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Mechanisms Mediating Functional Hyperemia in the Brain

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Abstract

Neuronal activity within the brain evokes local increases in blood flow, a response termed functional hyperemia. This response ensures that active neurons receive sufficient oxygen and nutrients to maintain tissue function and health. In this review, we discuss the functions of functional hyperemia, the types of vessels that generate the response, and the signaling mechanisms that mediate neurovascular coupling, the communication between neurons and blood vessels. Neurovascular coupling signaling is mediated primarily by the vasoactive metabolites of arachidonic acid (AA), by nitric oxide, and by K^+ . While much is known about these pathways, many contentious issues remain. We highlight two controversies, the role of glial cell Ca^{2+} signaling in mediating neurovascular coupling and the importance of capillaries in generating functional hyperemia. We propose signaling pathways that resolve these controversies. In this scheme, capillary dilations are generated by Ca^{2+} increases in astrocyte endfeet, leading to production of AA metabolites. In contrast, arteriole dilations are generated by Ca^{2+} increases in neurons, resulting in production of nitric oxide and AA metabolites. Arachidonic acid from neurons also diffuses into astrocyte endfeet where it is converted into additional vasoactive metabolites. While this scheme resolves several discrepancies in the field, many unresolved challenges remain and are discussed in the final section of the review.

Keywords

functional hyperemia; neurovascular coupling; astrocyte; cerebral blood flow; arachidonic acid; PGE₂; EETs

Introduction

The brain is a metabolically demanding organ. Although it accounts for only 2% of the total mass of the body, ~15% of cardiac output, ~20% of O_2 consumption, and ~25% of glucose utilization is devoted to nourishing the brain. Many mechanisms have evolved to ensure that the brain receives adequate O_2 and glucose to feed its neurons. When systemic blood pressure varies, autoregulatory mechanisms adjust the tone of brain blood vessels so that perfusion within the brain remains constant (Payne 2016). Also, as blood O_2 and CO_2

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changes, perfusion to the brain is varied accordingly to ensure that appropriate tissue gas levels are maintained (Rudzinski and others 2007). Neuronal projections from subcortical areas contribute to cortical blood flow regulation on a large spatial scale (Bekar and others 2012; Cohen and others 1996). At the local level, when brain activity increases, blood flow to that region rises, supplying active neurons with sufficient nutrients. This increase in local blood flow in response to neuronal activity is termed functional hyperemia.

This review focuses on the functional hyperemia response and the neurovascular coupling mechanisms that link neuronal activity to vessel dilation. We will discuss the functions of functional hyperemia, the types of vessels generating the functional hyperemia response, and the signaling mechanisms that generate the response. We will highlight recent studies that address two important and controversial issues: (1) the role of glial cell Ca^{2+} signaling in mediating neurovascular coupling and (2) the importance of capillaries in generating functional hyperemia.

The Function of Functional Hyperemia

Functional hyperemia was first described over a century ago, by Mosso in humans (see Raichle 2014) and by Roy and Sherrington (1890) in dogs. The hallmark of functional hyperemia is a spatially restricted increase in blood flow in response to a local increase in neural activity. The response occurs rapidly, with an onset time of less than a second (Silva and others 2000).

Since the discovery of functional hyperemia, it has been assumed that the response served to supply active neurons with needed O_2 and nutrients. When a region of the brain is activated, the metabolic rate of the tissue, along with glucose and O_2 utilization, go up (Shetty and others 2012). The increase in blood flow is thought to match the increased metabolic need of the tissue.

In fact, this eminently reasonable hypothesis has never been proven. Suppose that the functional hyperemia response was experimentally blocked without interfering with other brain processes. Would brain functions continue normally or would they be altered? Would neuronal health be compromised? Attempts at answering this question have been made. For example, when functional hyperemia was reduced by pharmacological block of nitric oxide (NO), prostaglandin E_2 (PGE_2), and epoxyeicosatrienoic acids (EETs) synthesis in mice, behavioral deficits were noted (Tarantini and others 2015). However, these deficits could have been due to nonselective effects of the blockers on neurons.

While experimental elimination of functional hyperemia remains challenging, there are natural conditions where the response is reduced or absent. Functional hyperemia is lacking in newborn humans (Anderson and others 2001) and neonatal mice (Kozberg and others 2016), suggesting that the response is not required for the health or functioning of the brain during early stages of development. Although these findings challenge the idea that functional hyperemia is essential for neuronal health, the needs of the neonatal brain may be very different from that of the adult.

A recent study in pericyte-deficient mice reported a reduction in functional hyperemia, a lowering of brain O₂ levels, and a loss of neurons in older animals (Kisler and others 2017). Although a reduction in capillary density was observed in these animals, which could have effects on the brain not directly related to the functional hyperemia response, the results indicate that functional hyperemia is important in maintaining neuronal health.

The importance of functional hyperemia is suggested by the association between loss of the response and many neuropathological disorders including Alzheimer's disease, hypertension, and ischemic stroke. In these conditions, loss of functional hyperemia is thought to contribute to disease pathology (Girouard and Iadecola 2006).

Functional hyperemia may actually be a protective mechanism against pathological conditions. The increases in blood flow seen in functional hyperemia are far higher than they need to be to meet the increased metabolic demand of neurons. For instance, whereas brain metabolism increases ~15% when the visual cortex is activated, blood flow increases as much as 65% (Lin and others 2010). The excessive blood flow increase might be needed to create a "safety margin" to ensure sufficient O₂ delivery even if the functional hyperemia response is reduced due to signaling errors or pathology (Leithner and Royl 2014). The large blood flow increase may also be needed to ensure that neuronal structures farthest from capillaries receive sufficient levels of O₂ (Devor and others 2011).

The Neurovascular Unit

Functional hyperemia is generated by signaling among cells within the neurovascular unit, which consists of neurons, astrocytes, vascular smooth muscle cells (VSMCs) surrounding arteries and arterioles, pericytes enveloping capillaries, and vascular endothelial cells (Fig. 1). As discussed below, signaling can occur directly from neurons to vessels or indirectly via astrocytes. In both VSMCs and pericytes, vasodilation is achieved largely by activation of K⁺ channels, leading to cell hyperpolarization, the closing of voltage-gated Ca²⁺ channels, and a lowering of cytosolic Ca²⁺ (Longden and others 2016). Vessel tone is also regulated by signaling from endothelial cells to VSMCs and pericytes. Endothelial cells synthesize a variety of vasoactive agents, including NO, PGE₂, and EETs (Roman 2002), and may contribute to neurovascular coupling (Chen and others 2014). However, relatively little is known about signaling from neurons to endothelial cells and this aspect of blood flow regulation will not be considered in this review.

Astrocytes are ideally situated to mediate neurovascular coupling as they contact both neurons and blood vessels (Fig. 2). Astrocyte processes surround many synapses within the CNS (Araque and others 1999) and are stimulated by the release of neurotransmitters and neuromodulators. Glutamate, GABA, norepinephrine, acetylcholine, ATP, and endocannabinoids all stimulate astrocytes, leading to increases in cytosolic Ca²⁺ levels (Zorec and others 2012). These transmitters act primarily by activating metabotropic receptors, although ionotropic P2X receptors may also contribute to Ca²⁺ increases (Mishra and others 2016). At the other end of the neurovascular unit, astrocyte endfeet envelope arterioles and capillaries, covering the vascular surface and directly contacting VSMCs and

pericytes. Vasoactive agents released from astrocyte endfeet lead to vessel dilation or constriction.

The Vessels that Actively Generate Functional Hyperemia

Early research on the cerebral vasculature showed that arteries and arterioles on the pial surface dilated and constricted under differing physiological and pathological conditions. These observations led to the conclusion that functional hyperemia was generated largely by dilation of pre-capillary vessels. This view has been challenged in recent years by observations that capillaries as well as arteries and arterioles dilate in response to neuronal activity.

The relaxation of VSMCs surrounding arterioles and arteries mediates dilation of these vessels (Fig. 1). Capillaries lack this smooth muscle cell covering. However, the circumferential processes of pericytes, a closely related contractile cell, wrap around capillaries (Trost and others 2016). Evoked neuronal activity as well as direct application of neurotransmitters results in pericyte relaxation and capillary dilation (Hamilton and others 2010). Measurements in the in vivo cortex (Hall and others 2014; Kisler and others 2017; Tian and others 2010) and retina (Biesecker and others 2016; Kornfield and Newman 2014) demonstrate that capillaries dilate in response to sensory stimulation.

Recent analyses of the cortical vasculature suggest that a large fraction of the vascular resistance lies within capillaries (Gould and others 2017). This conclusion suggests that active capillary dilation could be responsible for much of the blood flow increase seen during functional hyperemia. Indeed, one calculation estimated that a 6.7% dilation of cortical capillaries could account for 84% of the total blood flow increase seen during functional hyperemia (Hall and others 2014).

Recent studies demonstrate that in the cortex, capillaries dilate in response to sensory stimulation with a shorter latency than upstream penetrating arterioles, indicating that capillary dilations are actively generated rather than a result of passive stretch due to an increase in perfusion pressure (Hall and others 2014; Mishra and others 2016). The rapid dilation of capillaries also suggests that capillaries might be the primary recipient of neurovascular coupling signals and that arteriole dilation results from the upstream propagation of dilatory signals from capillaries, which can travel through vascular endothelial cells. However, selective inhibition of capillary dilation in the in vivo retina demonstrates that arterioles are capable of dilating in response to sensory stimulation even when capillaries fail to respond (Biesecker and others 2016).

Despite the evidence summarized above, the role of capillaries in generating functional hyperemia remains controversial. The capillary contribution to blood flow increases in the brain has not been measured experimentally. In addition, one study has challenged the ability of capillaries to dilate, suggesting that capillary pericytes do not contain the necessary contractile proteins and that the small vessels that dilate are actually pre-capillary arterioles rather than capillaries (Hill and others 2015). However, the criteria used to identify capillaries in this study has been challenged (Attwell and others 2016), and most evidence

indicates that true capillaries in both the cortex (Hall and others 2014; Mishra and others 2016) and retina (Biesecker and others 2016; Kornfield and Newman 2014) actively dilate and contribute to blood flow regulation.

Signaling Mechanisms Mediating Neurovascular Coupling

Metabolic By-Products

Roy and Sherrington (1890), in their seminal paper, suggested that neurovascular coupling was mediated by by-products of brain metabolism, stating “The chemical products of cerebral metabolism ... can cause variations of the calibre of the cerebral vessels.” A number of metabolic products could mediate neurovascular coupling, including a decrease in O_2 or glucose, or an increase in CO_2 , adenosine, or lactate.

Evidence suggests, however, that these metabolic signals are not major factors mediating neurovascular signaling. Functional hyperemia is not diminished when animals are exposed to 4 atmospheres of 100% O_2 , essentially clamping brain pO_2 to 100% (Lindauer and others 2010). Nor is neurovascular coupling diminished when blood glucose levels are varied (Powers and others 1996). It is also unlikely that increased CO_2 mediates neurovascular coupling. Although CO_2 produces a vasodilating acidification, which blocks VSMC Ca^{2+} channels, the brain parenchyma actually undergoes a net alkalization rather than an acidification following neuronal activation (Chesler 2003). Neuronal activity results in a net production of adenosine (Ko and others 1990) and lactate (Ido and others 2004), which both dilate vessels. These metabolites may contribute to neurovascular coupling, but are probably not the primary signals, as other mechanisms, described below, appear to generate the bulk of the functional hyperemia response.

If metabolic by-products are not the major contributors to neurovascular coupling, what are the important signals? It is now generally accepted that neurovascular coupling is mediated largely by substances produced directly or indirectly by neuronal activity. As discussed in the following sections, these include nitric oxide (NO), metabolites of arachidonic acid (AA), and K^+ .

Nitric Oxide

Nitric oxide is a potent vasodilator that acts directly on VSMCs surrounding arteries and arterioles. Nitric oxide is produced by neuronal nitric oxide synthase (nNOS) in neurons, by endothelial NOS in endothelial cells, and by inducible NOS, particularly in pathology. While NO produced by all forms of NOS cause vessel dilation, only nNOS is thought to contribute to neurovascular coupling. When neurons are activated, NO is produced in response to increases in cytoplasmic Ca^{2+} levels generated by Ca^{2+} influx through ion channels and Ca^{2+} release from internal stores (Ross 2012). NO is a membrane-permeant gas that diffuses across membranes and astrocytic endfeet to VSMCs where it activates guanylyl cyclase, ultimately resulting in the opening of BK Ca^{2+} dependent K^+ channels, cell hyperpolarization, and a reduction of cytosolic Ca^{2+} (Archer and others 1994).

In the cerebellum, genetic knockout of nNOS reduces functional hyperemia by 73%, demonstrating that NO plays a major role in mediating neurovascular coupling in this tissue

(Yang and others 2003). Similar results have been obtained in the cortex, where genetic and pharmacological inhibition of nNOS reduces functional hyperemia (Cholet and others 1997; Ma and others 1996). In the cortex, however, addition of an NO donor to effectively “clamp” NO to a high level reverses the effect of nNOS inhibition, restoring normal neurovascular coupling (Lindauer and others 1999). This finding demonstrates that NO likely functions as a permissive molecule rather than the mediator of neurovascular coupling in the cortex. In other words, NO must be present for vasodilation to occur, but it is not the active signaling molecule. NO donors do not rescue functional hyperemia following nNOS block in the cerebellum (Akgoren and others 1996; Yang and Iadecola 1997), demonstrating that NO is a true neurovascular signal in this tissue.

In addition to directly controlling vessel diameter by hyperpolarizing VSMCs, NO can also act indirectly to modulate vascular tone. NO inhibits the synthesis of 20-hydroxyeicosatetraenoic acid (20-HETE), an AA metabolite that constricts vessels (Roman 2002). Blocking 20-HETE synthesis results in vasodilation. This action may be a major mechanism by which NO acts permissively in the cortex. By removing the constricting effect of 20-HETE, NO allows vasodilating signals to act. NO has the opposite effect at high concentrations, blocking the synthesis of EETs, an AA metabolite which dilates vessels (Udosen and others 2003).

Arachidonic Acid Metabolites

A number of metabolites of AA are vasoactive (Fig. 3), either dilating or constricting blood vessels (Koehler and others 2009; Roman 2002). Two potent vasodilators are believed to mediate neurovascular coupling, PGE₂ and EETs. PGE₂ is produced by the action of two enzymes. AA is first converted to prostaglandin H₂ (PGH₂) by cyclooxygenase (COX). PGH₂ is further metabolized into PGE₂ by PGE synthase. PGE₂ acts on VSMCs by activating K⁺ channels (Serebryakov and others 1994), leading to cell hyperpolarization. PGE₂ also functions by activating EP₄ prostanoid receptors (Davis and others 2004), resulting in a decrease in myosin light chain phosphorylation and to vasodilation (Takata and others 2009). A second class of vasoactive AA metabolites, EETs, is produced by the action of cytochrome P450 epoxygenases, which convert AA into a number of vasodilating regioisomers, including 5,6-, 8,9-, 11,12-, and 14,15-EETs (Alkayed and others 1996a). EETs act on VSMCs by activating large conductance (BK) Ca²⁺-sensitive K⁺ channels.

Inhibition of either PGE₂ or EETs results in a substantial decrease in neurovascular coupling in the cortex. When prostaglandin synthesis is inhibited, arteriole dilations in the in vivo cortex are reduced by 50% to 80% (Takano and others 2006; Zonta and others 2003). Similarly, when EETs synthesis is blocked, arteriole dilations are reduced by 30% to 80% (Mishra and others 2011; Peng and others 2002; Peng and others 2004).

PGE₂ and EETs signaling can be mediated by astrocytes. When astrocytes are stimulated by neurotransmitters, the resulting increase in intracellular Ca²⁺ activates PLA2 or PLD2, resulting in the conversion of membrane phospholipids to AA. Increases in AA, in turn, lead to increases in the production of AA metabolites, including PGE₂ and EETs (Roman 2002). These chemicals, which are membrane permeant, diffuse directly from astrocyte endfeet to VSMCs where they cause vasodilation. All of the enzymatic machinery for PGE₂ and EETs

signaling, including PLA2, PLD2, COX1, PGE synthase, and cytochrome P450 epoxygenases, is found in astrocytes. Arachidonic acid and PGE₂ can also be synthesized in neurons, which express PLA2, PLD2, COX2, and PGE synthase. However, EETs is believed to be synthesized solely in astrocytes and not in neurons (Alkayed and others 1996b; Peng and others 2004).

When astrocyte Ca²⁺ levels are raised experimentally by photolysis of caged Ca²⁺ or by application of metabotropic receptor agonists, adjacent blood vessels dilate (Gordon and others 2008; Metea and Newman 2006; Takano and others 2006; Zonta and others 2003). These dilations are reduced by application of PLA2, COX, and epoxygenase inhibitors. Astrocyte-evoked vessel dilation occurs even when transmitter release from neurons is blocked (Metea and Newman 2006).

Calcium-Dependent Astrocyte Signaling

The evidence cited above lends strong support to the hypothesis that neurovascular coupling is mediated in large part by the production and release of vasodilating AA metabolites from astrocytes. Central to this hypothesis is that neuronal activity results in Ca²⁺ increases in astrocytes and that these Ca²⁺ increases precede vasodilation. Recent evidence, however, has challenged this hypothesis. Several studies have reported that arteriole vasodilation can occur in the absence of astrocyte Ca²⁺ increases (Bonder and McCarthy 2014; Nizar and others 2013). Furthermore, when Ca²⁺ signals are observed, they have long latencies, occurring after the initiation of vasodilation.

Experiments utilizing IP3 receptor 2 (IP3R2) null mice also challenge the Ca²⁺-dependent astrocyte hypothesis of neurovascular coupling. IP3R2 receptors are expressed almost exclusively in astrocytes and astrocyte Ca²⁺ signaling is largely abolished in IP3R2 null mice (Petravicz and others 2008). However, normal activity-dependent arteriole dilation and blood flow increases are observed in these transgenic animals (Bonder and McCarthy 2014; Nizar and others 2013; Takata and others 2013). Capillary dilation was not monitored in these experiments, however. These results strongly suggest that although astrocytes have the enzymatic machinery for dilating blood vessels, Ca²⁺-dependent astrocyte signaling is not responsible for neurovascular coupling. We are left with an apparent paradox. Although pharmacological experiments indicate that PGE₂ and EETs play a major role in neurovascular coupling, particularly in the cortex, the Ca²⁺ signals needed to initiate synthesis of these vasodilators in astrocytes is seemingly absent.

Two recent reports have helped to resolve this puzzle. Biesecker and others (2016) monitored Ca²⁺ signaling in Müller cells of the retinal eyecup while measuring arteriole and capillary dilation. Müller cells are a specialized type of astrocyte and the principal glial cells of the retina. Biesecker and others found that sensory stimulation evoked rapid Ca²⁺ increases, preceding vasodilation, in Müller cell endfeet contacting capillaries but not in endfeet contacting arterioles. Spontaneous or experimentally evoked Ca²⁺ increases in Müller cell endfeet resulted in capillary dilation. Furthermore, in IP3R2 null mice, where most Ca²⁺ signaling was blocked, stimulus-evoked capillary but not arteriole dilation was abolished. These results demonstrate that capillary but not arteriole dilation is mediated by a Ca²⁺-dependent astrocyte signaling mechanism in the retina.

Mishra and others (2016) reported similar results in the cortex. They studied capillary and arteriole dilation in cortical brain slices, where electrical stimulation of neurons resulted in arteriole and capillary dilation. Neuronal activity evoked rapid Ca^{2+} increases in astrocyte endfeet contacting capillaries. These Ca^{2+} increases were blocked by dialyzing the cells with BAPTA, a Ca^{2+} chelator. BAPTA nearly abolished stimulus-evoked capillary dilations but did not diminish arteriole dilations. Mishra and others also found that capillary dilation was mediated by PGE_2 and that inhibiting NOS production of NO had no effect on capillary dilations but abolished arteriole dilations. The effect of NOS inhibition on arteriole responses suggests that neurovascular arteriole coupling is mediated by NO signaling. However, the results are also consistent with NO being permissive but not the actual mediator of signaling, as demonstrated previously in the cortex (Lindauer and others 1999).

The results of these two studies indicate that neurovascular coupling onto capillaries is mediated by Ca^{2+} -dependent release of vasodilating AA metabolites from astrocyte endfeet. In contrast, neurovascular coupling onto arterioles is not mediated by Ca^{2+} increases in astrocytes. In the cerebellum, NO release from neurons following activation of nNOS is a major neurovascular signal (Yang and others 2003). In the cortex, NO is permissive but is probably not an important signal (Lindauer and others 1999). So what is the neurovascular signal for arterioles in the cortex? The studies cited above indicate that both PGE_2 and EETs are important vasodilatory signals at arterioles (Mishra and others 2011; Peng and others 2002; Peng and others 2004; Takano and others 2006; Zonta and others 2003). Yet, in astrocyte endfeet contacting arterioles, where EETs can be synthesized and released (Alkayed and others 1996b; Peng and others 2004), Ca^{2+} signals are absent or not necessary. This leaves us without a mechanism for EETs production and release onto arterioles.

We propose a signaling scheme where neurons and astrocytes work in concert to synthesize EETs as well as PGE_2 (Figure 4). Neurons express both PLA2 and PLD2, enzymes which convert membrane phospholipids to AA following Ca^{2+} increases (Cockcroft 2001). Thus, when neuronal Ca^{2+} rises following cell activation, AA as well as NO will be produced. Neurons also express PGE_2 synthesizing enzymes and will release PGE_2 when cells are active (Navamani and others 1997; Taylor and Hewett 2002). This PGE_2 will diffuse out of the neurons, through astrocyte endfeet, which completely envelop vessels, and onto VSMCs. Newly synthesized AA will also diffuse from neurons into astrocyte endfeet (Fig. 4, dashed line) where it will be converted into both PGE_2 and EETs before reaching the VSMCs. Thus, EETs is synthesized within astrocyte endfeet surrounding arterioles through increased substrate levels, even though Ca^{2+} increases are absent in this cellular compartment. In this way, neurons and astrocytes act cooperatively to mediate arteriole dilation. The synthesis of PGE_2 and EETs begins in activated neurons with the synthesis of AA and is completed in astrocyte endfeet, where the AA is converted into its vasoactive metabolites.

Potassium

Although NO and AA metabolites play essential roles in neurovascular coupling, blocking these signaling pathways does not eliminate neurovascular coupling entirely (Peng and others 2004; Tarantini and others 2015) and other mediators may also contribute to vessel dilation. The potassium ion (K^+) may be one such signal. Small increases in extracellular K^+

levels, up to ~12 mM from a basal level of ~4 mM, dilate blood vessels. Elevated K^+ increases the conductance of K_{ir} channels (Filosa and others 2006) and activates the electrogenic Na^+/K^+ ATPase (Haddy 1983) within VSMCs, leading to VSMC hyperpolarization and vessel dilation.

Paulson and Newman (1987) proposed that neurovascular coupling was mediated by an astrocytic K^+ siphoning mechanism (Newman and others 1984). In this scheme, K^+ released from active neurons flows into astrocytes and out astrocyte endfeet, directly onto blood vessels. Potassium siphoning through astrocytes does not play a major role in neurovascular coupling in retinal arterioles (Metea and others 2007). However, this signaling mechanism has not been evaluated in retinal capillaries or in the brain and may contribute to neurovascular coupling.

A different K^+ -mediated signaling mechanism was proposed by Nelson and colleagues (Filosa and others 2006), who suggested that Ca^{2+} increases in astrocyte endfeet open Ca^{2+} -dependent K^+ (BK) channels, resulting in release of K^+ from the endfeet onto VSMCs. This mechanism is predicated on Ca^{2+} increasing in astrocyte endfeet, however, and as we have seen, astrocyte Ca^{2+} increases are unlikely to mediate dilation at arterioles.

Remaining Challenges

Signaling Mechanisms

Multiple signaling cascades in both neurons and astrocytes contribute to neurovascular coupling. The relative importance of these signaling mechanisms and the relative contributions of neurons and astrocytes to these signals is unclear and will most likely vary in different brain regions and under different physiological and pathophysiological states. Understanding the mechanisms mediating functional hyperemia is essential for developing therapies for CNS pathologies affecting the vasculature. Determining the relative importance of these mechanisms is a major challenge.

Comprehensive pharmacological and molecular/genetic approaches will be needed to determine the relative importance of the neurovascular signaling cascades and the cells that regulate capillary and arteriole tone. Transgenic mice and viral vectors will be useful in selectively blocking expression of neuronal and astrocytic synthesis of NO, AA, PGE_2 , and EETs in order to elucidate the signaling cascades. Although PLA2 and PLD2 are important targets in these signaling cascades, they are involved in a large number of cellular functions and blocking these enzymes either genetically or pharmacologically would likely lead to a wide range of off-target effects, making it difficult to interpret any resulting changes to blood flow.

Neuronal activity in the retina leads to rapid Ca^{2+} increases in astrocyte endfeet contacting capillaries but not in endfeet contacting arterioles (Biesecker and others 2016). It remains to be determined whether this pattern of Ca^{2+} signaling is unique to the retina or holds throughout the brain. The mechanisms responsible for this differential pattern of Ca^{2+} activity in astrocyte endfeet must also be elucidated. Perhaps the receptors and channels expressed on the two classes of endfeet differ. A related question which must be addressed is

whether the release of PGE₂ and EETs from astrocyte endfeet onto arterioles is triggered by neuronal Ca²⁺ increases and the diffusion of AA from neurons to astrocyte endfeet.

Propagation of vasodilation and constriction along vessels is believed to be mediated by electrotonic conduction of hyperpolarizing and depolarizing signals through endothelial cells, which are coupled by gap junctions (Zhang and others 2011). Endothelial cells can also synthesize a number of vasodilating agents, including NO, PGE₂, and EETs (Roman 2002). However, little is known about the activation of endothelial cells by neurons (Chen and others 2014). An important challenge for future research will be to determine the contribution of endothelial cells to neurovascular coupling.

Capillary Contributions to Functional Hyperemia

The contribution of capillaries to functional hyperemia remains controversial. Several recent studies have demonstrated that small vessels, 4 to 8 μm in diameter, dilate (Biesecker and others 2016; Hall and others 2014; Kisler and others 2017; Kornfield and Newman 2014; Tian and others 2010). Yet these vessels have been identified by some investigators as pre-capillary arterioles rather than capillaries (Hill and others 2015). Additional studies are needed to resolve this dispute. Careful morphological and molecular characterization of arterioles, pre-capillary arterioles, and capillaries, as well as categorization of pericyte subtypes must be conducted.

Recent studies (Gould and others 2017) indicate that a large fraction of the total vascular resistance within the brain lies within capillaries. Small changes in capillary diameter would thus lead to large changes in vascular resistance and blood flow. Yet the contribution of active capillary dilation to functional hyperemia has not been measured directly. Existing computational analyses must be complemented by physiological experiments assessing the effect of capillary dilation on blood flow. Targeting capillary pericytes will be useful in answering this question (Kisler and others 2017).

The contribution of capillaries to BOLD-fMRI and other MR imaging techniques must also be clarified. BOLD signals largely reflect changes in O₂ saturation in the larger venules and veins. However, a significant fraction of the signal detected in spin echo BOLD is due to O₂ saturation and volume changes in capillaries (Gagnon and others 2015). If capillary responses reflect the activity of local groups of neurons, then MR imaging has the potential to detect the responses of restricted neuronal ensembles. However, the relationship between local neuronal activity and capillary responses must be elucidated to take full advantage of functional imaging technology.

Neuronal Specificity of Functional Hyperemia

Specific types of excitatory and inhibitory neurons may mediate different dilating and constricting components of functional hyperemia. For instance, PGE₂ released from pyramidal cells and NO released from inhibitory interneurons may dilate vessels (Lacroix and others 2015) while the activity of neuropeptide Y-containing interneurons may trigger vasoconstriction (Uhlirova and others 2016). These neurons may signal directly to VSMCs or indirectly, via astrocytes. Parsing the contributions of different types of neurons in initiating functional hyperemia is a major challenge for future research. Optogenetic

activation and inhibition of specific types of neurons will prove useful in addressing this issue (Anenberg and others 2015).

Other Functions of Functional Hyperemia

While accepting the concept that functional hyperemia plays an essential metabolic support role, several theories suggest additional functions of the response. One theory holds that increased blood flow may function as a thermoregulatory response, removing excess heat generated by active neurons (Sukstanskii and Yablonskiy 2006; Yablonskiy and others 2000). An alternative proposal, known as the hemo-neural hypothesis, suggests that increased blood flow could alter neuronal activity (Moore and Cao 2008). The concept that signaling can occur from blood vessels to neurons (instead of from neurons to vessels, as occurs in neurovascular coupling) is supported by recent work showing that experimentally induced changes in vessel diameter can induce alterations in neuronal activity (Kim and others 2016). These theories of functional hyperemia remain largely hypothetical. Future research will elucidate the complex relationships between neurons, astrocytes, and vessels and the role of functional hyperemia in the brain.

Summary

Functional hyperemia is believed to be essential for the health and normal function of the brain. Multiple neurovascular coupling mechanisms have evolved to mediate functional hyperemia, complementing each other and providing redundancy. Signaling occurs directly from neurons to vessels in some instances and from astrocytes to vessels in others. We propose that signaling can also occur cooperatively between neurons and astrocytes, with production of AA and its metabolites split between the two cellular compartments. In each of these signaling cascades, activity-evoked increases in astrocytic and neuronal Ca^{2+} play a key role in initiating neurovascular coupling. Future research will elucidate the contributions of these mechanisms to the generation of functional hyperemia.

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Figure 1.

Interactions between cells of the neurovascular unit regulate blood flow in the brain. The diameter of arterioles and arteries is controlled by the contractile state of smooth muscle cells, which form one or more continuous layers around the vessels. Capillary diameter is controlled by pericytes, whose longitudinal and circumferential contractile processes envelop capillaries. Astrocyte endfeet almost completely surround arterioles and capillaries and their contractile cells. Chemicals released from both astrocyte endfeet and neurons control the contractile state of smooth muscle cells and pericytes.

Figure 2.

Astrocytes are well suited to mediate signaling from neurons to blood vessels. This 1899 drawing by Santiago Ramon y Cajal illustrates that astrocytes, the darkly colored cells (A, B), form extensive contacts with neurons, the lightly colored cells (C, D), and with blood vessels (F). Astrocyte processes surround many synapses in the brain and generate intracellular Ca^{2+} increases in response to transmitter release from these synapses. Astrocyte endfeet envelope capillaries and arterioles. Astrocyte Ca^{2+} increases trigger the synthesis and release of vasoactive agents from the cell endfeet onto capillaries, inducing vessel dilation. Drawing courtesy of the Cajal Institute-CSIC, Madrid, Spain.

Figure 3.

Metabolites of arachidonic acid dilate and constrict blood vessels. Calcium increases in both astrocytes and neurons activate phospholipase A₂ and D₂, leading to the conversion of membrane phospholipids to arachidonic acid. Arachidonic acid, in turn, is converted to the vasodilators EETs and PGE₂. Arachidonic acid is also converted to the vasoconstrictor 20-HETE. Nitric oxide (NO) inhibits the synthesis of 20-HETE and EETs.

Figure 4.

Proposed neurovascular coupling pathways mediating vasodilation of capillaries and arterioles. At *capillaries*, neurotransmitters released from active neurons evoke Ca^{2+} increases in astrocyte endfeet by activation of metabotropic (GPCR) and ionotropic (P2XR) receptors. These Ca^{2+} increases result in the synthesis and release of vasodilating PGE_2 and EETs onto pericytes surrounding capillaries. Calcium increases may also open Ca^{2+} -activated K^+ channels (K_{Ca}), leading to the release of vasodilating K^+ . At *arterioles*, Ca^{2+} increases are evoked in neurons through activation of GPCRs, NMDA receptors (NMDAR) and through depolarization and activation of voltage-gated Ca^{2+} channels (VGCC). These Ca^{2+} increases result in the synthesis and release of nitric oxide (NO) through activation of neuronal nitric oxide synthase (nNOS) and PGE_2 . These vasodilators diffuse through astrocyte endfeet onto vascular smooth muscle cells surrounding arterioles. Nitric oxide also inhibits the synthesis of vasoconstricting 20-HETE within vascular smooth muscle cells, leading to vasodilation. Arachidonic acid (AA) produced in neurons also diffuses into astrocyte endfeet (dashed line) where it will be converted into PGE_2 and EETs. In this way neuronal Ca^{2+} increases trigger the synthesis and release of vasodilators from astrocyte

endfeet. Dilation of both capillaries and arterioles may also be mediated by a siphoning mechanism, where the release of K^+ from active neurons depolarizes astrocytes, leading to the release of K^+ onto vessels.

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