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Astrocytic calcium signaling: the information currency coupling neuronal activity to the cerebral microcirculation

Stephen V. Straub and Mark T. Nelson

Department of Pharmacology, University of Vermont, Burlington, VT 05405

Abstract

In the brain, increased neuronal synaptic activity is accompanied by an increase in local cerebral blood flow that serves to satisfy neuronal metabolic demands. This linkage between neuronal activity and local blood flow has been appreciated for over 100 years. Although this process has been exploited clinically in the form of functional imaging techniques to map brain function, the mechanisms by which increased synaptic activity is communicated to the cerebral microcirculation to generate a vasodilatory response are poorly understood. Recent studies, however, have illuminated a central role for astrocytic calcium (Ca^{2+}) signals as mediators of this process of neurovascular coupling. This review highlights recent evidence implicating astrocytes in the regulation of intracerebral arteriolar diameter, with particular emphasis on the putative signaling molecules and pathways proposed to exert changes on arteriolar physiology.

Introduction

Dynamic regulation of oxygen and glucose supply to match changes in metabolic demand is critical to the maintenance of cerebral homeostasis (Roy and Sherrington 1890). Specifically, increases in neuronal activity must be accompanied by rapid (within 1–2 seconds), spatially localized elevations of oxygen and glucose delivery such that ischemic conditions are averted (Anderson and Nedergaard 2003, Iadecola 2004). This coupling of neuronal activity to increased oxygen and glucose supply occurs as a result of enhanced cerebral blood flow at the level of the cerebral microcirculation and appears to depend on the generation of vasoactive substances in response to neuronal activity. Penetrating (parenchymal) cerebral arterioles receive neuronal innervation primarily from intrinsic neurons (Edvinsson and Hamel 2002, Hamel 2006). While the release of vasoactive substances from such neurons is clearly capable of inducing changes in arteriolar diameter through direct stimulation of vascular smooth muscle cells (SMCs) (Cauli et al. 2004, Rancillac et al. 2006), recent evidence suggests that Ca^{2+} -dependent signaling events in astrocytes also play a critical role in communicating neuronal activity to the cerebral microcirculation, resulting in profound changes in arteriolar diameter and local cerebral blood flow (Anderson and Nedergaard 2003, Filosa et al. 2004, Filosa et al. 2006, Iadecola 2004, Metea and Newman 2006, Mulligan and MacVicar 2004, Simard et al. 2003, Straub et al. 2006, Zonta et al. 2003a).

An individual astrocyte possesses a multitude of processes that surround thousands of neuronal synapses, forming a tripartite synapse with pre- and post-synaptic neurons (Araque et al. 1999). These same astrocytes send additional processes (endfeet) to encase the SMCs of

Correspondence: Stephen Straub, University of Vermont, Department of Pharmacology, 89 Beaumont Ave., Given Bldg. Rm. B303, Burlington, VT 05405, phone 802 656-8037, fax 802 656-4523, Stephen.Straub@uvm.edu

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intracerebral arterioles and endothelial cells of capillaries (Grosche et al. 1999, Kacem et al. 1998, Ventura and Harris 1999) (Figure 1). This astrocytic architecture suggests that an individual astrocyte may be capable of integrating the activity of multiple neurons and translating this information into physiological signals, including those that regulate cerebral blood flow.

Mechanisms Underlying Ca^{2+} Signals in Astrocytes

Astrocytes act as vital regulators of neuronal function, serving to modulate extracellular potassium concentration ($[\text{K}^+]_o$) and extracellular volume, and remove neurotransmitters from synapses (Diamond 2005, Kofuji and Newman 2004). These cells possess receptors for a range of neurotransmitters and neuropeptides (Porter and McCarthy 1997). Glutamate-dependent generation of astrocytic Ca^{2+} signals is a common mechanism by which neuronal information is transmitted to the cerebral vasculature. Specifically, activation of metabotropic glutamate receptors (mGluRs) located on astrocytic projections that surround synapses of glutamatergic neurons results in an increase in astrocytic cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) that propagates through astrocytic processes, ultimately resulting in a $[\text{Ca}^{2+}]_i$ increase in the endfoot (Cornell-Bell et al. 1990, Zonta et al. 2003a). These $[\text{Ca}^{2+}]_i$ increases likely occur through activation of the phospholipase C/inositol trisphosphate (InsP_3) cascade, and release of Ca^{2+} from InsP_3 sensitive channels located on the endoplasmic reticulum (Beck et al. 2004, Golovina and Blaustein 1997, 2000, Perea and Araque 2005, Verkhratsky et al. 1998). Several studies using cultured astrocytes suggest that Ca^{2+} release from ryanodine receptors may also be involved in generating the astrocytic Ca^{2+} signal (Golovina and Blaustein 1997, 2000). In addition to glutamate, neurotransmitters such as GABA, somatostatin, and vasoactive intestinal peptide released from interneurons may likewise generate vasoactive effects through generation of astrocytic Ca^{2+} signals, in addition to direct innervation of vessels (Cauli et al. 2004, Kang et al. 1998, Straub et al. 2006).

To understand the mechanisms that underlie communication between perivascular endfeet and cerebral arterioles, the spatiotemporal properties of Ca^{2+} signals within endfeet in cortical brain slices were recently characterized (Straub et al. 2006). Endfeet were found to possess InsP_3 receptors (InsP_3Rs) that could be activated by local photorelease of InsP_3 within the endfoot or by signals arising from activated neurons. Ryanodine receptors do not appear to play a role in generating Ca^{2+} increases in endfeet, as determined by a lack of effect of ryanodine and caffeine on endfoot Ca^{2+} signals. InsP_3R in endfeet and perivascular astrocytic processes were found to generate heterogeneous Ca^{2+} signals, with spatially restricted regions of elevated Ca^{2+} generated throughout the endfoot and perivascular processes in response to neuronal activity. These findings suggest that Ca^{2+} signaling within endfeet is highly dynamic in nature, and therefore likely to exert local control over Ca^{2+} -dependent vasoactive signaling pathways involved in neurovascular coupling.

Astrocytic Ca^{2+} Increases are Involved in Dynamic Regulation of the Cerebral Vasculature

While much effort has been focused on understanding the role of astrocytic Ca^{2+} signals in communicating information between adjacent astrocytes and neurons, only recently has astrocytic Ca^{2+} signaling been implicated in the dynamic regulation of the cerebral vasculature. In a seminal study, Zonta and colleagues (Zonta et al. 2003a) linked astrocytic Ca^{2+} changes to alterations in arteriolar diameter. In this study, astrocytic Ca^{2+} changes induced by neuronal activity or application of an mGluR agonist were measured in rat cortical brain slices and correlated with alterations in vessel diameter. Electrical stimulation of glutamatergic neurons induced consistent increases in astrocytic Ca^{2+} that were significantly attenuated, though not completely abrogated, in the presence of the mGluR antagonists MPEP and LY367385.

Separate experiments showed that vasodilation occurred in a similar time frame to the increases in astrocytic Ca^{2+} , suggesting that astrocytic Ca^{2+} changes are coupled to changes in vessel diameter. In addition, utilizing laser Doppler flowmetry to measure cerebral blood flow *in vivo*, Zonta et al. found that systemic administration of MPEP and LY367385 significantly decreased the hyperemic response to somatosensory stimulation. These findings suggest that astrocyte-derived factors, generated in response to elevations in astrocytic Ca^{2+} , are capable of engaging cerebral arterioles, modulating arteriolar diameter, and dynamically regulating local cerebral blood flow.

In addition to vasodilation, astrocytic Ca^{2+} increases have also been shown to induce vasoconstriction. Mulligan and MacVicar (2004) found that elevation of Ca^{2+} within an individual astrocyte, induced through photolysis of the caged divalent ion chelator DMNP-EDTA, resulted in constriction of arterioles in brain slices. Cerebral arteriolar diameter was affected only when a large Ca^{2+} increase (approximately 185% increase in fractional fluorescence of rhod-2) propagated into an endfoot. No change in the state of the arterioles was seen when a Ca^{2+} increase was selectively induced within an astrocytic cell body, or when a small Ca^{2+} increase was generated in an endfoot. Similar to the effects of photoreleasing Ca^{2+} , application of norepinephrine to slices induced an increase in astrocytic Ca^{2+} that was accompanied by vasoconstriction. Although this study and that of Zonta et al. draw different conclusions about the vasodynamic consequences of astrocytic signaling, they nevertheless strongly support the assertion that Ca^{2+} -dependent, vasoactive signaling processes are localized within astrocytic endfeet.

The Importance of Vascular Tone

While the studies of Zonta et al. (2003a) and Mulligan and MacVicar (2004) illustrate a central role for astrocytic Ca^{2+} signals in neurovascular coupling, at first glance it is unclear how these studies could generate such disparate results. One likely explanation for these divergent findings lies in the resting state of the arterioles within the brain slices. Cerebral arteries and arterioles *in vivo* exhibit myogenic tone in response to intravascular pressure (Bayliss 1902). This tone results from SMC depolarization (Harder 1984) and subsequent Ca^{2+} influx through voltage-gated Ca^{2+} channels (VDCC) (Knot and Nelson 1995, 1998). This partially constricted state of cerebral arteries and arterioles *in vivo* allows for vasodilation or vasoconstriction, as required, to maintain a constant level of cerebral perfusion in the face of changing intravascular pressure. Thus, an experimental preparation that recapitulates the dynamic range of the vessel in its native setting – that is, one in which both vasodilation and vasoconstriction can occur – is a prerequisite. Although not attributed to differences in arteriolar diameter by the authors, an elegant example of the effects of vascular tone on the outcome of astrocytic stimulation can be seen in the study of Metea and Newman (2006), who investigated neurovascular coupling mechanisms in whole retinal mounts. When levels of the vasomodulator nitric oxide (NO) were manipulated with NO donors or scavengers, vasodilatory responses could be converted into vasoconstrictions, and vice versa. Similarly, in the study of Mulligan and MacVicar, when vascular tone was induced (by inhibition of nitric oxide synthase), vasodilation was observed in response to endfoot Ca^{2+} increases. These findings highlight the profound influence of smooth muscle membrane potential, $[\text{Ca}^{2+}]_i$, and vascular tone on the outcome of activity-induced vascular responses.

The effect of Ca^{2+} release through endfoot-resident InsP_3R on the diameter of pre-constricted cerebral arterioles has been directly probed by generating endfoot-delimited Ca^{2+} increases through spatially restricted photorelease of InsP_3 within an individual endfoot (Straub et al. 2006). These endfoot-delimited Ca^{2+} signals induced rapid, local vasodilation of adjacent arterioles that reached 50% of maximum within 1.6 seconds of the uncaging event, and was limited to a short segment of the arteriole (~ 30 μm) centered on the endfoot. These findings

suggest that endfeet serve as individual vasoregulatory units in the brain, with the spatial extent of vasodilation dictated by the number of endfeet activated in response to neuronal stimulation. Consistent with these findings, Takano et al. (2006) observed vasodilation of arterioles *in vivo* in response to endfoot-delimited photorelease of caged Ca^{2+} .

Astrocytic Ca^{2+} Signals are Linked to Changes in SMC Calcium

If astrocytic Ca^{2+} signals play a critical role in facilitating changes in cerebral arteriolar diameter, obvious questions arise: what are the chemical mediators of this process, and what alterations in the resting arteriolar signaling processes are brought about by these mediators? Recent evidence suggests that astrocytic Ca^{2+} signals induce alterations in arteriolar SMC Ca^{2+} signaling processes (Filosa et al. 2004). Utilizing fluorescence confocal microscopy to provide high temporal and spatial resolution, Filosa et al. (2004) simultaneously measured changes in astrocytic and SMC Ca^{2+} in cortical slices loaded with the Ca^{2+} sensitive dye fluo-4 AM. Since arteriolar tone is regulated by SMC membrane potential (Knot and Nelson 1995), which in turn dictates SMC Ca^{2+} levels (Knot and Nelson 1998), these measurements allow for investigation of the dynamic interplay between astrocytic and SMC Ca^{2+} signals, with the hypothesis that any astrocytic signal that modulates arteriolar diameter must do so, at least in part, through a manipulation of SMC Ca^{2+} levels.

With the use of the thromboxane A₂ receptor agonist U46619 (100 nM) to precontract arterioles, a condition that mimics the vascular tone found *in vivo* (Brown et al. 2002) and allows both vasodilation and vasoconstriction to be measured, it was observed that arterioles in rat cortical slices exhibit regular oscillations in SMC Ca^{2+} , which are accompanied by synchronized vasomotion (Filosa et al. 2004). This phenomenon of vasomotion has been well documented *in vivo* (Mayhew et al. 1996, Nilsson and Aalkjaer 2003) and is a fundamental physiological property of mature blood vessels (Filosa et al. 2004, Gokina et al. 1996, Haddock and Hill 2002, Lefer et al. 1990, Lovick et al. 2005, Nilsson and Aalkjaer 2003) that is typically not observed in young, immature vessels, which possess few circumferential SMCs. Importantly, the extent of vasoconstriction induced by U46619 was comparable to that seen in isolated arterioles under physiological pressure. Induction of neuronal synaptic activity via electrical stimulation routinely resulted in a rapid (within ~ 1 second) inhibition of SMC Ca^{2+} oscillations that was prevented by tetrodotoxin, indicating a requirement for neuronal activity in this process. Simultaneous measurements of astrocytic and SMC Ca^{2+} signals illustrated that the inhibition of SMC Ca^{2+} oscillations was preceded by a rise in astrocytic Ca^{2+} , providing further evidence that Ca^{2+} dependent astrocytic signaling processes play a role in altering SMC Ca^{2+} levels. Importantly, inhibition of SMC Ca^{2+} oscillations was accompanied by vasodilation of cerebral arterioles. Thus, a dynamic interplay exists between astrocytic and SMC Ca^{2+} signals, in which a Ca^{2+} dependent astrocytic signal alters SMC Ca^{2+} dynamics to generate a change in vascular diameter.

Potential Mediators of Neurovascular Coupling – Signals Linked to Arachidonic Acid

Several signals based on arachidonic acid (AA) metabolism have been proposed to modulate vessel diameter in response to neuronal activity (Harder et al. 1998, Mulligan and MacVicar 2004, Takano et al. 2006, Zonta et al. 2003a). Specifically, elevation of astrocytic Ca^{2+} can lead to the Ca^{2+} -dependent activation of phospholipase A₂ (PLA₂) and the resultant production of AA, which can be metabolized by a host of different enzymes both within astrocytes and SMCs to generate vasoactive substances (Alkayed et al. 1996, Harder et al. 1998).

Zonta et al. (2003a) provided evidence that the vasodilatory factor released from astrocytes following the astrocytic Ca^{2+} increase was prostaglandin E₂ (PGE₂), a product of

cyclooxygenase (COX) metabolism of AA (Figure 2). This proposal was based primarily on a decrease in the number of vessels that dilated to neuronal stimulation in the presence of the COX inhibitor acetylsalicylic acid. Consistent with this finding, direct application of PGE₂ to brain slices resulted in vasodilation similar to that induced by the mGluR agonist t-ACPD. Additionally, this group has previously shown that PGE₂ is released in a pulsatile manner from cultured astrocytes (Zonta et al. 2003b). In support of this mechanism, Takano et al. (2006) found that the non-selective COX inhibitor, indomethacin, and the COX-1-selective inhibitor SC-560 applied to the surface of the intact brain reduced vasodilation associated with elevation of endfoot Ca²⁺ *in vivo*. In contrast, Iadecola and colleagues found that selective inhibition or ablation of COX-1 did not alter whisker stimulation-induced elevations in CBF (Niwa et al. 2001). Importantly, in the studies of Zonta et al. and Takano et al., COX inhibition only partially suppressed vasodilation, an observation consistent with the view that multiple mechanisms contribute to the neuronal regulation of arteriolar diameter.

AA can also be converted to epoxyeicosatrienoic acids (EETs) by the action of cytochrome P450 (CYP450) epoxygenases, to produce the vasoactive components 11,12 and 14,15 EET, among others (Alkayed et al. 1996). Harder and colleagues have shown that EETs, which are potent vasodilators (Harder et al. 1998, Roman 2002), activate Ca²⁺-sensitive, large conductance potassium channels (BK channels) in smooth muscle as well as astrocytes (Gebremedhin et al. 2003). Interestingly, Earley et al. (2005) have recently shown that EETs activate SMC BK channels indirectly through a novel mechanism involving Ca²⁺ influx mediated by the vanilloid TRP cation channel family member TRPV4, which activates RyRs (Ca²⁺ sparks), and then subsequently stimulates BK channels (Earley et al. 2005). These findings are consistent with a previous observation that TRPV4 channels are a target of EETs (Vriens et al. 2005). In support of a potential role for EETs in neurovascular coupling, Lovick et al. (2005) recently implicated epoxygenase products in maintaining vasodilation of cortical arterioles following astrocytic AMPA receptor stimulation.

In concert with the theme of BK channel regulation by AA-derived vasoactive substances, Mulligan and MacVicar (2004) postulated a role for 20-hydroxyeicosatetraenoic acid (20-HETE) in generating vasoconstriction of cerebral arterioles in response to neuronal activity. It was suggested that 20-HETE is synthesized by CYP450 hydroxylases in SMCs following diffusion of AA from astrocytic endfeet to SMCs. This proposal was based on the ability of PLA₂ and CYP450 hydroxylase inhibitors to prevent vasoconstriction induced by photolysis of caged Ca²⁺ in astrocytes. In this scenario, 20-HETE is suggested to inhibit BK channels in SMCs, leading to depolarization and constriction. However, as previously noted, the arterioles measured in this study were not pre-constricted, likely existing in a maximally dilated state. Under such conditions SMC BK channels are not engaged in the regulation of vessel diameter (Brayden and Nelson 1992).

It should be noted that blockers of PLA₂ and AA metabolism may have direct effects on SMC potassium channels. For example, in isolated SMCs, miconazole and quinacrine have been shown to inhibit K_V and BK currents, respectively (Vanheel et al. 1999, Yuan et al. 1995). In addition, since the endothelium of cerebral arteries and arterioles is a rich source of AA products that are involved in setting the basal level of vascular tone and generating vasodilation (Andresen et al. 2006), drug-induced alterations in endothelial function will have profound effects on vascular reactivity. Thus, interpretation of pharmacological interventions aimed at inhibiting AA metabolism in astrocytes is significantly complicated by the potential disruption of endothelium-SMC signaling. Therefore, an attenuation of neurovascular coupling in the presence of inhibitors of AA pathways does not necessarily indicate that the AA metabolites involved are astrocyte derived.

Potential Mediators of Neurovascular Coupling – A Role for Extracellular Potassium

Potassium ions (K^+) are among the most potent vasodilatory signals in the cerebral vasculature. Elevation of $[K^+]_o$ activates strong inward rectifier potassium (Kir) channels (Knot et al. 1996, McCarron and Halpern 1990), in particular the Kir2.1 channel, and the Na^+/K^+ -ATPase in SMCs of extracerebral (pial) arteries (Bradley et al. 1999, Knot et al. 1996, Zaritsky et al. 2000). Activation of SMC Kir channels results in SMC hyperpolarization (Bradley et al. 1999, Zaritsky et al. 2000), which closes voltage-dependent Ca^{2+} channels in the SMCs and leads to decreased $[Ca^{2+}]_i$ and vasodilation (Knot and Nelson 1998, Knot et al. 1996). Interestingly, astrocytes have been proposed to play a critical role in the spatial buffering of K^+ within the brain (Newman 1986). Specifically, the K^+ siphoning hypothesis put forth by Newman and colleagues suggests that astrocytes function to move K^+ from regions of elevated $[K^+]_o$ around synapses to regions of lower $[K^+]_o$ around the vasculature.

Consistent with a potential role for K^+ in regulating cerebrovascular function, recent evidence suggests that K^+ release from astrocytic endfeet plays a critical role in generating rapid vasodilation of cerebral arterioles in response to neuronal activity (Filosa et al. 2006). Specifically, elevation of astrocytic Ca^{2+} leads to a vasodilation of pre-constricted arterioles in cortical brain slices that is significantly reduced (~70 % reduction) by treatment with the BK channel blockers iberiotoxin or TEA, or by block of Kir channels with barium. This vasodilation is rapid, typically occurring within 2 seconds following onset of electrical stimulation to induce neuronal activity, consistent with the functional hyperemic response observed *in vivo*. Approximately 40% of the astrocytic K^+ current (at 0 mV) in the absence of neuronal stimulation is carried through BK channels, which are preferentially expressed in endfeet (Price et al. 2002). In addition, neuronal stimulation dramatically increases single channel activity consistent with BK channels in “on-endfoot” patch clamp recordings from endfeet within cortical slices. SMCs isolated from parenchymal arterioles exhibit a high density of Kir currents, suggesting that Kir channels in parenchymal arterioles may be a target of K^+ released from endfeet through BK channels. Furthermore, neurovascular coupling was diminished in cortical slices from mice in which BK channels were genetically ablated, but not from slices preincubated with the COX inhibitor indomethacin (10 μ M). Interestingly, in the presence of indomethacin, treatment with barium or TEA completely abolished vasodilation. Consistent with these *in situ* data, Gerrits et al. (2002) showed a decrease in whisker stimulation-evoked blood flow changes following block of BK channels with topical application of TEA or iberiotoxin or intravenous administration of TEA, suggesting a critical role for BK channel activity in the functional hyperemic response. Since neither TEA nor iberiotoxin affected neuronal activity, these findings are consistent with the assertion that BK channels in astrocytes and/or SMCs have a critical role in functional hyperemia.

These observations support a model of rapid neurovascular coupling in which neuronal activity is communicated to the vasculature through the Ca^{2+} dependent activation of endfoot BK channels and release of K^+ into the perivascular space, which induces hyperpolarization of SMCs through activation of SMC Kir channels, to promote vasodilation (Figure 2). An interesting feature of this model is that extracellular K^+ is capable of accounting for both rapid vasodilation, as described above, and vasoconstriction, depending upon its concentration in the perivascular space and the SMC membrane potential. Specifically, elevation of $[K^+]_o$ above ~15 mM, as might occur with excessive neuronal stimulation associated with pathological conditions such as spreading depression (Chuquet et al. 2007, Quayle et al. 1997), results in constriction of extracerebral arteries due to depolarization-induced activation of voltage-dependent Ca^{2+} channels (VDCCs) (Knot et al. 1996).

Limitations of Commonly Utilized Experimental Preparations

Investigations of the mechanisms underlying neurovascular coupling typically utilize either *in situ* tissue slices or *in vivo* imaging of astrocytes and cortical arterioles of anesthetized animals. While each technique has its advantages, each also has disadvantages that can limit interpretability of experimental findings. One commonly-cited limitation of brain slice studies is that blood vessels in these preparations lack intraluminal pressure and flow, therefore limiting the activation of autoregulatory mechanisms. Under these conditions, vessels lack normal vascular tone and exist in a maximally dilated state. For this reason, studies employing this preparation typically utilize a vasoconstricting agonist to recapitulate the level of vascular tone generated by physiological pressure, allowing for vasodilation and vasoconstriction as occurs *in vivo*. Vasoconstricting agonists produce similar levels of tone and vasomotion as are observed when intraluminal pressure and flow are applied to arterioles within slices through cannulation of individual vessels (Lovick et al. 2005). Furthermore, pre-constricted arterioles in brain slices retain their characteristic responsiveness to extracellular K^+ (Nakahata et al. 2006), and possess a functional complement of SMC K^+ channels (Filosa et al. 2006, Nakahata et al. 2003, Nakahata et al. 2006). One obvious caveat to using this technique is that the level of vascular tone induced by the vasoconstricting agonist should mimic the level of tone generated *in vivo* in response to physiological pressure.

In vivo imaging of cerebral parenchymal arterioles and astrocytes allows for the study of neurovascular coupling in the presence of physiological levels of intraluminal pressure and flow, suggesting that all physiologically relevant vascular signaling mechanisms should be functional. However, such experimental techniques require the use of anesthetics throughout the course of the experiment, which have been shown to alter vascular tone and other cardiovascular parameters (Jong et al. 2002, Kim et al. 2007, Ueki et al. 1992). In addition, since imaging is performed through a cranial window, cellular access is limited and precludes the use of this preparation with electrophysiological methodologies which are required to understand the ionic basis of vascular function. Furthermore, pharmacological agents must be applied to the exposed surface of the brain rather than at the desired site of action, requiring the application of potentially toxic drug concentrations and yielding unknown concentrations at the recording site. Despite the limitations associated with both techniques, *in vivo* imaging studies have been largely in concert with findings made in the *in situ* condition which suggest that astrocytic Ca^{2+} signals generate vasodilation of arterioles with physiological levels of tone (Filosa et al. 2006, Takano et al. 2006, Zonta et al. 2003a). Furthermore, findings from both approaches support the central role of endfoot Ca^{2+} signals in the induction of vasodilation through the Ca^{2+} -dependent generation of COX products and K^+ signals (Filosa et al. 2006, Gerrits et al. 2002, Takano et al. 2006, Zonta et al. 2003a) (Figure 2).

Since intracerebral blood vessels are the targets of neurovascular coupling, it is critical that both brain slice and *in vivo* approaches are complemented with appropriate studies on isolated intracerebral arterioles and SMCs. Such studies are required for elucidation of the cellular mechanisms that underlie neurovascular coupling. Furthermore, the judicious use of transgenic mouse models, especially those incorporating tissue-specific genetic manipulations, represents a powerful tool for mechanism formulation, elucidation of potential therapeutic targets, and testing drug specificity.

Future Directions

To define the neuronally-induced signaling events that occur within astrocytes, particularly within astrocytic endfeet, is paramount. These include, but are certainly not limited to, investigations of Ca^{2+} -dependent signaling domains within endfeet and investigation of endfoot ion channel expression and activity *in situ* and *in vivo*. Since the process of neurovascular coupling involves numerous cell types, an integrative approach should be

utilized, and should include the investigation of as many cellular constituents as possible. This will most certainly require utilization of multiple experimental approaches and tissue/cellular preparations to elucidate biologically relevant findings. Going forward, it will be especially important that putative messengers and mechanisms be validated at the SMC and arteriole level, both within the intact (slice/*in vivo*) preparation and in isolated arterioles and SMCs. Ideally this will involve the use of animal models in which the putative arteriolar target or signaling mechanism is genetically crippled.

In conclusion, recent evidence clearly supports a central role for astrocytic Ca^{2+} signals in the generation of vasoactive factors involved in neurovascular coupling. While the identity of the astrocyte derived vasoactive factors involved in this process is still the subject of intense debate, any mechanism proposed to play a role in neurovascular coupling must be capable of inducing rapid vasodilation as is observed *in vivo*. In addition, each mechanism must have a clearly defined site of action within the vasculature that can account for the effects of neuronal activity on the cerebral circulation. One such mechanism utilizes K^{+} release from astrocytes through BK channels to promote vasodilation via activation of SMC Kir channels. This mechanism satisfies the temporal requirements of functional hyperemia observed *in vivo*, and in addition is capable of inducing both vasodilation and vasoconstriction, seemingly accounting for contradictory findings reported in the literature. Importantly, this mechanism is distinct from the previously reported COX-dependent pathway, suggesting that multiple vasoactive pathways likely act in concert to dynamically regulate cerebrovascular responses *in vivo*.

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Figure 1.

Astrocytic Ca²⁺ signals couple neuronal activity to changes in vascular function. Neuronally-released glutamate (G) binds metabotropic glutamate receptors (mGluR) on astrocytes inducing production of inositol trisphosphate (InsP₃) and increasing astrocytic cytosolic Ca²⁺ concentration. Ca²⁺ released from inositol trisphosphate receptors (InsP₃R) in astrocytic endfeet, which encase cerebral arterioles, activates Ca²⁺ dependent signaling mechanisms leading to the generation and/or release of several putative vasoactive factors including potassium ions (K⁺) and arachidonic acid (AA) metabolites such as prostaglandin E₂ (PGE₂). These vasoactive factors interact with vascular targets, leading to a decrease in SMC [Ca²⁺]_i and vasodilation. PLA₂: phospholipase A₂, BK: Ca²⁺ sensitive large conductance K⁺ channel, EETs: epoxyeicosatrienoic acids, Kir: strong inwardly rectifying K⁺ channel (Kir2.1), K_{ATP}: ATP sensitive K⁺ channel, Na⁺~K⁺: sodium-potassium ATPase.

Figure 2.

Putative vasoactive signaling pathways involved in neurovascular coupling. Neuronal activity generates elevation of $[Ca^{2+}]_i$ in the astrocytic endfoot, through activation of $InsP_3R$, which activates astrocytic BK channels. BK channel activation leads to the local release of K^+ ions from endfeet surrounding cortical blood vessels, which activates Kir channels in arteriolar SMCs. Local concentrations of K^+ ions between 3–15 mM increase SMC Kir channel conductance, causing SMC membrane potential hyperpolarization, closing voltage-dependent Ca^{2+} channels (VDCC), and leading to vasodilation. Higher local concentrations of K^+ (e.g. >20 mM) would cause membrane potential depolarization, and vasoconstriction. Through an alternate pathway, increased astrocytic $[Ca^{2+}]_i$ is postulated to activate phospholipase A_2 (PLA_2) to generate arachidonic acid (AA) and subsequently prostaglandin E_2 (PGE_2) through a cyclooxygenase (COX)-dependent mechanism. Activation of EP receptors by PGE_2 would lead to increased cAMP in SMCs, ultimately decreasing SMC $[Ca^{2+}]_i$ and generating vasodilation through an as yet unidentified mechanism. Modified from (Filosa et al. 2006).