Astrocytes have recently been shown to be essential participants in the control of cerebral blood flow (CBF) through their prominent control of cerebral vessel diameter. Although the unique close relationship of astrocytes with cerebral blood vessels has long been recognized it is only within the last few years that evidence has shown how astrocytes might translate information to the vasculature on the activity level and energy demands of neurons. These findings suggest that astrocytes are key players in the system for the delivery and clearance of molecules important to brain function.
Astrocytes possess the necessary signaling capability to induce both vasoconstriction as well as vasodilation in response to elevations in astrocyte end-feet Ca^{2+}. Both types of vasomotor responses are initiated by the generation of arachidonic acid (AA) in astrocytes by Ca^{2+}-sensitive phospholipase A_2 (PLA_2). Subsequent to AA formation, vasoconstriction occurs as a result of the generation of 20-hydroxyeicosatetraenoic acid (20-HETE), while vasodilation ensues from the production of epoxyeicosatrienoic acid (EET) or prostaglandin E_2 (PGE_2). Notably, the level of nitric oxide (NO) seems to control which of these two routes is utilized, either by the inhibition of critical enzymes by NO or by an indirect effect on vessel tone. In addition to the Ca^{2+}-activated PLA_2 pathway, the activation of large conductance Ca^{2+}-activated K^+ channels in astrocyte end-feet has been proposed to induce vasodilation by hyperpolarizing smooth muscle cells (SMCs) through the effect of increased external [K^+] on SMC Kir channels. This large array of possibilities highlights the importance of astrocytes as well as the need for additional experimentation to fully delineate their contributions to vascular dynamics.

18.1 Functional Hyperemia

More than 100 years ago Roy and Sherrington first discovered that brain tissue is intrinsically capable of controlling CBF within a specific, localized region (Roy and Sherrington, 1890). This regional phenomenon is termed functional hyperemia, whereby vessel diameter is enlarged to augment CBF in response to energy demands that result from enhanced synaptic transmission and neuronal firing. The purpose of functional hyperemia is to augment the delivery of oxygen-rich hemoglobin, glucose, and other nutrients to the working cells, while simultaneously clearing metabolic products such as CO_2. Understanding the mechanistic physiology of how the cerebrovasculature changes diameter may be critical for developing effective treatments for an array of neurological afflictions such as stroke, hemorrhage, focal ischemia, and migraine. In addition to these pathologies, understanding cerebrovascular control within the context of brain energy metabolism is important for the correct interpretation of data obtained from high resolution, functional magnetic resonance imaging (fMRI), which uses the magnetic signal of deoxyhemoglobin as an indirect measure of CBF and brain activity. This technology, as well as with other imaging strategies, is widely used to identify volumes of activated brain tissue during both normal and pathological cerebral functioning. Some of the “implicit” understanding surrounding fMRI signals is now being refined as we learn more about how the brain consumes oxygen during activity and how this relates to changes in CBF. However, there is still much to learn because the cellular mechanisms involved in transforming changes in neuronal activity to changes in CBF are incompletely understood. There are many molecules that have effects on cerebral vessel tone, the number of different cell types that participate in neurovascular coupling is increasing and the source of certain vasoactive substances is controversial. In spite of this, there has been a noteworthy focus on one cell type – the astrocyte. These cells
have been hailed as the missing element coupling changes in neuronal activity to alterations in CBF via influencing cerebral vessel diameter. In this chapter we focus on the role of astrocytes, and in particular that of astrocyte end-feet Ca\(^{2+}\) signaling, in the control of brain blood vessel girth. The different experimental models and approaches used to test astrocyte involvement, the possible signaling molecules participating, and the potential mechanisms of astrocyte action are highlighted.

## 18.2 Astrocytes and Functional Hyperemia: Origins and Revisions

Roy and Sherrington (1890) originally posited the idea that a local accumulation of metabolic products triggered a homeostatic dilation of nearby vessels to augment flow. The increased CBF would then clear these catabolic substances and CBF would presumably return to an appropriate rate. For functional hyperemia though, recent evidence argues against such a hypothesis, primarily because of the rapid time to onset for vessel dilation and increased CBF from the time of enhanced neural activity (Lou et al., 1987). Observations made in vivo suggest that ~1–2 s is required to make the transition from forepaw stimulation to vessel response in corresponding regions of the somatosensory cortex (Zonta et al., 2003b). When considering that neuronal processes can be located at an average maximal distance of 60 μm from the nearest vessel (Lokkegaard et al., 2001), the local accumulation and diffusion of metabolic by-products is an unsatisfactory explanation for the rapid coupling of neural activity to changes in CBF. Furthermore, the reestablishment of vessel tone after metabolic products have been shuttled away is too passive and parsimonious an explanation for what is, essentially, a constriction process. There must be other aspects about brain physiology unconsidered in Roy and Sherrington’s original idea.

Astrocytes recently have been demonstrated to be active participants in the coupling of neuronal activity to blood flow control and may in fact be the critical component providing fast and dynamic control of blood vessels. As early as 1913 Ramon y Cajal recognized that astrocytes can form a physical bridge from neurons to the cerebrovasculature. Processes from a single astrocyte interact with an enormous number of synapses (Ventura and Harris, 1999; Bushong et al., 2002; Haber et al., 2006), adjacent astrocytes (Massa and Mognaini, 1982; Fischer and Kettenmann, 1985) and, through the use of specialized end-feet, the microvessels of the brain (Simard et al., 2003). End-feet are enlarged compartments located distally on astrocytic processes that are specialized for close interactions with endothelial cells, SMCs, and possibly pericytes. The diameter of arterioles, and thereby blood flow, is regulated by contracting and relaxing SMCs. Pericytes recently have also been shown to constrict capillaries (Peppiatt et al., 2006) although the contribution of this interaction to the regulation of cerebral blood flow is still unknown. Collectively, end-feet from numerous astrocytes wrap all cerebral blood vessels in the CNS. A current hypothesis for functional hyperemia that is receiving much interest is that astrocytes can directly sense changes in synaptic activity and relay this information...
to the cerebrovasculature. In this model, CBF can be augmented more quickly to meet the demands of activated tissue compared to waiting for the accumulation and diffusion of specific vasoactive by-products of metabolism.

18.3 Astrocytic Characteristics for Cerebrovascular Control

Discoveries over the past 25 years have shown that astrocytes possess a vast repertoire of ion channels, receptors, and signaling pathways that enable them to detect and convey synaptic information to vessels. In the early 1990s work with calcium indicator dyes showed that exogenous glutamate application to cultured astrocytes caused oscillating increases in internal free Ca\(^{2+}\) resulting from the activity of intracellular Ca\(^{2+}\) stores (Cornell-Bell et al., 1990). The most notable finding was that the Ca\(^{2+}\) oscillations propagated as a wave through connected astrocytes. These data indicated that membrane bound metabotropic glutamate receptor (mGluR), could elicit novel, long-range signaling cascades incorporating networks of astrocytes. Further studies demonstrated a similar effect using more physiological preparations and from synaptically released glutamate (Dani et al., 1992; Porter and McCarthy, 1995, 1996; Pasti et al., 1997). These data inspired nearly two decades of research into how this Ca\(^{2+}\) signal propagates and what is it used for in the physiology of the animal. Here there is a focus on astrocyte Ca\(^{2+}\) signals, particularly those that occur in the end-feet, and how these signals utilize the molecular machinery of the end-feet to initiate changes to the cerebrovasculature and ultimately CBF.

18.3.1 From End-Foot to Vasomotion: Molecular Players

End-feet are endowed with many features thought to be important for cerebrovasculature control. For instance, metabotropic P2Y purinoceptors and the gap junction protein connexin-43 are highly expressed in astrocytic end-feet in situ (Simard et al., 2003). These proteins are thought to initiate and allow the release of adenosine 5’-triphosphate (ATP), respectively, and cooperatively can cause an end-foot Ca\(^{2+}\) signal to spread distances greater than 50 mm along a vessel’s outer surface (Simard et al., 2003). ATP, once released, can be broken down by ecto-ATPase and ecto-5-nucleotidase into adenosine, a nucleoside that has a dilating influence on the cerebrovasculature during functional hyperemia (Shi et al., 2008). Adenosine can also act on astrocytes to enhance Ca\(^{2+}\) wave propagation in both the cerebellum (Jimenez et al., 1998) and the retina (Newman, 2003). All of these elements may operate together to ensure the astrocyte end-foot signal propagates to a sufficient number of SMCs in an apposed vessel to elicit a desirable vasomotor response.

In addition to the metabotropic glutamate and P2Y receptors, end-feet also possess functional adrenoceptors (Paspalas and Papadopoulos, 1996), which, when activated, cause prominent elevations in end-foot Ca\(^{2+}\) (Mulligan and MacVicar, 2004). With such strong and seemingly pervasive astrocyte Ca\(^{2+}\) signals, what intracellular
molecules might this signal target and to what end? Admittedly, an increase in intracellular \( \text{Ca}^{2+} \) has a plethora of potential targets but one molecule of interest is soluble \( \text{PLA}_2 \), which is abundantly expressed in astrocyte end-feet (Farooqui et al., 1997). Once activated by \( \text{Ca}^{2+} \) this enzyme leads to the production of multiple vasoactive substances (Fig. 18.1). \( \text{PLA}_2 \) generates diffusible AA from the plasma membrane, which can be converted into a number of compounds, some which induce vasodilation and others induce vasoconstriction. Dilating products include \( \text{PGE}_2 \) from the action of cyclooxygenase (COX) enzymes (Niwa et al., 2001; Zonta et al., 2003b; Takano et al., 2006) and several EETs (5,6-EET; 8,9-EET; 11,12-EET; and 14,15-EET) (Ellis et al., 1990; Gebremedhin et al., 1992) from the activity of a subtype of cytochrome P450 (CYP450) enzymes. Constricting molecules consist of \( \text{PGF}_2 \) (Ellis et al., 1983) and thromboxane \( \text{A}_2 \) (Ishimoto et al., 1996; Benyo et al., 1998a, b; Filosa et al., 2004) from COX activity, endothelin peptide (Faraci, 1989; MacCumber et al., 1990), as well as 20-HETE (Lange et al., 1997; Mulligan and MacVicar, 2004) from the conversion of AA by a different CYP450 enzyme than that mentioned for EETs.

In cultured astrocytes, stimulating soluble \( \text{PLA}_2 \) causes the release of AA (Stella et al., 1997). However, AA can also be converted while still within the cells. Cultured astrocytes express COX-1 and can be triggered to express COX-2 (Koyama et al., 1999; Luo et al., 2001), which can generate \( \text{PGE}_2 \), both in response to the \( \text{Ca}^{2+} \) ionophore ionomycin (Oomagari et al., 1991) and glutamate agonists (Zonta et al., 2003a). Similarly, the AA metabolites EETs can be released from astrocyte cultures.

![Fig. 18.1](image)

**Fig. 18.1** Astrocyte end-feet control of cerebrovasculature diameter by the generation of astrocyte AA via \( \text{Ca}^{2+} \) sensitive \( \text{PLA}_2 \). Two separate end-feet making contact with an arteriole show possible constriction (left end-foot) and dilation (right end-foot) mechanisms (See Color Plates).
when this preparation is treated with exogenous glutamate agonists (Alkayed et al., 1997). These in vitro data indicate astrocytes are capable of releasing a variety of vasoactive products.

In addition to PLA$_2$ activation, an increase in free intracellular Ca$^{2+}$ within astrocytes may also stimulate Ca$^{2+}$-activated K$^+$ channels (K$_{Ca}$). Activation of mGluRs in cultured hippocampal astrocytes triggers the opening of K$_{Ca}$ channels, leading to K$^+$ efflux (Gebremedhin et al., 2003). This effect was blocked by mGluR antagonists and, interestingly, by inhibition of cytochrome P450 arachidonate epoxygenase. Large conductance Ca$^{2+}$-activated K$^+$ channels (BK channels; also termed slo or MaxiK channels) have also been identified in situ, with notable localization to the perivascular astrocyte end-feet (Price et al., 2002). K$^+$ efflux from these channels after opening would cause local elevations in the extracellular concentration of K$^+$ around vessels, which may influence the membrane potential of SMCs or facilitate Kir channel opening.

Aquaporin 4 water channels show a similar distribution pattern with the highest expression level in astrocyte end-feet with lower levels in astrocyte processes that ensheath glutamatergic synapses (Amiry-Moghaddam and Ottersen, 2004), often localizing with Kir channels (Nagelhus et al., 2004). In contrast, neurons show limited expression of aquaporins, a result that has led to the idea that astrocytes, as opposed to neurons, are responsible for volume changes and volume homeostasis in the brain (Simard and Nedergaard, 2004; Andrew et al., 2007). As volume changes, such as the cell swelling induced from elevated neural activity, require a set point, the opening of volume regulated anion channels (VRACs) is thought to fulfill this role by allowing Cl$^-$ and K$^+$ efflux, thereby limiting the maximum extent of the swell (Pasantes-Morales, 1996). VRACs are also permeable to amino acid gliotransmitters (Basarsky et al., 1999) and thus provide another potential route for astrocyte influence on vessel diameter and CBF.

Other characteristics include the expression of Nitric Oxide (NO) synthesizing enzymes. However, whether NO synthase (NOS) is expressed in end-feet and what isoform is expressed, i.e., eNOS (Wiencken and Casagrande, 1999) vs. sNOS (Calka and Wolf, 2003), remains controversial. Given the ubiquitous importance of NO modulation of vessel tone (more below), and that astrocyte processes may generate it (Murphy et al., 1993), the role of astrocyte-derived NO in functional hyperemia will be a crucial issue for future studies. All of these characteristics collectively have lead to the concept of the “neurovascular unit,” in which astrocytes are well-enough endowed, both anatomically and physiologically, to transmit relevant information about the extracellular environment from neurons to vessels.

### 18.4 Astrocytes Control Cerebrovascular Diameter

As stated above, astrocytes respond to glutamate with an increase in intracellular Ca$^{2+}$ through the activation of mGluRs. The first evidence this process was a major component of astrocyte-mediated neurovascular coupling was demonstrated by the
Carmignoto laboratory (Zonta et al., 2003b). Activating astrocytes indirectly by disrupting membranes with patch electrodes or by applying mGluR agonists triggered the release of diffusible factors that then acted on SMCs to cause dilation of arterioles. Further, an elevation of extracellular glutamate from enhanced synaptic activation evoked Ca\textsuperscript{2+} increases in astrocyte end-feet (via mGluR) and caused vasodilation. The dilation was reduced by interfering with COX activity with aspirin, suggesting products from cyclooxygenase activity such as PGE\textsubscript{2} and/or prostacyclin were involved. This data followed from a previous report showing that mGluR-mediated Ca\textsuperscript{2+} oscillations in astrocytes results in the pulsatile release of prostaglandin (Zonta et al., 2003a). The results from the vessel experiments, which were obtained from acute brain slices, were also extended to the intact animal. Using Doppler flowmetry to measure CBF, cortical functional hyperemia was induced by forepaw stimulation. The ensuing vasodilation initiated quickly and was dramatically reduced by mGluR antagonists. An important control was performed to show there were no attenuating effects on evoked synaptic potentials in the presence of the antagonists, suggesting incoming signals from the periphery were still relayed to the cortical region examined. However, the block of functional hyperemia by aspirin in the slice condition was not tested in vivo.

Experiments that broadly affect the COX enzymes have been conducted to test their role in cerebrovascular control. Pharmacological inhibition or knockout (KO) of COX-2 dramatically attenuates functional hyperemia-induced vasodilation in response to whisker stimulation (Niwa et al., 2000). In contrast, the same inhibitory actions on COX-1 fail to affect this form of functional hyperemia, but these treatments do attenuate a form of acetylcholine-mediated vasodilation (Niwa et al., 2001). Because COX-2 expression is thought to be low in astrocytes, these results point to neuronal derived COX activity and COX products that are responsible for functional hyperemia.

However, a recent in vivo study conducted by the Nedergaard laboratory, specifically examining astrocytes Ca\textsuperscript{2+} signals in functional hyperemia, supports a role for COX-1 rather than COX-2 (Takano et al., 2006). This study described the effects of uncaging Ca\textsuperscript{2+} in astrocyte end-feet on vasomotor responses in arteries of the somatosensory cortex. Astrocytes were loaded with the Ca\textsuperscript{2+} indicator dye Rhod2-AM and caged Ca\textsuperscript{2+} DM-nitrophen. Uncaging Ca\textsuperscript{2+} within astrocyte end-feet triggered an intracellular Ca\textsuperscript{2+} rise that was followed by the dilation of the adjacent penetrating arteriole. Using this protocol few constrictions were observed. Dilations were found to be blocked by inhibitors of COX-1 but not COX-2 enzymes. Immunohistochemical staining for COX-1 showed intense reactivity at cerebral blood vessels, which was suggested to overlap with glial fibrillary acidic protein (GFAP) positive end-feet. However, it is difficult to rigorously ascertain that COX-1 proteins were indeed in the end-feet vs. 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 positive processes that were far from the vessels, suggesting that COX-1 expression is, at the very least, localized to the vessel region. Nevertheless, these data support the idea that focal elevation of end-foot Ca\textsuperscript{2+} results in vessel dilation mediated by the AA conversion to vasoactive COX products such as PGE\textsubscript{2}. 
Similar to the work by Zonta and Carmignoto, the Nelson laboratory has demonstrated that afferent stimulation in acute brain slices causes an increase in astrocyte soma and end-foot Ca\(^{2+}\) levels, which can be mitigated by mGluR antagonists (Filosa et al., 2004). However, in the absence of any treatment Nelson’s group observed spontaneous and repetitive vasomotion timed with a fluctuating Ca\(^{2+}\) signal in SMCs. When stimulating afferents, rather than showing an increase in vessel diameter from a smaller resting state, they show a reduction in the contractile phase of the motor rhythmicity and in the spontaneous Ca\(^{2+}\) oscillations. Interestingly, application of mGluR agonist mimicked both observations, but in the presence of mGluR antagonists, only the astrocyte Ca\(^{2+}\) signal was significantly reduced when afferents were stimulated. That the reduction in SMC Ca\(^{2+}\) oscillations persisted suggests that either there was insufficient block of the mGluRs, that other glutamate receptors were involved or that non-glutamatergic inputs and transmitters were participating in relaying information about the state of the cellular environment to vessels through astrocytes.

The MacVicar laboratory examined the effects of elevations in astrocyte Ca\(^{2+}\) alone, without incorporating the involvement of membrane-bound receptors by uncaging Ca\(^{2+}\) using two photon photolysis. This technique allowed them to increase Ca\(^{2+}\) within the discrete volume of astrocytes without provoking other cell types. Astrocytes were identified in transgenic mice that expressed enhanced green fluorescent protein driven by the GFAP promoter and these cells were loaded with the AM form of Rhod-2, the Ca\(^{2+}\) sensitive dye. Under typical brain slice recording conditions in the hippocampus and cortex, Ca\(^{2+}\) uncaging in astrocytes induced a Ca\(^{2+}\) wave that propagated throughout the astrocyte syncytium and invaded end-feet, and the end-feet Ca\(^{2+}\) signal was immediately followed by a constriction of adjacent arterioles. Mulligan and MacVicar found a strong, positive relationship between the extent of the constriction and the extent of the Ca\(^{2+}\) signal within and among the end-feet. Astrocyte-mediated vasoconstrictions were blocked by inhibiting the Ca\(^{2+}\)-dependent enzyme PLA\(_2\), and preventing the release of the diffusible factor AA. Arteriole constrictions were found to be caused by the generation of 20-HETE within SMCs from the astrocyte derived AA by a CYP450 enzyme (Fig. 18.2). In other studies, 20-HETE has been shown to depolarize SMCs by blocking K\(^+\) channel opening (Lange et al., 1997), and by enhancing Ca\(^{2+}\) entry through voltage-gated calcium ion channels (VGCCs) (Gebremedhin et al., 1992). While cultured astrocytes have been demonstrated to synthesize 20-HETE (Nithipatikom et al., 2001), in the brain the w-hydroxylase enzyme that synthesizes 20-HETE is principally expressed within SMCs (Gebremedhin et al., 2000).

A study from the Newman laboratory conducted in retinal explants has examined the effect of Ca\(^{2+}\) elevations within retinal glia by using UV photolysis of caged Ca\(^{2+}\) and caged 1,4,5-inositol-trisphosphate (IP\(_3\)) (Metea and Newman, 2006). Notably, in response to Ca\(^{2+}\) uncaging constrictions as well as dilations were observed, while increases in free IP\(_3\) produced mostly dilation. Constrictions, as with the results obtained from the MacVicar laboratory, were dependent on the generation of astrocyte AA and its conversion to 20-HETE. Dilations were also caused by astrocyte AA but instead of its conversion to the potent constrictor molecule, AA was converted
to EET by another CYP450 enzyme (Fig. 18.2). These data indicate an intriguing complexity in the dialog between glial cells and arterioles with respect to the potential factors determining the polarity of the response (see below) and the different outcomes observed depending of the method of Ca²⁺ liberation within glia. The EET-induced dilations are supported by other reports in the CNS. In vivo cortical application of 5,6-EET, more so than other epoxyeicosatrienoic acids, causes a large increase in cerebral arteriole diameter (Amruthesh et al., 1993). Others have reported that 8,9-EET and 11,12-EET, rather than 5,6-EET, elicited dose-dependent relaxation of cerebral arteries through activation of SMC K⁺ channels (Gebremedhin et al., 1992; Hu and Kim, 1993). Furthermore, pharmacological blockade of the EET generating enzyme P450 reduces resting CBF as measured in vivo (Alkayed et al., 1996).
18.5 NO: An Important Modulator of Astrocyte-Mediated Cerebral Vessel Control

As stated earlier, the first study implicating astrocyte Ca\textsuperscript{2+} signals in the relaxation of vascular tone as a model for functional hyperemia was conducted by the Carmignoto laboratory (Zonta et al., 2003b). In a subset of their experiments blood vessels were preconstricted by incubating the brain slices with N(G)-nitro-L-arginine methyl ester (L-NAME) to block NOS and reduce the level of endogenous NO to ultimately enhance the vasodilations observed. This is interesting when compared the results obtained by the MacVicar laboratory, in which arterioles in untreated slices always displayed constriction when astrocytes were stimulated (Mulligan and MacVicar, 2004). Only when Mulligan and MacVicar incubated in L-NAME did they observe vasodilations in response to the mGluR agonist (1S, 3R)-1-aminocyclopentane-1, 3-dicarboxylic acid (t-ACPD).

On the basis of these dichotomous results Metea and Newman speculated that the levels of NO dictated the type of vasomotor response (Metea and Newman, 2006). This was hypothesized to occur via regulation of the enzymatic conversion of AA to either EET or 20-HETE. Consistent with this idea, vessels that upon first test showed dilations were transformed into constrictions in the presence of the NO donor S-nitrosol-N-acetylpenicillamine (SNAP), an outcome thought to be due to the NO sensitivity of the EET producing enzyme CYP450 (Fleming, 2001). In the presence of the NO scavenger-phenyl-4,4,5,5-ketramethyl-imidazoline-1-oxyl-3-oxide (PTIO), the opposite was true: vasoconstrictions were converted to vasodilations. In line with this latter experiment in which NO is limiting, blocking all forms of NOS with L-NAME produced only vasodilations when the preparation was stimulated, similar to the results of the Carmignoto and MacVicar laboratories.

An in vivo study conducted by the Nedergaard laboratory also examined a role for NO in astrocyte-mediated dilations by applying L-NAME to block NOS (Takano et al., 2006). This treatment failed to affect the degree of dilation induced when Ca\textsuperscript{2+} was uncaged in astrocyte end-feet. This negative result may be explained by a lower level of endogenous NO in the intact animal. However, NO donors were not tested and it would be interesting to see if by elevating NO levels in vivo the vasomotor response changes polarity from dilation to constriction when astrocytes are stimulated – as has been demonstrated in in vitro experiments.

The work outlined above show that astrocytes wield the necessary physiology to initiate both vasoconstriction and vasodilation mechanisms and that the level of NO may dictate the polarity of the vasomotor response. However, there are many unknowns in the role of NO in mediating the above processes. First, little is known of the importance of astrocyte-derived NO over that of neurons and endothelial cells in functional hyperemia. Second, studies testing the impact of NO on synaptic transmission have demonstrated that NO enhances the release of several neurotransmitters, including glutamate (Prast and Philippu, 2001). This notion also corresponds with data collected in vivo showing a prominent role for enhanced NO release in function hyperemia (Iadecola et al., 1995; Yang et al., 1999). These demonstrations
need to be reconciled both with the in vitro data showing higher NO levels promote astrocyte-mediated vasoconstriction and with the recent in vivo data showing astrocyte-mediated vasodilations do not rely on NO production.

18.6 K⁺ and Vascular Control by Astrocytes

18.6.1 K⁺ Siphoning Through Kir Channels

It was first proposed 20 years ago by the Newman laboratory that K⁺ efflux from Kir channels in the end-feet 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 end-feet of Muller cells (Newman, 1984). However, this hypothesis has recently been disproved by Newman’s laboratory 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 K⁺ through open Kir channels. Under these conditions, they failed to observe any change in vessel diameter. Second, they investigated the extent of K⁺-induced vascular effects in the retina in control vs. the Kir KO mouse. When the vasomotor physiology was compared between the two mouse strains, no difference was observed in the degree of K⁺-induced dilation. This result was also corroborated by verifying the loss of the inwardly rectifying channel with electrophysiological recordings.

18.6.2 Ca²⁺-Activated K⁺ Release

A role for 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 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 Ca²⁺ 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 end-feet. Different from the Newman lab, the effect here is thought to involve end-feet BK channels (Price et al., 2002), which open in response to higher levels of intracellular Ca²⁺ to allow the efflux of K⁺ (Fig. 18.2). 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 conflicting with the data from the Newman lab showing that there is no difference in K\(^{+}\)-induced dilations between wild type and Kir KO 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.

18.7 Astrocyte Ca\(^{2+}\) Signals: Functional Significance?

18.7.1 Astrocyte Ca\(^{2+}\): Initiation and Spread

To initiate Ca\(^{2+}\) signals, astrocytes express a variety of membrane bound receptors. As discussed previously, the major contribution from glutamate receptors is attributable to G\(_q\) coupled group I mGluRs (Pearce et al., 1986), particularly subtype mGluR5 (Balazs et al., 1997), which has a prominent role in functional hyperemia (Zonta et al., 2003b; Filosa et al., 2004; Mulligan and MacVicar, 2004) (see below). Also pertinent are the ionotropic and metabotropic purinoceptors, which respond to elevated concentrations of extracellular ATP (Jimenez et al., 2000; Kukley et al., 2001). Astrocyte Ca\(^{2+}\) signals occur via Ca\(^{2+}\) influx through Ca\(^{2+}\) permeable ionotropic P2X receptors (Walz et al., 1994) or when Ca\(^{2+}\) is released from intracellular stores subsequent to the activation of metabotropic P2Y receptors that couple to phospholipase C and IP\(_3\) generation (Nakahara et al., 1997). Further studies have expanded these findings to include other neurotransmitters in the induction of Ca\(^{2+}\) signals in astrocytes. These include the release of acetylcholine in the hippocampus from cholinergic septal afferents (Araque et al., 2002), the exogenous effects of norepinephrine (Duffy and MacVicar, 1995) and gamma-aminobutyric acid (GABA) (Kang et al., 1998; Serrano et al., 2006) in the hippocampus, as well as the actions of the nonclassical transmitter NO on the Bergmann glial cells of the cerebellum (Matyash et al., 2001) and on astrocytes of the cortex (Bal-Price et al., 2002).

Long-range Ca\(^{2+}\) signals in astrocytes are made possible by two principle mechanisms (1) the intracellular diffusion of IP\(_3\) through gap junctional channels (Sneyd et al., 1994; Venance et al., 1997), which allow electrical and cytoplasmic connectivity between adjacent astrocytes and (2) the extracellular paracrine actions of glial-derived ATP (Guthrie et al., 1999). ATP release has been found to occur in continuous waves coinciding with Ca\(^{2+}\) wave propagation (Wang et al., 2000), and
also in isolated bursts that were highly localized and separated by large distances (Arcuino et al., 2002). The mechanism of ATP release from glial cells is incompletely understood. There are likely many initiating routes including purinergic P2Y receptors (James and Butt, 2002), α₁-adrenoceptors (Gordon et al., 2005) and group I mGluRs (Cornell-Bell et al., 1990), as well as mechanisms of release, which may include vesicular fusion (Bal-Price et al., 2002; Pascual et al., 2005) or hemichannel (Cotrina et al., 1998; Braet et al., 2003) and P2X7 receptor (Duan and Neary, 2006) opening. ATP release from cultured astrocytes has also been shown to be triggered by hypo-osmotic-induced astrocyte swelling through a multidrug resistance protein pathway (Darby et al., 2003). Ca²⁺ waves are thought to be essential contributors relaying synaptic information to vessels. Recent data shedding light on this area has revealed that the situation may be more complicated, which is where we now turn our attention.

18.7.2 Enigmatic Astrocyte Ca²⁺ Signaling

From the studies described above it is clear that astrocyte Ca²⁺ transients, and, in particular, those within the end-feet, have important albeit complex actions on vascular tone. Of paramount importance to all this work, however, is whether a rise in astrocyte intracellular Ca²⁺ is indeed the signal responsible for relaying information on the metabolic state of neurons to the vasculature in order to affect vessel diameter and ultimately CBF. There are several observations concerning the very nature of astrocyte Ca²⁺ signaling that makes it a worthwhile endeavor to entertain this query. As CBF changes occur in a graded manner in order to match graded changes in synaptic activation, it is expected that astrocyte Ca²⁺ will mirror these changes if this signal is indeed the key factor in mediating functional hyperemia and other forms of neurovascular coupling. However, there are several lines of evidence that reveal a curious inconsistency with this idea. First, Ca²⁺ increases in astrocytes often occur independent of neuronal activity (Nett et al., 2002). These spontaneous Ca²⁺ oscillations have been reported both in vivo (Hirase et al., 2004) and in vitro (Parri and Crunelli, 2001; Parri et al., 2001). Second, the astrocyte Ca²⁺ waves thought to transduce important information toward vessels are not faithfully observed in vivo, suggesting this signaling process may actually be attributable to aspects of the slice or culture condition, or that it manifests more easily in pathophysiological conditions such as epilepsy (Tashiro et al., 2002; Balazsi et al., 2003), rather than being the “normal” method of signaling for neurovascular coupling. In brain slices and in vivo, astrocyte Ca²⁺ signals are far more discrete. Glutamate uncaging on single astrocytes in the hippocampus activates a limited number of astrocytes in a discrete yet complex astrocytic network; complex in the sense that a neighboring astrocyte’s proximity to the stimulated one is not an indicator of activation (Sul et al., 2004). Subtle afferent stimulation can cause a highly localized rise in Ca²⁺ in an astrocyte process (Pasti et al., 1997), while stimulating at a higher frequency can activate a few local astrocytes (Porter and McCarthy, 1996). Regardless
of the method a broad reaching Ca\textsuperscript{2+} is not generated. However, afferent stimulation can produce what appears to be a discrete Ca\textsuperscript{2+} wave that propagates down astrocytic processes to invade end-feet causing cerebral vessel dilation (Zonta et al., 2003b), suggesting Ca\textsuperscript{2+} waves are utilized in the intact brain on a small scale. Finally, recent work for the Helmchen lab has described a novel two photon scanning method that is capable of measuring Ca\textsuperscript{2+} signals at frequencies up to 10 Hz in hundreds of cells simultaneously (Gobel et al., 2007). This technique has demonstrated that neuronal activity fails to consistently increase astrocyte Ca\textsuperscript{2+} with a temporal profile that follows the timing and patterning of neuronal activation. However, the lack of an obvious Ca\textsuperscript{2+} signal that propagates from synapses to a vessel does not necessarily mean astrocyte Ca\textsuperscript{2+} signals (end-feet excluded) are meaningless with regard to neurovascular coupling. Instead, this may simply reflect our ignorance of why such timing differences arise and how the vastly different astrocyte signals preserve neural information, which we cannot currently decode. It is apparent that more experimentation is needed to tease out the true nature of astrocyte Ca\textsuperscript{2+} signals in the control of the cerebrovasculature.

### 18.8 Norepinephrine and Astrocyte-Mediated Cerebrovascular Control

One set of experiments that has provided clues toward the functional impact of astrocyte Ca\textsuperscript{2+} signals in controlling CBF comes from the rise in end-feet Ca\textsuperscript{2+} induced by norepinephrine (NE) and the subsequent vessel constriction (Mulligan and MacVicar, 2004). Vasomotor responses cannot be completely abolished by mGluR antagonists when synapses become activated (Filosa et al., 2004) suggesting that transmitters other than glutamate can participate in neurovascular coupling. Along this line, an electron microscope examination of arterioles has revealed that the majority of noradrenergic terminals originating from the locus coeruleus that associate with vessels, synapse on astrocyte end-feet rather than SMCs (Cohen et al., 1997).

Previous in vivo work has demonstrated that activation by NE causes a decrease in CBF (Raichle et al., 1975), an effect that may help maintain CBF at a constant rate at higher blood pressures. In vitro work has shown that NE triggers robust intracellular Ca\textsuperscript{2+} increases in astrocytes via activation of \( \alpha \) and \( \beta \) adrenergic receptors (Duffy and MacVicar, 1995). Consistent with these results, experiments conducted by Mulligan and MacVicar (2004) showed that NE-mediated Ca\textsuperscript{2+} increases within astrocyte end-feet temporally preceded prominent vasoconstrictions. When astrocytes were loaded with BAPTA-AM to chelate rises in intracellular Ca\textsuperscript{2+}, vascular constrictions generated by NE were drastically reduced, suggesting Ca\textsuperscript{2+} was critical for the astrocyte-mediated effect. These data add another level of complexity to astrocyte-mediated neurovascular coupling by expanding the realm of vasoactive transmitters beyond that of glutamate.
18.9 Astrocytes in Spreading Depression and Cerebrovascular Constriction

Cortical spreading depression (CSD) is a self-propagating wave of transient cellular depolarization and ionic redistribution followed by synaptic depression. The cellular mechanisms responsible for this phenomenon are incompletely understood, despite both the passage of over 60 years since Leao (1944) first observed the phenomenon and vested scientific interest due to its implications for migraine and the progression of brain tissue injury after stroke or trauma. Of interest here is the in vivo observation that CSD occurs coincident with a reduced CBF in cortical arterioles and capillaries (Hadjikhani et al., 2001; Chuquet et al., 2007). Due to their seemingly uniform anatomical interconnectedness, an ability to generate long distance Ca$^{2+}$ waves and their intimate association with the cerebrovasculature, astrocytes have been implicated in CSD (Smith et al., 2006). Earlier reports show that an astrocyte Ca$^{2+}$ wave temporally precedes a change in the intrinsic optical signal associated with a wave of spreading depression (Basarsky et al., 1998; Kunkler and Kraig, 1998). However, eliminating the astrocytic Ca$^{2+}$ wave with a Ca$^{2+}$ free external solution did not eliminate the CSD wave (Basarsky et al., 1998). A very recent study utilizing in vivo two-photon Ca$^{2+}$ imaging in both neurons and astrocytes also demonstrates the Ca$^{2+}$ wave to precede the CSD wave, but the Ca$^{2+}$ signal increases first in neurons and second in astrocytes, suggesting – as others have – that astrocytes are not driving CSD (Chuquet et al., 2007). However, a consequence of the astrocyte Ca$^{2+}$ wave was a Ca$^{2+}$ increase in astrocyte end-feet, which caused pronounced arteriole constriction. Whereas other studies conducted in vitro and in vivo have shown astrocyte-mediated vasodilation when end-foot Ca$^{2+}$ was elevated during functional hyperemia (Zonta et al., 2003b; Takano et al., 2006), this CSD-induced vasoconstriction was similar to that obtain by Mulligan and MacVicar (2004) in which the focal liberation of Ca$^{2+}$ within end-feet elicited a pronounced decrease in vessel diameter. The reduced CBF that results from this action is consistent with other recent reports showing there is severe hypoxia coincident with CSD because of an increased metabolic demand that exceeds blood supply delivery (Takano et al., 2007).

18.10 Astrocyte-Mediated Vasodilations or Vasoconstrictions?

The fact that end-feet 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 above as a potential factor dictating the vessel response profile, playing a potential role by modulating the efficacy of critical enzymes such as the CYP450s. Another important consideration is the amount of myogenic tone in the vessels under study. In the in vivo preparation, cerebral arterioles generally exhibit a degree of partial
constriction, whereas in vitro the lack of blood flow and accompanying shear stress fails to produce this to the same extent (Resnick et al., 2003). Thus, vessels equilibrate toward a slightly more relaxed state in acute brain slices compared to the intact brain. This may account for some of the observed differences between the two preparations as dilating factors will be less effective on already dilated vessels and the same for constricting factors on constricted vessels. However, as indicated by the NO results obtained from the Newman laboratory, the situation is more complicated than this. For instance, throughout the “middle portion” of the vessel’s full dynamic range, vessel diameter could change in either direction, even if slightly dilated or slightly constricted, which depended on the relative presence or absence of NO (Metea and Newman, 2006). This raises an interesting question as to how other factors or physiological circumstances will influence the polarity of vasomotion during functional hyperemia. For instance, are there factors that dictate what is released from the astrocyte when end-foot Ca\(^{2+}\) becomes elevated, i.e., constricting or dilating agent? From which cell type do such factors arise: astrocytes, SMCs, endothelial cells, or is there a complex interplay between all parties? Alternatively, could both constricting and dilating agents be released from astrocytes simultaneously, with some selection of the preferred agent performed at the SMC level, perhaps influenced by the level of myogenic tone? These will be central questions for the future studies on brain vasculature control.

With the vasoconstrictions observed during CSD we have seen that certain pathophysiological states of the tissue may be important for determining the polarity of vasomotor responses when end-foot Ca\(^{2+}\) is elevated. Other influences 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 other. How these disparate effects are selected for as a function of distance from the center of the functional hyperemic response is not known.

## 18.11 Astrocytes in Brain Energetics and the Link to Blood Flow

It is now appreciated that changes in synaptic activity and neuronal spiking correlate with changes in blood oxygen content (Mukamel et al., 2005). These parameters are also associated with regional alterations in CBF (Vogel and Kuschinsky, 1996; Devor et al., 2005) and glucose consumption (Iadecola et al., 1996; Hu and Wilson, 1997b), but the degree and temporal characteristics of these changes are not intuitively obvious. Astrocyte energetics may play an important role linking changes in
the metabolic demand of the tissue to changes in CBF. Immediately after the initiation of activity there is transient reduction in vessel oxygen content, which precedes any increase in CBF, suggesting a rapid utilization of oxygen (Ances et al., 2001). This observation is supported by a recent metabolic imaging study, which utilized two-photon microscopy to examine the intrinsic fluorescence of the ubiquitous electron carrier reduced β-nicotinamide adenine dinucleotide (NADH) as a measure of brain metabolic redox state. Examination of this signal showed that at the onset of activity, dendritic oxidative metabolism is enhanced (Kasischke et al., 2004), which is consistent with a rapid consumption of O$_2$. Interestingly though, this is followed by a prolonged increase in astrocyte glycolytic metabolism. There are a few lines of evidence in support of this dichotomy between the different type of metabolism recruited by neurons and astrocytes. First, although CBF changes occur in close proportion to cerebral glucose utilization, the proportion of increased O$_2$ consumption is much less (Fox and Raichle, 1986; Fox et al., 1988), suggesting an energy contribution from glycolysis. In the vasculature, the ensuing increase in CBF actually overcompensates for the needed O$_2$, resulting in an oversupply of oxygenated blood, which is responsible for the blood-oxygen-level-dependent (BOLD) signal observed in fMRI (Ogawa et al., 1990). The recruitment of the glycolytic pathway is also supported by a localized increase in lactate, an end product of anaerobic metabolism (Fellows et al., 1993; Hu and Wilson, 1997a). That astrocytes are the primary anaerobic players and the source of the lactate is supported by the idea that astrocytes have far fewer mitochondria than neurons by virtue of the fact that fine astrocyte processes are too thin to contain them and that these finely ramified extensions comprise the majority of the astrocytes volume. While this may only suggest astrocyte’s energy needs are inferior to that of neurons, astrocytes are the predominant source of glycogen in the brain (Ignacio et al., 1990), which is thought to be important for rapid, on-demand supply of ATP via glycogenolysis and subsequent glycolysis (Brown and Ransom, 2007). This supports the notion that astrocytes are inherently primed for glycolysis. Finally, is the concept of the astrocyte–neuron lactate shuttle, in which synaptic glutamate release triggers glutamate and Na$^+$ ion co-transport via enhanced astrocyte transporter activity? Energy-dependent Na$^+$–K$^+$ ATPases work to restore the perturbed ionic gradients, which drive astrocyte glycolysis to generate more ATP. Enhanced glycolysis results in the production and accumulation of lactate, a molecule that can be consumed as fuel in oxidative metabolism. Fittingly, the astrocyte-derived lactate is released and taken up into neurons for this purpose through monocarboxylate transporters (Pellerin and Magistretti, 1994; Pellerin et al., 1998). This hypothesis, while supported, is not without controversy (Pellerin et al., 2007). For instance, the proportionally greater amount of glucose uptake compared to that of O$_2$ consumption, is not accounted for by an equivalent generation of lactate (Madsen et al., 1999). A recent metabolic imaging study shows that the protracted overshoot in NADH fluorescence observed in response to synaptic activation does not represent the initiation of glycolytic metabolism but instead represents the production of NADH from oxidative efforts (Brennan et al., 2006). The fast dip and subsequent overshoot in the NADH signal have also been suggested to be an artifact of the slice preparation, where O$_2$ is diffusion
limited, because this characteristic response profile is not observed in vivo (Turner et al., 2007). There may be alternative targets for the lactate as well. As mentioned above, the local accumulation of metabolic products is not likely to be the sole mechanism responsible for functional hyperemia, but several studies have shown lactate can affect vessel tone and CBF. In the retina, lactate has direct effects on SMCs via activation of NOS and opening of K⁺-ATP channels causing vessel relaxation (Hein et al., 2006). In the CNS, an increase in CBF triggered in response to activity is potentiated by increasing the lactate/pyruvate ratio (Mintun et al., 2004).

The astrocyte–neuron lactate shuttle hypothesis is interesting in light of a recent in vivo study conducted in the olfactory bulb. Using 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, Gurden et al. (2006) studied 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 ionotrophic or metabotropic glutamate receptors. Instead, their findings implicated glutamate uptake through astrocyte transporters as the major contributor (Gurden et al., 2006). These data are consistent with the idea that glutamate uptake is an important trigger for blood vessel dilation. As the astrocyte–neuron lactate shuttle is thought to be initiated by glutamate uptake, the generation and release of lactate may be important for both a neural metabolic substrate and the control of CBF. However, what proportion of the measured olfactory bulb 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 volume changes (MacVicar and Hochman, 1991). Clearly, more experiments are necessary to determine the precise role of glutamate uptake and oxidative vs. glycolytic metabolism in the control of CBF.

18.12 New Players: Pericytes and Vasoactive Interneurons

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 them. 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 pericytes to relax the capillary wall (Peppiatt et al., 2006). Ischemia also results in focal capillary constrictions that correspond 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).

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 (Tong and Hamel, 2000; Cauli et al., 2004). 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 nitric oxide synthase elicited dilations. Here again we see a dichotomy in the control of vasomotor responses. This is a stark reminder that the original assumptions underlying functional hyperemia – that the degree of blood flow is a simple function of the metabolic state and therefore the level of neuronal activation – need reconsideration.

18.13 Conclusion

The work described here indicates that astrocytes are capable of eliciting changes in vessel diameter in both directions. While there appears to be an important initiating role for glutamate through the activation of group I mGluRs, which raises intraglial Ca\textsuperscript{2+}, other inputs and transmitters are likely involved in neurovascular coupling. An increase in intracellular free Ca\textsuperscript{2+} and the subsequent activation of Ca\textsuperscript{2+} sensitive PLA\textsubscript{2} in astrocytes can trigger a surprisingly diverse array of vasoactive metabolites after the initial production of AA. Constriction occurs when AA is converted to 20-HETE, while dilation results from the conversion of AA to PGE\textsubscript{2} 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 Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in astrocyte end-feet and the efflux of K\textsuperscript{+} has also been suggested to relax vascular tone by hyperpolarizing SMCs via Kir channels. Astrocytes are proving to be important mediators of neurovascular coupling, but a comprehensive understanding of their part is far from complete. Novel experiments and techniques are unfurling much complexity in processes such as functional hyperemia, which historically was thought to occur by a simple correspondence to neuronal activation.
References


Madsen PL, Cruz NF, Sokoloff L, Diener GA (1999) Cerebral oxygen/glucose ratio is low during sensory stimulation and rises above normal during recovery: Excess glucose consumption during stimulation is not accounted for by lactate efflux from or accumulation in brain tissue. J Cereb Blood Flow Metab 19:393–400
Ogawa S, Lee TM, Kay AR, Tank DW (1990) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci USA 87:9868–9872

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine 5′-triphosphate</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>CSD</td>
<td>Cortical spreading depression</td>
</tr>
<tr>
<td>CYP450</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>EET</td>
<td>Epoxyeicosatrienoic acid</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glial fibrillary acidic protein</td>
</tr>
<tr>
<td>20-HETE</td>
<td>20-Hydroxyeicosatetraenoic acid</td>
</tr>
<tr>
<td>IOS</td>
<td>Intrinsic optical signal</td>
</tr>
<tr>
<td>IP₃</td>
<td>1,4,5-Inositol-trisphosphate</td>
</tr>
<tr>
<td>KO</td>
<td>Knockout</td>
</tr>
<tr>
<td>L-NAME</td>
<td>N’(G)-Nitro-L-arginine methyl ester</td>
</tr>
<tr>
<td>mGluR</td>
<td>Metabotropic glutamate receptor</td>
</tr>
<tr>
<td>NADH</td>
<td>Reduced β-nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>NO synthase</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td>PLA₂</td>
<td>Phospholipase A₂</td>
</tr>
<tr>
<td>SMCs</td>
<td>Smooth muscle cells</td>
</tr>
<tr>
<td>t-ACPD</td>
<td>(1S, 3R)-1-Aminocyclopentane-1, 3-dicarboxylic acid</td>
</tr>
<tr>
<td>VGCCs</td>
<td>Voltage-gated calcium channels</td>
</tr>
<tr>
<td>VRACs</td>
<td>Volume-regulated anion channels</td>
</tr>
</tbody>
</table>