

# Astrocyte Control of the Cerebrovasculature

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## KEY WORDS

cerebrovasculature; astrocytes; arachidonic acid; calcium

## ABSTRACT

The control of cerebral vessel diameter is of fundamental importance in maintaining healthy brain function because it is critical to match cerebral blood flow (CBF) to the metabolic demand of active neurons. Recent studies have shown that astrocytes are critical players in the regulation of cerebral blood vessel diameter and that there are several molecular pathways through which astrocytes can elicit these changes. Increased intracellular  $\text{Ca}^{2+}$  in astrocytes has demonstrated a dichotomy in vasomotor responses by causing the constriction as well as the dilation of neighboring blood vessels. The production of arachidonic acid (AA) in astrocytes by  $\text{Ca}^{2+}$  sensitive phospholipase  $\text{A}_2$  (PLA<sub>2</sub>) has been shown to be common to both constriction and dilation mechanisms. Constriction results from the conversion of AA to 20-hydroxyeicosatetraenoic acid (20-HETE) and dilation from the production of prostaglandin  $\text{E}_2$  (PGE<sub>2</sub>) or epoxyeicosatrienoic acid (EET) and the level of nitric oxide (NO) appears to dictate which of these two pathways is recruited. In addition the activation of  $\text{Ca}^{2+}$  activated  $\text{K}^+$  channels in astrocyte endfeet and the efflux of  $\text{K}^+$  has also been suggested to modify vascular tone by hyperpolarization and relaxation of smooth muscle cells (SMCs). The wide range of putative pathways indicates that more work is needed to clarify the contributions of astrocytes to vascular dynamics under different cellular conditions. Nonetheless it is clear that astrocytes are important albeit complicated regulators of CBF. © 2007 Wiley-Liss, Inc.

## OVERVIEW

The regulation of cerebral blood flow (CBF) has been known to be modulated by factors intrinsic to the brain for over 110 years (Roy and Sherrington, 1890). Although a great deal of inquiry has identified many factors that have direct consequences on cerebral vessel tone, including PGE<sub>2</sub> (Ellis et al., 1979), EETs (Ellis et al., 1990; Gebremedhin et al., 1992), 20-HETE (Gebremedhin et al., 2000), adenosine (Dirnagl et al., 1994; Ngai et al., 2001), NO (Lindauer et al., 1999), and  $\text{K}^+$  (Filosa et al., 2006), there is still much to be understood about the precise physiological conditions under which these molecules induce changes in vessel diameter. One such condition that is fundamental to normal brain operation is functional hyperemia, which is the active process of enlarging vessel diameter in response to the rising metabolic demands of enhanced synaptic transmission and neuronal spiking. This occurs so that CBF may be augmented

to supply additional oxygen and nutrients to the working cells, while at the same time clearing metabolic products such as  $\text{CO}_2$ . Changes in blood flow and the oxygenation levels of hemoglobin that result from local alterations in neuronal activity can be detected using several imaging strategies (Boas et al., 2004; Chen et al., 2003; Malonek et al., 1997; Shariff et al., 2006) to provide maps of functional activation of discrete brain volumes in the CNS—tools that have proven immensely helpful to the study of both normal and pathological brain functioning. The implicit assumption underlying the mapping of brain function by imaging CBF changes is that the degree of blood flow is a simple function of neuronal activation. However, several studies have hinted that the link between activity and vasomotor responses is highly complex, a situation that is only exacerbated by an incomplete understanding of the cellular mechanisms responsible for coupling changes in neuronal activation to changes in CBF.

In spite of this however, progress has recently been made towards explaining this important phenomenon by examining astrocytes as the critical link between neurons and brain vasculature. Astrocytes are in an ideal anatomical position to enable the transfer of information on the state of the extracellular environment from neural structures directly to vessels. Processes from a single astrocyte can associate with numerous synapses (Bushong et al., 2002; Haber et al., 2006; Ventura and Harris, 1999), contact adjacent astrocytes to form a glial syncytium (Fischer and Kettenmann, 1985; Massa and Mugnaini, 1982), and form perivascular ‘endfeet’ on arterioles and capillaries (Simard et al., 2003). Endfeet are enlarged astrocytic compartments that appear to be specialized for the direct interaction with vessels. Together with the collective arrangement of many other astrocyte processes, all cerebral arterioles in the brain are encased by endfeet. In addition to this anatomical propensity that suggests a prominent role in vasculature physiology, astrocytes also wield an equally impressive signaling capability. This includes but is not limited to long-range inter-astrocytic  $\text{Ca}^{2+}$  waves (Cornell-Bell et al., 1990; Newman and Zahs, 1997) and an ability to sense transmitters such as glutamate (Cornell-Bell et al., 1990), GABA (Kang et al. 1998),

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Received 23 May 2007; Accepted 13 June 2007

DOI 10.1002/glia.20543

Published online 20 July 2007 in Wiley InterScience (www.interscience.wiley.com).

norepinephrine (Duffy and MacVicar, 1995) and ATP (Newman and Zahs, 1997), which may enable the transmission of relevant information about the extracellular environment to vessels. Towards this aim, astrocytes release numerous vasoactive substances such as ATP (Guthrie et al., 1999; Queiroz et al., 1999), adenosine, (Zhang et al., 2003) NO (Murphy et al., 1993), PGE<sub>2</sub>, and EETs (Amruthesh et al., 1993). Furthermore, the endfeet are themselves endowed with many elements speculated to partake in cerebrovasculature control. Endfeet are thought to express noradrenergic receptors (Mulligan and MacVicar, 2004; Paspalas and Papadopoulos, 1996), soluble PLA<sub>2</sub> (Farooqui et al., 1997), nitric oxide synthase (NOS) (Calka and Wolf, 2003) and hemichannels (Nagy et al., 1999; Simard et al., 2003), while the concentration of aquaporin 4 water channels (Nicchia et al., 2000), Ca<sup>2+</sup> activated K<sup>+</sup> channels (Price et al., 2002) and purinergic receptors (Simard et al., 2003) is higher here than in any other astrocytic compartment. These characteristics suggest that astrocytes and particularly the endfeet are well equipped to produce diffusible, vasoactive molecules to communicate directly to arteriole smooth muscle cells (SMCs) and thus contribute to the physiology of blood vessel control.

Because of these unique anatomical and physiological characteristics, the impact of astrocyte Ca<sup>2+</sup> signaling on cerebral blood vessel diameter has been intensively examined in several recent publications. The results have shown that astrocyte Ca<sup>2+</sup> transients in endfeet can lead both to constrictions and to dilations. In this review we will examine the functional roles of astrocyte Ca<sup>2+</sup> signaling, highlight the different experimental models and approaches that may have lead to these disparate results, as well as discuss the importance of NO, K<sup>+</sup>, vasoactive interneurons and pericytes in the control of cerebrovascular dynamics.

#### DIFFERENT TRANSMITTERS CAN HAVE OPPOSING ACTION ON VASOMOTOR RESPONSES

Astrocytes have been shown to respond to numerous transmitters including glutamate, which causes an increase in intracellular Ca<sup>2+</sup> through the activation of the mGluR subtype (Cornell-Bell et al., 1990). It has been suggested that astrocytes sense the increased extracellular glutamate from synaptic activation and the evoked Ca<sup>2+</sup> increases in the endfeet trigger the release of diffusible factors that then act within SMCs to cause dilation of arterioles (Zonta et al., 2003). Application of mGluR agonists has been shown in several studies to activate astrocytes and in turn, affect vasomotor tone (Filosa et al., 2004; Mulligan and MacVicar, 2004; Zonta et al., 2003). However, evidence also points to the importance of other inputs to astrocytes in addition to glutamatergic. Filosa et al. has demonstrated that in the presence of a thromboxane analog to increase vascular tone, electrical stimulation of the tissue increased endfoot Ca<sup>2+</sup> and reduced spontaneous Ca<sup>2+</sup> signals in SMCs, but neither of these

effects were completely blocked by mGluR antagonists (Filosa et al., 2004).

Along this line, it is clear from the anatomical arrangement of vasoactive nerve terminals that the totality of the neurovascular link is more complicated than simply sensing local fluctuations in glutamate concentration. An electron microscope analysis of noradrenergic terminals originating from the locus coeruleus has showed that the majority of synapses associated with cerebral blood vessels occur on astrocyte endfeet, rather than SMCs (Paspalas and Papadopoulos, 1996). NE is a potent activator of intracellular Ca<sup>2+</sup> increases in astrocytes via activation of  $\alpha_1$  and  $\beta$  adrenergic receptors (Duffy and MacVicar, 1995) and previous *in vivo* work has revealed that activation by NE causes a decrease in CBF (Raichle et al., 1975). Our experiments, in which Ca<sup>2+</sup> signals were measured within the discrete volumes of individual astrocytes and astrocytic endfeet, showed that NE-mediated Ca<sup>2+</sup> increases temporally preceded prominent vasoconstrictions (Mulligan and MacVicar, 2004). Loading astrocytes with BAPTA-AM to chelate and dampen rises in intracellular Ca<sup>2+</sup> greatly reduced vascular constrictions generated by NE, suggesting Ca<sup>2+</sup> was a critical element for the astrocyte-mediated effect.

Collectively, these experiments indicated that (1) astrocytes possess the necessary physiology to both constrict and dilate arterioles and (2) the increases in endfeet Ca<sup>2+</sup> observed in response to different transmitters can have opposing effects on vessel girth. It was also clear from these tests that the basic model for functional hyperemia and the role astrocytes play in this process may be more complex than originally speculated. The first course of action for our lab was to determine to role of Ca<sup>2+</sup> itself, without incorporating the involvement of membrane bound receptors. From our results from the NE experiments we hypothesized that increased Ca<sup>2+</sup> in astrocytes could lead to vasoconstriction.

#### CONSTRICTIONS VERSUS DILATIONS *In Vitro*

The most direct way to test the roles for Ca<sup>2+</sup> in astrocytes is to uncage Ca<sup>2+</sup> using two-photon photolysis, because it allows one to increase Ca<sup>2+</sup> selectively within the volume of astrocytes without affecting the surrounding tissue. In our study (Mulligan and MacVicar, 2004), astrocytes were identified in transgenic mice that expressed EGFP driven by the GFAP promoter. The astrocytes were loaded with the-AM form of Rhod-2, the Ca<sup>2+</sup> sensitive dye. Rhod-2 is a good Ca<sup>2+</sup> indicator in two-photon laser scanning microscopy experiments because there is sufficient two-photon cross section to achieve signals from excitation wavelengths of 730–900 nm.

Under conditions typical of brain slice recordings, Ca<sup>2+</sup> uncaging in astrocytes in our lab induced Ca<sup>2+</sup> waves that propagated throughout the astrocyte syncytium and invaded endfeet. When the Ca<sup>2+</sup> wave fully invaded the endfeet we observed constrictions of adjacent arterioles in both hippocampal and cortical slices. The extent of the

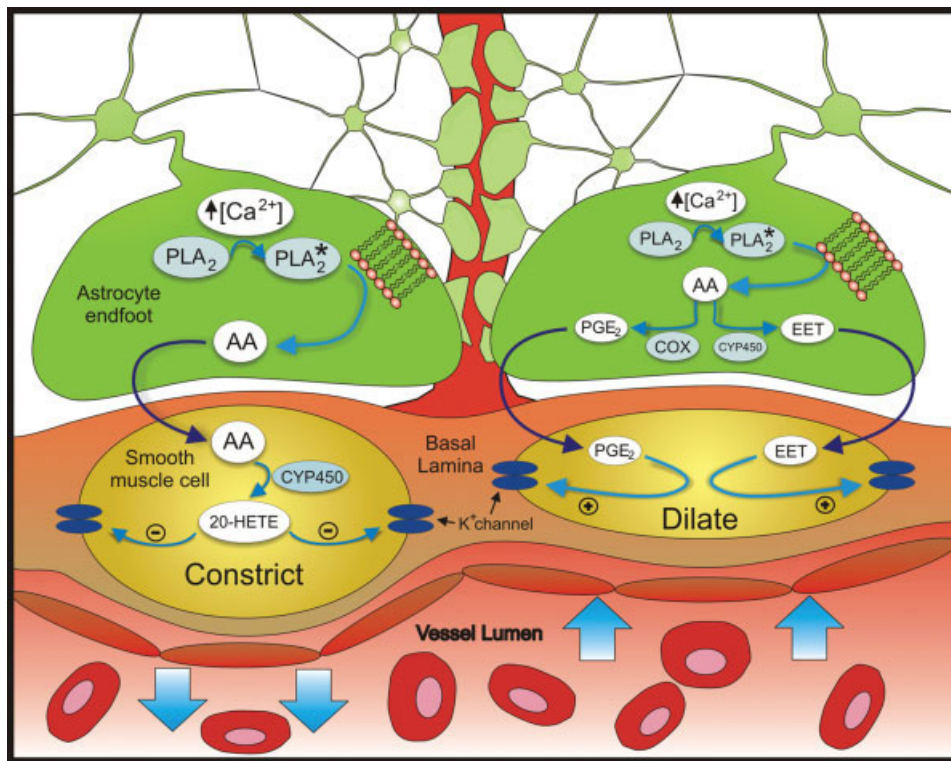


Fig. 1. Astrocyte endfeet control cerebrovasculature diameter. Two separate endfeet making contact with an arteriole show possible constriction (left endfoot) and dilation (right endfoot) mechanisms. The site of vasoactive substance synthesis in the diagram is hypothesized based on the location of the highest expression of the enzymes.

constriction followed the extent of the  $Ca^{2+}$  wave in endfeet and the peak  $[Ca^{2+}]_i$  increase in the endfeet preceded the initial onset of vessel constriction by  $2.7 \pm 0.5$  s. Both of these observations suggest a pattern and timing associated with the generation of second messengers and release of a diffusible factor from astrocytes. The pharmacological sensitivity of this response supported this conclusion. Constrictions were blocked by inhibiting the  $Ca^{2+}$  dependent enzyme  $PLA_2$  and preventing the release of AA. The constrictions of the arterioles are caused by contractions of SMCs which appears to result from the generation of 20-HETE from the astrocyte derived AA by CYP4504a (Gebremedhin et al., 2000). A selective CYP4504a inhibitor also blocked constrictions.

In the first study showing potential relationships between astrocyte  $Ca^{2+}$  signals and changes in vascular tone in brain slices, stimulating astrocytes indirectly by afferent stimulation, disrupting membranes with patch electrodes or by applying mGluR agonists elicited modest but consistent vasodilation (Zonta et al., 2003). The authors found that dilations were observed far less frequently in the presence of the COX inhibitor aspirin, suggesting COX products like  $PGE_2$  or prostacyclin were involved. In a subset of experiments blood vessels were precontracted by incubating brain slices with L-NAME to inhibit NOS and reduce NO levels. In this condition, vasodilations were much more pronounced. As detailed earlier, our lab observed consistent vascular constrictions when the mGluR agonist t-ACPD was applied under untreated conditions (Mulligan and MacVicar, 2004). However, when our lab blocked NO synthesis with L-NAME, like in Zonta et al., mGluR application now produced dilation.

In retinal explants, Newman's lab showed that  $Ca^{2+}$  uncaging in retinal astrocytes using UV excitation caused constriction in arterioles (Metea and Newman, 2006). This effect was shown to be generated by astrocyte AA generation and conversion to 20-HETE. However, in contrast to our laboratory, who only observed constrictions, in a substantial portion of experiments, Metea et al. observed that  $Ca^{2+}$  uncaging in astrocytes also caused dilation. This indicates an intriguing complexity in the communication of astrocytes with arterioles. The dilations were caused also by AA release from astrocytes which was then converted to EET by another CYP450 enzyme (Fig. 1 for the possible astrocyte-mediated constriction and dilation mechanisms). In these experiments the retina levels of NO were again speculated to dictate the type of vasomotor response by regulating the enzymatic conversion of AA to either EET or 20-HETE. Consistent with this idea, in the presence of the NO donor SNAP, vasodilations were transformed into vasoconstrictions, an outcome likely due to a NO sensitivity of CYP450 (Fleming, 2001; Roman, 2002) enzymes responsible for generating vasodilating EETs.

Clearly these studies show that astrocytes can induce constriction or dilation of adjacent arterioles and that the level of NO or the degree of tonic constriction may alter the response of the vessels. The cellular source of NO that is influencing the polarity of astrocyte-mediated vasomotor responses is an important issue needing clarification. There is evidence for the participation of neuronal NO in functional hyperemia, rather than that derived from endothelial cells (Ayata et al., 1996; Ma et al., 1996), but NO from astrocytes (Gibson et al., 2005) has not yet

been thoroughly examined in this process. Also, most studies examining the impact of NO on synaptic transmission have demonstrated that NO induces marked enhancements in the release of a variety of neurotransmitters, including glutamate (Prast and Philippu, 2001). This idea needs to be reconciled with the vessel data showing higher NO levels promote astrocyte-mediated vasoconstriction. Reconciliation may only occur after the source, spread, and timing of NO production is better delineated for functional hyperemia.

A recent set of publications has placed a new role on different subtypes of cortical, GABAergic interneurons whose processes can make close apposition with the walls of microvessels (Cauli et al., 2004; Tong and Hamel, 2000). Notably, depending on the subtype, which was determined by single cell RT-PCR, interneurons induced constrictions or dilations. Activity in somatostatin-expressing interneurons triggered constrictions while activity in interneurons expressing vasoactive intestinal polypeptide or NOS elicited dilations. Here again we see a dichotomy in the control of vasomotor responses. This is a stark reminder that the primary assumptions which underlie functional hyperemia—which states the degree of blood flow is a simple function of the metabolic state and therefore the level of neuronal activation—need reconsideration.

### *In Vivo*

A recent article described the effects of  $\text{Ca}^{2+}$  uncaging in astrocyte endfeet *in vivo* on the diameter of arteries in somatosensory cortex (Takano et al., 2006). Astrocytes were loaded with the  $\text{Ca}^{2+}$  indicator dye Rhod-2 and caged  $\text{Ca}^{2+}$  DM-nitrophen. Uncaging using a UV laser increased  $\text{Ca}^{2+}$  in the endfeet of astrocytes and evoked mainly dilations and some constrictions. Because of the infrequency of the observation, constrictions were not further characterized, whereas dilations were analyzed with respect to their pharmacological sensitivity. Application of inhibitors to COX-1 but not COX-2 prevented the dilation from astrocyte  $\text{Ca}^{2+}$  uncaging. Immunohistochemical staining for COX-1 showed intense reactivity at cerebral blood vessels which was suggested to overlap with GFAP +ve astrocyte endfeet. However, it is difficult to rigorously ascertain that COX-1 proteins were indeed in the endfeet versus being located in closely apposed perivascular microglia or cells of the blood vessels such as endothelial cells. The immunostaining also revealed a lack of COX-1 in GFAP+ve processes that were far from the vessels, suggesting that COX-1 expression is, at least, localized to the vessel region. This study also examined a role for NO and adenosine in astrocyte-mediated dilations by applying L-NAME to block NOS and caffeine to nonspecifically block adenosine receptors. Neither treatment affected the degree of induced dilation when  $\text{Ca}^{2+}$  was uncaged in astrocyte endfeet. NO donor molecules were not examined and it would be interesting to see if vessels *in vivo*, when subjected to higher levels of NO, show a tendency towards vasoconstriction when astrocytes are stimulated—

as observed in the *in vitro* preparations (Metea and Newman, 2006; Mulligan and MacVicar, 2004).

An alternative *in vivo* strategy that can be used to address similar questions on the control of brain blood flow is intrinsic optical imaging, in which alterations in metabolism and CBF can be detected by changes in the reflectance of light off the brain when illuminated. Recently, Gurden et al. (2006) used this technique in glomeruli of the olfactory bulb to study the mechanisms involved in generating intrinsic optical signals (IOSs) evoked by physiological odor presentation. Notably, the authors found no link between odor evoked IOSs and the activation of ionotropic or metabotropic glutamate receptors. Instead, their findings implicated glutamate uptake through astrocyte transporters as the major contributor to odor evoked IOSs (Gurden et al., 2006). While an interesting result, what proportion of the measured IOSs actually represent a change in CBF due to an increase in vessel diameter is not known. *In vitro* studies suggest glutamate clearance via transporter activity can induce astrocyte swelling (Hansson et al., 1994). If a similar effect is occurring in the glomeruli when an odor is presented, an appreciable fraction of the IOS change observed may be the result of alterations in light scattering due to astrocyte swelling (MacVicar and Hochman, 1991).

### **$\text{K}^+$ AND VASCULAR CONTROL BY ASTROCYTES $\text{K}^+$ Siphoning Through Kir Channels**

It was first proposed 20 years ago by Newman that  $\text{K}^+$  efflux from Kir channels in the endfeet of glia in the retina could lead to blood vessel dilations (Newman et al., 1984; Paulson and Newman, 1987). This was the first hypothesis to point to a possible mechanism by which glial cells could control vascular tone. The hypothesis was based on the observation by Newman that high levels of Kir channels are expressed on the endfeet of Muller cells (Newman, 1984). However, this hypothesis has recently been disproved by Newman by two separate tests (Metea et al., 2007). First, they recorded from single glia in close proximity to a vessel and elicited large depolarizations that would be more than sufficient to permit efflux of  $\text{K}^+$  through open Kir channels. Under these conditions they failed to observe any change in vessel diameter. Second, they investigated the extent of  $\text{K}^+$  induced vascular effects in the retina in control versus the Kir KO mouse. When the vasomotor physiology was compared between the two mouse strains, no difference was observed in the degree of  $\text{K}^+$  induced dilation. This result was also corroborated by verifying the loss of the inwardly rectifying channel with electrophysiological recordings.

### **$\text{Ca}^{2+}$ Activated $\text{K}^+$ Release**

A role for  $\text{K}^+$  channels in astrocyte-mediated regulation of vascular tone may exist through a different mechanism proposed by the Nelson laboratory. In a recent publication they demonstrate that modest increases in

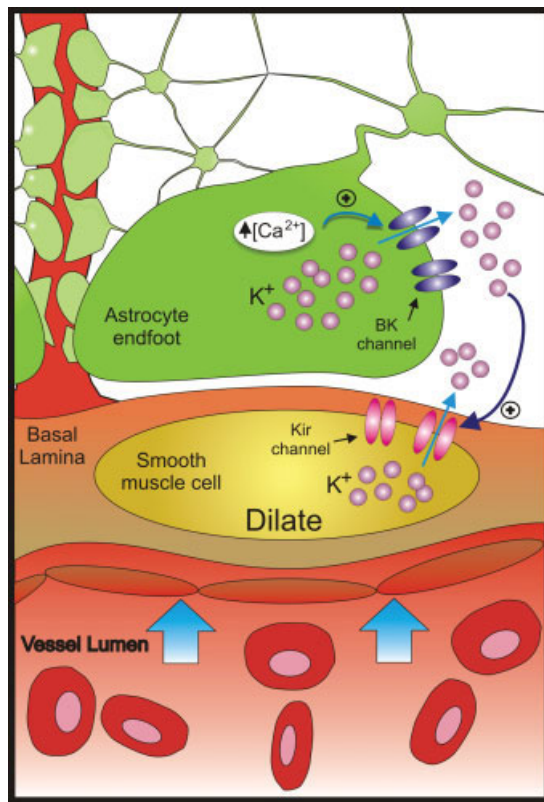


Fig. 2. Astrocyte endfeet-mediated cerebrovasculature dilation by the efflux of potassium from BK channels. Elevated extracellular potassium subsequent to BK activation opens Kir channels on SMCs, which causes vessel dilation via hyperpolarization and subsequent SMC relaxation.

external  $K^+$  promote dilation of vessels through Kir channels in SMCs (Filosa et al., 2006). Higher extracellular  $K^+$  causes hyperpolarization of SMCs by enhancing the Kir conductance, which leads to decreased  $Ca^{2+}$  entry, a relaxation of SMCs, and the consequent dilation. As with the Newman hypothesis, the high extracellular  $K^+$  trigger that initiates this process is proposed to come not from neurons but directly from astrocyte endfeet. Different from the Newman lab, the effect here is thought to involve endfeet large-conductance  $Ca^{2+}$  activated  $K^+$  channels (BK channels) (Price et al., 2002), which open in response to higher levels of intracellular  $Ca^{2+}$  to allow the efflux of  $K^+$  (Fig. 2). It is unclear how rises in endfeet  $Ca^{2+}$  and subsequent activation of BK channels would coexist with constriction mechanisms that also rely on an elevation of endfeet  $Ca^{2+}$ . One potential caveat to this work is the sole use of barium ions to determine a role for the Kir channels. Though barium is commonly used to block Kir channels, this treatment also elicits pronounced depolarizations of astrocytes (Anderson et al., 1995) and can out compete  $Ca^{2+}$  for the pore of L-type voltage gated  $Ca^{2+}$  channels, which are prominent in cerebral SMCs (Alborch et al., 1995). The additional use of an imidazole compound to block Kir channels would help support the author's conclusions (Favaloro et al., 2003). Placing an important role on SMC Kir channels is likely not conflict-

ing with the data from the Newman lab showing that there is no difference in  $K^+$  induced dilations between wild type and Kir knockout mice because of the difference in the Kir subtypes involved. The predominant family of Kir channels in SMCs is 2.0 (Quayle et al., 1996), while it is the Kir 4.1 subtype that is absent in the Newman KO study (Metea et al., 2007). These data from the Nelson lab also suggest that  $K^+$  channels are not the only players in  $K^+$  or activity induced vasodilations because a portion of the dilation response was blocked by inhibitors of cyclooxygenase enzymes, suggesting vasoactive PGEs were also contributing.

### PERICYTE CONTROL OF CAPILLARY BLOOD FLOW

A new player in CBF control has recently been described: the pericyte (Peppiatt et al., 2006). Pericytes are small, oval cells that contain contractile proteins and are in direct contact with the endothelial cells comprising the wall of capillaries (Herman and D'Amore, 1985). Individual pericytes are solitary, keeping a fairly regular distance between themselves. Each cell has processes that project around and encircle the girth of the capillary, enabling a focused control of capillary diameter. Pericytes can elicit pronounced constrictions in response to electrical stimulation, NE and ATP, whereas glutamate triggers pericyte to relax the capillary wall (Peppiatt et al., 2006). Ischemia also results in focal capillary constrictions that corresponded to the location of pericytes, suggesting these cells may be responsible for a portion of the reduced CBF observed during this pathological condition. Notably, in spite of a lack of dye coupling between neighboring pericytes, the constriction observed in response to the electrical stimulation of one cell, was also observed at distant pericyte-controlled regions after a few tens of seconds (Peppiatt et al., 2006). This interesting result suggests that pericytes either release their own diffusible factors which travel appreciable distances to affect adjacent pericytes, or pericytes are communicating to each other by utilizing other cell types, which may include the endothelial cells of the capillary to which pericytes are physically connected via gap junctions (Wu et al., 2006) or the surrounding astrocyte syncytium. The latter possibility may explain why pericytes are sensitive to ATP, which is a ubiquitous astrocyte transmitter utilized for long-range paracrine signaling (Guthrie et al., 1999).

### FUNCTIONAL IMPACT OF ASTROCYTE $Ca^{2+}$ ON CEREBRAL BLOOD VESSELS

It is apparent from the studies described here that  $Ca^{2+}$  transients in astrocyte endfeet can have complex actions in modifying vascular tone. A burning question asked by many of the earlier studies is whether  $Ca^{2+}$  elevation in astrocytes is indeed the signal that transduces enhanced neuronal activity into dilated vessels—that is, functional hyperemia. Undoubtedly these experiments

indicate that astrocytes can alter vascular tone and that increases in astrocytic intracellular  $\text{Ca}^{2+}$  is a critical factor in mediating vasomotor responses, but the direct connection to functional hyperemia is unclear. CBF changes can be graded so that increased synaptic activation of neurons progressively increases CBF. Therefore, a central tenet of the hypothesis that astrocyte  $\text{Ca}^{2+}$  is the key link to functional hyperemia is that changes in astrocyte  $\text{Ca}^{2+}$  would be tightly coupled to synaptic activation and would progressively increase with increasing synaptic activation (Pasti et al., 1997; Zonta and Carmignoto, 2002). However, the curious inconsistency with this idea is that fluctuations in astrocyte  $\text{Ca}^{2+}$  fail to track faithfully that of neuronal activity. First,  $\text{Ca}^{2+}$  increases often occur independent of neuronal activity (Nett et al., 2002) and spontaneous  $\text{Ca}^{2+}$  oscillations have been reported both *in vivo* (Hirase et al., 2004) and in brain slices (Parri and Crunelli, 2001; Parri et al., 2001). Second, is the observation that neuronal activity does not consistently increase astrocyte  $\text{Ca}^{2+}$  with a time course that follows the time of activation. A recent report describing a new method of simultaneously recording neuronal and glial  $\text{Ca}^{2+}$  signals at frequencies up to 10 Hz in a large volume of cortical tissue shows the striking differences (Gobel et al., 2007). Furthermore, the  $\text{Ca}^{2+}$  waves thought to be important for relaying neuronal information through the astrocyte syncytium towards vessels are not always observed *in vivo*, suggesting this phenomenon may be attributable to aspects of the slice or culture condition, or that this effect will be more easily observed in the intact animal under pathological conditions such as epilepsy (Balazsi et al., 2003; Tashiro et al., 2002).

One set of experiments that has provided clues towards the functional impact of astrocyte  $\text{Ca}^{2+}$  signals in controlling CBF comes from the rise in endfeet  $\text{Ca}^{2+}$  induced by NE and the subsequent vessel constriction. For instance, autoregulation is process of maintaining CBF at a constant rate over a wide range of blood pressures. Shifting autoregulation into the upper range of blood pressures is thought to require increased vascular tone (Goadsby and Edvinsson, 2002); therefore, the vasoconstriction initiated by NE may represent a functional method for providing the necessary tone to brain vasculature to accomplish this feat.

The fact that endfeet  $\text{Ca}^{2+}$  signals are capable of initiating constriction or dilation of cerebral blood vessels suggests there are precise physiological circumstances in which each mechanism is recruited. The effect of NO has already been described earlier as a potential factor dictating the vessel response profile. Other factors may include the distance a particular section of vessel is located from the source of enhanced neural activity. Recently it has been demonstrated that cerebral vessels located in the center of a functional hyperemic region of brain tissue dilate but, interestingly, this core is surrounded by a concentric volume of tissue where the residing vessels constrict (Devor et al., 2005). This surround inhibition of vessel diameter is thought to enhance CBF to the oxygen requiring core and may represent the physiological condition in which both astrocyte-mediated constrictions and dilations are simultaneously utilized juxtaposed to each

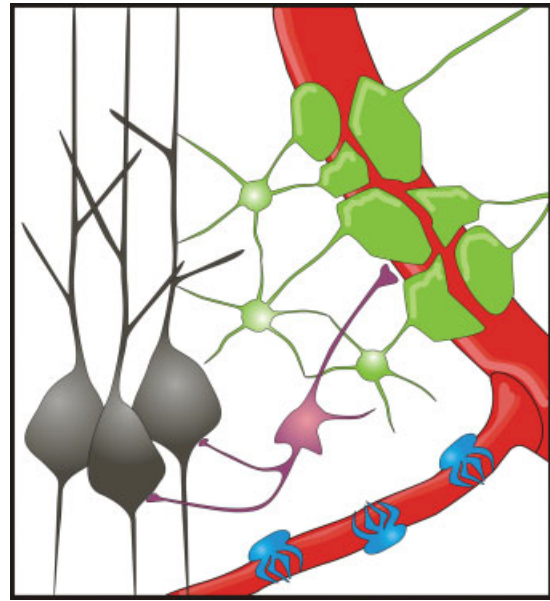


Fig. 3. Cell types thought to contribute to cerebrovasculature control. Arteriole and smaller capillary shown in red; principal neurons shown in gray; vasoactive interneuron shown in purple with a process in close apposition with the arteriole; pericytes shown in blue poised around the capillary; astrocytes shown in green with processes projecting to the neurons and to the arteriole. Arteriole astrocyte processes become enlarged endfeet and together, cover the arteriole surface.

other. How these disparate effects are selected for as a function of distance from the center of the functional hyperemic response is not known. One possibility may be differences in the level of brain gases such as  $\text{O}_2$  and/or  $\text{CO}_2$ , closer to versus farther away from the source of activity. This is an interesting prospect that is amenable to experimentation and has not yet been examined.

## CONCLUSION

As the control of CBF is critical in maintaining a functioning internal brain environment, a more comprehensive understanding of the precise physiological conditions, cell types, and signaling molecules involved in the control of cerebral vasculature is of fundamental importance. Confidence in notion that astrocytes and several molecular pathways are essential to vasomotor responses is rising. In particular, astrocytes appear capable of eliciting changes in vessel diameter in both directions, which rely on the initial activation of  $\text{Ca}^{2+}$  sensitive  $\text{PLA}_2$  and the production of AA. Constriction occurs when AA is converted to 20-HETE, while dilation results from the conversion of AA to PGE<sub>2</sub> or EET. The enzymes governing the production of these vasoactive products are sensitive to NO, suggesting NO levels may dictate the direction of the vessels response. In addition, a role for  $\text{Ca}^{2+}$  activated  $\text{K}^+$  channels in astrocyte endfeet and the efflux of  $\text{K}^+$  has also been suggested relax vascular tone by hyperpolarizing SMCs via Kir channels. With the addition of new players, pericytes and interneurons that produce vasoactive substances (Fig. 3), there is a rapidly expand-

ing complexity surrounding the overall control of CBF that is incompletely understood. Furthermore, the potential drawbacks of acute slices versus *in vivo* preparations are also hurdles to be overcome so that consensus can be achieved on the mechanisms of neurovascular coupling. From the above work it has become clear that while astrocytes are proving to be important mediators, a full understanding of processes such as functional hyperemia will be met with many more scientific challenges and successes as further complexity is unfolded with additional experimentation.

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