important mechanism in setting basal tone during the very long-lasting (2 h) decrease in CBF following cortical spreading depression (Fig. 2; Fordsmann et al. 2013). Dynamic changes in NO synthesis also contribute to basal tone regulation. Interestingly, mitochondria are enriched in astrocyte endfeet (Busija et al. 2016; Göbel et al. 2020). A recent report showed that NO synthesis from astrocytic mitochondria via a NOS-independent pathway requiring reduction of NO_2^- underlies vasodilation during mild hypoxia (Christie et al. 2023). Other reports have suggested that hypoxic dilation depends, to some extent, on NO production by mitochondrial NOS (Lacza et al. 2001), potentially from astrocyte endfeet.

Astrocytes also generate vascular tone by the tonic release of ATP (Kur and Newman 2014). Hippocampal astrocytes tonically release ATP, resulting in extracellular ATP levels of ~10 μM (Pascual et al. 2005) while astrocytes and retinal Müller cells release ATP in response to cellular Ca^{2+} increases (Newman 2001). Released ATP tonically constricts arterioles by activating P2X1 receptors on vascular smooth muscle cells. Enzymatic degradation of extracellular ATP, blockage of P2X1 receptors, and selective poisoning of glial cells with the toxin fluorocitrate reduced vascular tone (Kur and Newman 2014). Additionally, cortical astrocytes in brain slices tonically release dilatory prostaglandins via COX1 activity (Rosenegger et al. 2015) and this

mechanism can even be initiated in response to vasoconstriction (Haidey et al. 2021). Chemogenetically induced arteriole constriction triggered a small, but constant elevation of astrocyte-free Ca^{2+} in a TRPV4-dependent manner, which triggered COX1 activity to mitigate vasoconstriction through the production of a vasodilator (Haidey et al. 2021). In other work in brain slices, astrocytes were shown to be required for sustaining luminal pressure-induced vasoconstriction. When astrocytes were filled with BAPTA to buffer their free Ca^{2+} and luminal pressure was applied, steady-state vascular tone was reduced after an equilibration period, suggesting the loss of an astrocyte-derived vasoconstrictor (Kim et al. 2015).

The discoveries of steady-state vascular tone control by astrocytes have opened new avenues to explore how astrocytes contribute to CBF regulation. Notably, arterioles and low-order capillaries are rarely static in vivo and instead their diameter oscillates at ~0.1 Hz in a process called vasomotion (Mateo et al. 2017). Recent work suggests astrocytes may be important players contributing to this rhythmic vascular activity. In awake mice with an acute cranial window, astrocyte endfoot Ca^{2+} levels oscillate in a manner that is anticorrelated to arteriole diameter: peak vasoconstriction correlated with higher endfoot Ca^{2+}, whereas peak dilation correlated with lower endfoot Ca^{2+} (Haidey et al. 2021). Interestingly, astrocyte-selective knockdown of COX1 using floxed PTGS1 mice amplified vasomotor oscillations in arteriole diameter compared to controls (Fig. 3). This is consistent with astrocyte endfeet limiting the degree of vasoconstriction in a Ca^{2+} and COX1-dependent manner, resulting in larger downward oscillations when COX1 levels are lower.

Finally, astrocytes contribute to CBF regulation through CO_2 sensing. CO_2 has long been recognized as a potent vasodilator and may arise either through brain-generated CO_2 from metabolism or systemic elevations that travel via the blood to the brain. In acute brain slices, local elevation of CO_2 evoked a Ca^{2+} increase in astrocytes and downstream COX1-dependent vasodilation (Howarth et al. 2017). Glutathione was identified as a key mediator in this pathway due to the dependence of microsomal prostaglandin E synthase-1 on this cellular antioxidant. More recently, systemic CO_2 elevation was shown to occlude functional hyperemia using fMRI, and the effects could not be explained by a ceiling effect (Hosford et al. 2022). In the same work, using direct O_2 measurements in the cortex as a proxy, functional hyperemia was blocked by selectively knocking out sodium bicarbonate cotransporters in astrocytes, a protein critical for CO_2 sensing via conversion to bicarbonate. This intriguing mechanism points to a Ca^{2+}-independent form of NVC through astrocytes, and further suggests that multiple known NVC pathways could converge onto CO_2 signaling (Fig. 4).

ASTROCYTES AND BLOOD FLOW DYSREGULATION IN PATHOLOGY

Alzheimer's Disease

Alzheimer's disease (AD) is characterized classically by extracellular accumulation of amyloid β (Aβ) plaques and intracellular inclusions of neurofibrillary tau tangles (Alzheimer 1907; Kosik et al. 1986; Goedert et al. 1988; Graeber and Mehraein 1999). In mouse models of AD, many physiological and phenotypic changes are also observed in the cells surrounding plaques, including abnormal synchronous neuronal hyperactivity, decreased glutamate uptake by astrocytes (Hefendehl et al. 2016), and signs of profound microglia activation (Terry et al. 1991; Wyss-Coray 2006; Venneti et al. 2008; Akiyama et al. 2000). Additionally, astrocytes exhibit spontaneous intercellular Ca^{2+} waves and higher resting Ca^{2+} levels (Kuchibhotla et al. 2009; Lines et al. 2022), while reactive astrocytes around Aβ plaques show enhanced Ca^{2+} transients due to their abnormally high expression of the metabotropic P2Y1 receptors (Delekate et al. 2014). The alterations in both neuronal and astrocyte Ca^{2+} signaling could synergistically lead to impaired vascular control and possibly vasoconstrictions and tissue hypoxia (Mulligan and MacVicar 2004; Gordon et al. 2008; Attwell et al. 2010). Aβ may also disrupt vasoregulation by increasing reactive oxygen species (Park et al. 2004, 2005; Nortley et al. 2019) that could be enhanced during transient hypoxia (Zhang

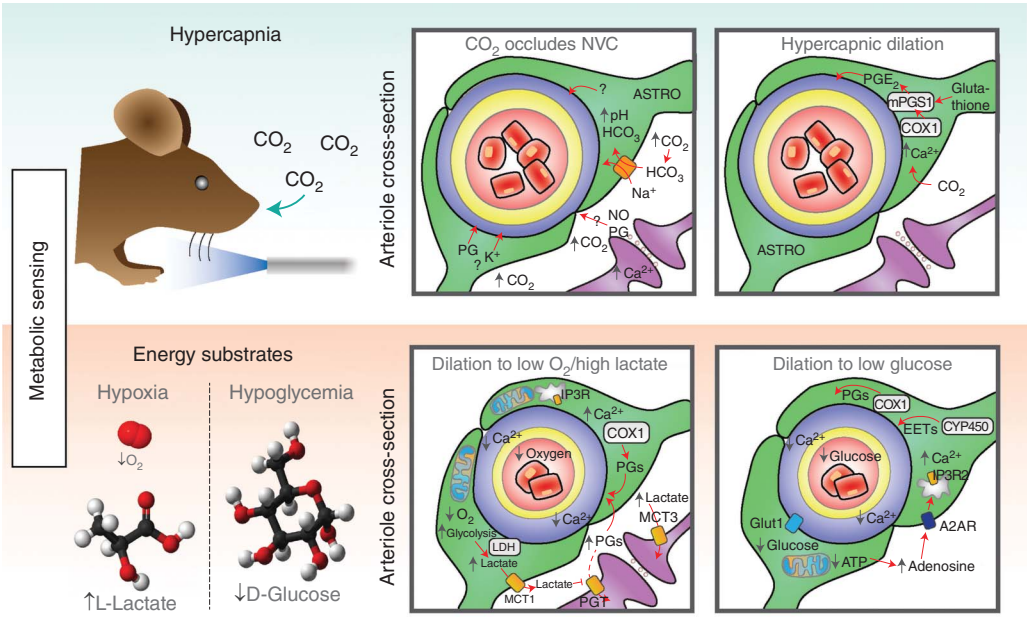

Figure 4. Diagram showing signaling pathways in astrocytes involved in cerebral blood flow (CBF) regulatory mechanisms related to metabolism and metabolites. *Upper* panels show two different mechanisms for how elevated CO_2 (hypercapnia) causes vasodilation through astrocytes. *Top middle* panel depicts a mechanism involving the sodium bicarbonate cotransporter, which is highly expressed by astrocytes. The mechanism mediating vasodilation is unclear. *Top right* panel shows a pathway involving Ca^{2+} elevation by CO_2 and the activation of COX1 and mPGS1 to generate vasodilatory PGE_2. *Lower* panels depict two mechanisms showing how energy substrates control CBF through astrocytes. *Bottom middle* panel shows how low O_2/high lactate (hypoxia) causes dilation by elevating PG signaling, in part by hindering PG uptake. *Bottom right* panel shows how low blood glucose (hypoglycemia) causes vasodilation through astrocytes via adenosine elevation, A2A receptors, and the Ca^{2+}-dependent generation of PGs and EETs. (A2AR) Adenosine A2 receptor, (ASTRO) astrocytes, (ATP) adenosine 5′-triphosphate, (CO_2) carbon dioxide, (COX1) cyclooxygenase 1, (CYP450) cytochrome 450 enzymes, (EETs) epoxyeicosatrienoic acid, (Glut1) glucose transporter 1, (HCO3) bicarbonate, (IP3R2) inositol 1,4,5-trisphosphate receptor 2, (LDH) lactate dehydrogenase, (MCT1) monocarboxylate transporter 1, (MCT3) monocarboxylate transporter 3, (mPGS1) microsomal prostaglandin E synthase-1, (NO) nitric oxide, (PG) prostaglandins, (PGE_2) prostaglandin E2.

et al. 2014). Neurovascular dysfunction is emerging as an early symptom in AD-related dementia, preceding even Aβ and tau pathologies (Iturria-Medina et al. 2016). Thus, disruptions in neurovascular signaling by astrocytes as well as neurons could be important in the development of Alzheimer's disease and vascular dementia (Iadecola 2013).

Diabetes and Hypoglycemia

Vascular pathology is the most serious manifestation of diabetes (Coucha et al. 2018) and the regulation of CBF is compromised in the disease. Functional hyperemia in the retina is disrupted in patients with diabetic retinopathy, a serious complication of diabetes. In healthy individuals, flickering light dilates retinal arterioles by ∼7% (Polak et al. 2002; Garhöfer et al. 2004a). This response is reduced by ∼60% in patients with type 1 or type 2 diabetes (Garhöfer et al. 2004b; Nguyen et al. 2009; Pemp et al. 2009) and in an animal model of type 1 diabetes (Mishra and Newman 2010, 2012).

Functional hyperemia may be lost in the diabetic retina due to the disruption of signaling

from glial cells to blood vessels. Glial-evoked dilation of blood vessels in the retina is reduced by NO (Metea and Newman 2006). In early stages of diabetic retinopathy, there is an up-regulation of iNOS (Du et al. 2002; Mishra and Newman 2010), leading to increased NO levels (Kowluru et al. 2000) and a reduction in glial-evoked vessel dilation (Mishra and Newman 2010). Inhibition of iNOS in diabetic animals to reduce NO production restored both glial-evoked and light-evoked vessel dilation to control levels (Mishra and Newman 2010, 2012).

Hypoglycemia, a reduction in blood glucose concentration, is a serious complication of insulin treatment for diabetes (Johnson-Rabbett and Seaquist 2019). Hypoglycemia induces vessel dilation and a global increase in CBF (Neil et al. 1987), mediated, in part, by the Ca^{2+}-dependent release of PGE_2 and EETs from astrocytes. Astrocyte Ca^{2+} signaling in the mouse somatosensory cortex increases as blood glucose falls and hypoglycemia-induced arteriole dilation is decreased when astrocyte Ca^{2+} signaling is reduced or PGE_2 and EETs synthesis is blocked (Nippert et al. 2022). These findings implicate astrocytes in hypoglycemia-evoked CBF increases (Fig. 4).

Epilepsy

Epileptic events, consisting of aberrant, high-frequency, synchronized neuronal activity, are associated with profound changes in vessel diameter, CBF, and oxygenation (Zhao et al. 2009; Gómez-Gonzalo et al. 2011; Farrell et al. 2016). While many studies have observed hyperemic responses in the seizure focus during ictal activity, pathological drops in CBF have been reported after the termination of long-duration ictal events (Farrell et al. 2016). This causes dangerously low levels of O_2 (<10 mgHg) persisting for ~1 h. Sustained vasoconstriction was also observed in vivo in mice (Tran et al. 2020), with correlated Ca^{2+} signals in astrocytes and vascular smooth muscles. A follow-up study found that generation of AA from breakdown of the endocannabinoid 2-AG and subsequent metabolism by COX2 into PGE_2 caused the vasoconstriction by acting on the EP1 receptor (Farrell et al. 2021). This is an intriguing new pathway for CBF regulation. While PGE_2 is mostly studied as a vasodilator during functional hyperemia, which it achieves by acting on EP4 receptors at low doses, it can also vasoconstrict at high doses by acting on EP1 receptors (Dabertrand et al. 2013; Czigler et al. 2020). The role of COX2 and 2-AG suggests that neurons are the most likely source of high PGE_2 in epilepsy. However, astrocytes may be involved to limit the degree of vasoconstriction through TRPV4-COX1 feedback dilation pathways (Haidey et al. 2021). An in vivo study of a 4-AP model of epilepsy found that high endfoot Ca^{2+} was associated with reduced vessel diameter, whereas lower endfoot Ca^{2+} was associated with larger vessel diameters during ictal events (Zhang et al. 2019). It is not clear whether these astrocyte Ca^{2+} signals exacerbate the reduction in CBF or attempt to minimize it.

Stroke

Stroke is a vascular disorder caused by either the blockage (ischemic stroke) or rupture (hemorrhagic stroke) of a cerebral blood vessel. In patients surviving ischemic stroke, brain-wide impairments in cerebral autoregulation, hypercapnic hyperemia and NVC have been reported (Krainik et al. 2005; Lin et al. 2011; Salinet et al. 2015). A recent rodent study found that the reduction in NVC after stroke is caused by increased synthesis of 20-HETE, one of the vasoconstrictors produced by astrocytes (Li et al. 2021). Increased 20-HETE is also reported in both ischemic and hemorrhagic stroke patients and predicts worse outcomes (Crago et al. 2011; Donnelly et al. 2015; Yi et al. 2017). Other groups have reported that astrocytes react to ischemic events by exhibiting large Ca^{2+} signals (Ding et al. 2009; Rakers and Petzold 2017), which may increase 20-HETE synthesis (Haidey and Gordon 2021). Observations that attenuating reactive astrogliosis mitigates CBF defects and improves neurological outcomes after ischemic stroke further support this hypothesis (Begum et al. 2018).

Hemorrhagic stroke also disrupts cerebral autoregulation (Koide et al. 2021) and impairs NVC in animal models. Interestingly, after a hemorrhage, activity-dependent responses of arterioles switch from vasodilations to constric-

tions, resulting in decreased CBF (Balbi et al. 2017). This inversion of NVC has been attributed to abnormally large purinergic-mediated Ca^{2+} signals in astrocytes (Pappas et al. 2015, 2016), which increase BK channel activity and lead to large K^+ efflux from endfeet, causing vessel constriction (Koide et al. 2012).

Cortical spreading depolarization, which is common after both ischemic and hemorrhagic strokes, also results in large Ca^{2+} waves in astrocytes (Chuquet et al. 2007), and is followed by a wave of vasoconstriction and NVC inversion (Chuquet et al. 2007; Major et al. 2017). These effects are partly mediated by an increase in 20-HETE (Fordsmann et al. 2013), although the exact role of astrocytes in this process has yet to be determined.

CONCLUSION

The regulation of CBF is essential for proper brain function. Astrocytes contribute to the regulation of CBF in several ways. Ca^{2+}-dependent synthesis of AA metabolites by astrocytes can bidirectionally modulate CBF: synthesis of PGE_2 and EETs dilates blood vessels while 20-HETE constricts vessels. Ca^{2+}-dependent release of K^+ also contributes to bidirectional vascular regulation. The precise physiological contexts in which astrocytes participate in NVC as well as the mechanisms underlying astrocyte Ca^{2+} signaling related to this process continue to be refined. While astrocytes may help control capillary bed perfusion or amplify sustained elevations in CBF to neuronal activation, new roles for astrocytes outside functional hyperemia have also been proposed. These include vascular tone setting, vasomotion, and metabolic sensing. Under pathological conditions, including Alzheimer's disease, epilepsy, stroke, and diabetic retinopathy, disruption of normal astrocyte physiology can compromise CBF regulation. This can exert a negative impact on tissue health and contribute to neurodegeneration.

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