The role of neuronal signaling in controlling cerebral blood flow

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Abstract

Well-regulated blood flow within the brain is vital to normal function. The brain’s requirement for sufficient blood flow is ensured by a tight link between neural activity and blood flow. The link between regional synaptic activity and regional cerebral blood flow, termed functional hyperemia, is the basis for several modern imaging techniques that have revolutionized the study of human brain activity. Here, we review the mechanisms of functional hyperemia and their implications for interpreting the blood oxygen level-dependent (BOLD) contrast signal used in functional magnetic resonance imaging (fMRI).

Keywords: Functional hyperemia; Cerebral blood flow; Neurovascular unit; BOLD

1. Introduction

Although it is only 2% of total body weight, the brain uses 20% of the total energy consumed by the body (Sokoloff, 1989). Well-regulated blood flow within the brain is vital to maintain energy-dependent processes and to clear metabolic byproducts produced by neuronal activity, such as CO₂, excess lactate, other metabolites and heat. The dependence of the brain on blood flow is highlighted by the fact that even relatively small reductions in cerebral blood flow (CBF) can have deleterious effects on the brain. Specifically, a 20% reduction in CBF inhibits cerebral protein synthesis (Hossmann, 1994). A 50% CBF reduction leads to extracellular accumulation of glutamate and lactate, and water shift from intra- to extra-cellular compartments (Hossmann, 1994). CBF reductions greater than 50% impair ATP synthesis and decrease the ability of neurons to fire action potentials. If flow is reduced by 80%, neurons lose ionic gradients, a process termed anoxic depolarization, and undergo irreversible damage (Hossmann, 1994). Thus, even small reductions in CBF negatively affect neuronal function, and large CBF reductions, such as are seen in cerebral ischemia, can produce massive damage to the brain. Moreover, cerebrovascular dysregulation is associated with Alzheimer’s disease and other neurodegenerative conditions (see Iadecola, 2004 for review).

The brain’s requirement for sufficient blood flow is ensured by a tight link between neural activity and blood flow. This link between regional synaptic activity and regional CBF, termed functional hyperemia, is the basis for several modern imaging techniques that have revolutionized the study of human brain activity (Logothetis & Wandell, 2004; Raichle, 1998). Here, we review the mechanisms of functional hyperemia and their implications for interpreting functional magnetic resonance imaging (fMRI) data.

1.1. Neuroanatomical basis of functional hyperemia: the neurovascular unit

In both normal and injury conditions, neurons, glia and blood vessels exhibit a close anatomical and functional coupling (del Zoppo & Mabuchi, 2003; Woolsey et al., 1996). These cells can be considered a functional entity,
termed the neurovascular unit (Lo, Dalkara, & Moskowitz, 2003). As shown in Fig. 1, the cellular constituents of the neurovascular unit vary with the type, size and location of blood vessels.

On the surface of the brain, large cerebral arteries give rise to smaller arteries and arterioles known as pial arteries (Jones, 1970). Pial arteries are composed of an endothelial cell layer, a smooth muscle cell layer and an outer layer of leptomeningeal cells (adventitia), which is separated from the brain by the Virchow-Robin space (Peters, Palay, & Webster, 1991). Pial arteries are densely innervated by perivascular nerves that originate from autonomic and sensory ganglia, mainly the sphenopalatine, trigeminal and superior cervical ganglia (Edvinsson & Hamel, 2002). These nerves contain several neurotransmitters and neuropeptides that can either constrict or dilate arteries and arterioles (Edvinsson & Hamel, 2002). Because of the diffuse nature of the innervation and the relatively large size of the vessels innervated, perivascular innervation by peripheral autonomic nerves is unlikely to play a role in the highly localized changes in flow initiated by functional brain activity. Rather, these neurons are likely to modulate global increases in CBF that occur during epileptic seizures, hypertension, or following transient interruption of CBF (Iadecola, 1998).

As pial arteries and arterioles penetrate deeper into the brain parenchyma, they are still separated from the substance of the brain by a virtual space (the Virchow-Robin space) (Fig. 1). However, recent work (Takano et al., 2006) has shown that arterioles are closely associated with astrocytic end-feet, which can influence arteriole diameter. Cerebral endothelial cells are specialized to form the blood–brain barrier: they lack fenestrations and are connected by small adhesions known as tight junctions. Endothelial cells are further connected by gap junctions, which allow transmission of intracellular responses between adjacent cells (Segal, 2000; Sokoya et al., 2006). Endothelial cells can release a variety of vasoactive
substances (Busse & Fleming, 2003). These include the vasodilators nitric oxide, reactive oxygen species (which can cause vascular dysregulation at high concentrations (Faraci & Heistad, 1998)) prostacyclin, and endothelium-derived hyperpolarizing factor, and the vasoconstrictors endothelin and endothelin-derived constrictor factor (Faraci & Heistad, 1998; Golding, Marrelli, You, & Bryan, 2002). In addition, carbon monoxide, possibly derived from the endothelium, has been proposed to produce vasodilation in the piglet cerebral microcirculation (Kanu, Whitfield, & Leffler, 2006). Astrocytes also can release a factor derived from lipoxygenase metabolism that produces endothelium-dependent relaxation of cerebral arteries (Murphy, Rich, Orgren, Moore, & Faraci, 1994). The final targets of these vasoactive substances, whether released from endothelial cells, astrocytes, or neurons, are the smooth muscle cells and pericytes. Studies in the retinal microcirculation suggest that pericytes respond to vasoactive signals by constricting or relaxing to alter vascular diameter (Kawamura et al., 2003; Wu, Kawamura, Sakagami, Kobayashi, & Puro, 2003). Smooth muscle cells also respond directly to increased intravascular pressure with constriction (Prewitt, Rice, & Dobrian, 2002). This property allows smooth muscle cells to counter pressure-induced changes in the rate of blood flow, and contributes to the ability of cerebral blood vessels to maintain flow in the face of changes in arterial pressure (cerebrovascular autoregulation). Additionally, gap junctions between smooth muscle cells facilitate propagation of vascular signals along the vessel (Kawamura et al., 2003; Lagaud, Karicheti, Knot, Christ, & Laher, 2002).

As vessels penetrate deeper into the brain, the Virchow-Robin space is no longer found and vessels become intraparenchymal arterioles and capillaries. At this level the vascular basal lamina, and to a lesser extent smooth muscle cells and pericytes, directly contact neural processes and astrocytic end-feet (Cohen, Bonvento, Lacombe, & Hamel, 1996; Rennels & Nelson, 1975) (Fig. 1). Intracerebral arterioles and capillaries are likely to be involved in the local regulation of microvascular flow, and chemical signals to these vessels from astrocytes and neurons are important influences on local flow. Astrocytic end-feet occupy a much greater proportion of the vascular surface than do neural processes (Jones, 1970; Maynard, Schultz, & Pease, 1957), and evidence has emerged for a direct role of astrocytes in influencing vascular diameter (Hirase, 2005; Mulligan & MacVicar, 2004; Zonta & Angulo et al., 2003a). Neuronal processes that contact intraparenchymal blood vessels originate from local interneurons or from neurons whose cell bodies are located in subcortical regions and project to neocortex (Fig. 1). To date, subcortical regions known to contribute to cortical perivascular innervation include the basal forebrain, raphe, ventral tegmental area and locus coeruleus (Van Lieshout, Wieling, Karemaker, & Secher, 2003).

Thus, the close anatomical and functional associations between microvessels, neurons, and astrocytes in the brain suggest the importance of conceptualizing them as a neurovascular unit. The ability of this unit to regulate CBF involves several types of interactions between all of the components.

2. Mechanisms of functional hyperemia

2.1. Diffusion of synaptically released neurotransmitters

More than a century ago, Roy and Sherrington (Roy & Sherrington, 1890) proposed that working neurons release vasoactive agents in the extracellular space, and these agents reach blood vessels by diffusion and produce relaxation of vascular smooth muscles. Considerable evidence has since accumulated supporting vasoactive consequences of neurotransmitter release, in particular for the synthetically released fast transmitters glutamate and GABA, but the mechanisms are far more complex and indirect than simple diffusion to vascular targets.

2.1.1. Glutamate

There is substantial evidence from studies of cerebellum, hippocampus and neocortex that glutamate influences blood flow. In cerebellar cortex, the Purkinje cells, which are the only cerebellar output neurons, receive their major excitatory synaptic inputs from parallel fibers and climbing fibers. The parallel fibers are known to be glutamatergic (Ito, 1991), and the climbing fibers utilize an excitatory amino acid such as glutamate, aspartate or N-acetyl-aspartyl-glutamate (Ross, Bredt, & Snyder, 1990). Focal activation of these excitatory inputs leads to an increased blood flow that is blocked by antagonists at non-NMDA-type glutamate receptors, and is mimicked by exogenous glutamate application (Yang & Iadecola, 1996; Yang & Iadecola, 1997). Glutamate also has vasodilatory actions in neocortical and hippocampal slice preparations. Exogenous glutamate or selective glutamate receptor agonists dilate pial arterioles and/or precapillary microvessels. In contrast to cerebellum, this effect in hippocampus and neocortex does involve NMDA-type glutamate receptors (Fergus & Lee, 1997a; Lovick, Brown, & Key, 1999).

Glutamate does not act directly on smooth muscle cells to produce vasodilation (Faraci & Breese, 1993). Rather, this neurotransmitter increases CBF by inducing the release of vasoactive factors from other cells through several calcium-dependent mechanisms (Fig. 2). In all brain areas studied, the vasoactive actions of glutamate are attenuated by blocking nitric oxide synthase (NOS) (Akgören, Fabricius, & Lauritzen, 1994; Faraci & Breese, 1993; Fergus & Lee, 1997a; Li & Iadecola, 1994; Lovick et al., 1999; Yang, Chen, Ebner, & Iadecola, 1999) or in NOS null mice (Yang, Zhang, Ross, & Iadecola, 2003). Neuronal NOS (NOS I) is present in neurons and glia, and is the synthetic enzyme for nitric oxide (NO), a potent vasodilator that is released during synaptic activity (Strijbos, 1998). Several region-specific
effects of glutamate are also seen. In cerebellum, pharmacological and genetic studies have shown that NOS-dependent CBF increases are responsible for the vast majority of the response to functional activation (Li & Iadecola, 1994; Yang et al., 1999; Yang et al., 2003), whereas in somatosensory cortex, NOS-related mechanisms are less prominent (Lindauer, Megow, Matsuda, & Dirnagl, 1999). Cyclooxygenase-2 (COX-2), an enzyme that synthesizes prostaglandins from arachidonic acid and is associated with glutamatergic synapses (Kaufmann, Worley, Pegg, Bremer, & Isakson, 1996), is also involved in the regulation of CBF during synaptic activity (Niwa, Araki, Morham, Ross, & Iadecola, 2000). In neocortex, glutamate-induced increases in intracellular calcium also activate the synthesis of epoxygenase products from cytochrome P450 epoxygenase (Alkayed et al., 1997).

Recent evidence suggests a prominent role for astrocytes in glutamate-mediated vasooactivity. Astrocytes are known to possess metabotropic glutamate receptors (mGluRs) and to respond to glutamate with a transient increase in intracellular Ca++ that spreads to more distant portions of the cell (Kang, Jiang, Goldman, & Nedergaard, 1998; Pasti, Volterra, Pozzan, & Carmignoto, 1997; Porter & McCarthy, 1996). Zonta et al. (Zonta & Angulo et al., 2003a) found that in somatosensory cortex slices, neuronal activity or certain subtype-selective mGluR agonists trigger astrocytic Ca++ waves that correspond temporally with activity-induced vasodilation of local arterioles. Antagonism of the same mGluR subtypes diminishes astrocytic Ca++ waves without affecting neurons, and direct stimulation of individual astrocytes produces rapid vasodilation of nearby arterioles. The astrocytic effect is largely dependent on a COX product, perhaps the powerful vasodilator prostaglandin E2, which is released from somatosensory cortical astrocytes by Ca++ waves (Zonta & Sebelin et al., 2003b). It was suggested that synthetically released glutamate spills over from the synapse and activates nearby astrocytes; if the resulting intracellular Ca++ wave is sufficient to reach an end-foot contacting a microvessel, vasodilation will occur (Zonta & Angulo et al., 2003a). On the other hand, others have shown that decreases in Ca++ in astrocytes produce vasoconstriction rather than vasodilation by releasing P450 metabolites (Mulligan & MacVicar, 2004). The discrepancy could be explained by the fact that Zonta et al. preconstricted the vessels in the slice with L-NAME, a blocker of NO synthesis (Zonta & Angulo et al., 2003a).
Although the observed effects in slices may not completely mimic in vivo responses, given the limitations inherent in the slice model, these studies clearly show that glutamate can have direct effects on astrocytes that influence vascular tone.

2.1.2. GABA

Exogenous GABA, acting via GABA_A receptors, dilates precapillary microvessels in hippocampus and neocortex (Fergus & Lee, 1997b). Furthermore, local neurons mediate the neocortical vasodilatation resulting from cerebellar stimulation (Iadecola, Arneric, Baker, Tucker, & Reis, 1987). However, in the cerebellar cortex itself GABA is not involved in activation-induced increases in flow (Li & Iadecola, 1994; Mathiesen, Caesar, Akgoren, & Lauritzen, 1998). The vasoactive effects of GABA in the forebrain are interesting in light of continued controversy over whether inhibitory neurotransmission contributes to brain energy use or to functional imaging signals. In cerebral cortex, GABAergic interneurons substantially innervate microvessels, suggesting they may act as integrators of local vascular responses (Vaucher, Tong, Cholet, Lantin, & Hamel, 2000). Interestingly, some GABAergic interneurons also contain NOS (Cauli et al., 2004), and NOS-containing neurons are known to contact large cerebral arteries and microvessels (Estrada & De, 1998; Iadecola et al., 1993).

2.1.3. Other vasoactive mediators released by neural activity

During neurotransmission, potassium ions are released during neuronal repolarization and astrocytic spatial buffering; the latter process particularly involves perivascular endfoot, where K+ conductance is greatest (Iadecola, Li, Yang, & Xu, 1996). Activity-evoked rises in extracellular K+ have been shown to moderate increases in blood flow (Caesar, Akgoren, Mathiesen, & Lauritzen, 1999; Iadecola & Kraig, 1991), an effect in part mediated by NO (Dreier et al., 1995). Neural activity also produces extracellular increases in hydrogen ions, as well as in adenosine produced by ATP catabolism (Iliff, D’Ambrosio, Ngai, & Winn, 2003).

2.2. Vascular and glial innervation

In addition to diffusion of synaptically released vasoactive agents, central neurons could influence vascular tone through direct contacts with arterioles, capillaries, and perivascular glial processes (Fig. 2). These neurons contain neuromodulators and neuropeptides, which contrast with GABA and glutamate in that they can be released from non-synaptic portions of the neuron to act on nearby cellular targets. The neuromodulators identified in terminals forming perivascular contacts have been shown to influence vascular diameter to regulate CBF, suggesting important roles in controlling blood flow in the normal brain.

Many studies have suggested vasoactive roles for monoaminergic and cholinergic neurons in the brain. Serotonergic axons and terminals contact parenchymal microvessels (Reinhard, Liebmann, Schlosberg, & Moskowitz, 1979). These neurons originate in the brainstem raphe and project diffusely throughout the brain. Serotonin has a pronounced vasoconstrictor effect, although it can be vasodilatory under some conditions. This dual effect is consistent with the complex distribution of serotonin receptors to both neurons and astrocytes, the large number of serotonin receptor subtypes, and the demonstrated ability of serotonin to affect vessels directly as well as indirectly through astrocytes (Cohen et al., 1996). Norepinephrine-containing terminals from locus coeruleus contact scattered cortical microvessels (Cohen, Molinatti, & Hamel, 1997; Raichle, Hartman, Eichling, & Sharpe, 1975), particularly capillaries (Cohen et al., 1997), and norepinephrine applied in vivo produces vasoconstriction and a reduced CBF (Raichle et al., 1975). These terminals more frequently contact astrocytic end-feet that are associated with microvessels (Cohen et al., 1997; Papasalas & Papadopoulos, 1996). The functional relevance of this association is suggested by recent evidence that norepinephrine can produce vasoconstriction that is tightly associated with direct norepinephrine-induced effects on astrocytes (Mulligan & MacVicar, 2004). Dopaminergic projections from ventral tegmental area contact penetrating arterioles and cerebral capillaries in cerebral cortex. These afferents terminate either directly on the vascular basal lamina or on perivascular astrocytic end-feet, or are near capillary pericytes (Krimer, Muly, Williams, & Goldman-Rakic, 1998). In cortical slice preparations, iontophoretic microapplication of dopamine near cerebral microvessels produces vasoconstriction in approximately 50% of the microvessels studied (Krimer et al., 1998). Similarly, in isolated cerebral arteries and in pial arterioles in situ, dopamine produces vasoconstriction (Sharkey & McCulloch, 1986). Finally, axons and terminals containing choline acetyl transferase, the acetylcholine synthetic enzyme, are apposed to the basal laminae of capillaries and small arterioles, and to capillary endothelial cells (Arneric et al., 1988; Parnavelas, Kelley, & Burnstock, 1985). Neurons in the basal forebrain are a major source of this cholinergic perivascular innervation, which is particularly prominent in cerebral cortex (Arneric, 1989; Arneric et al., 1988). The functional significance of cholinergic innervation is suggested by findings that stimulation of basal forebrain neurons produces vasodilation and increases blood flow in the cerebral cortex (reviewed in (Sato & Sato, 1992)). This increase in CBF is not associated with increased cortical energy metabolism (Sato & Sato, 1992) and is likely to involve production of NO in endothelial cells (Zhang, Xu, & Iadecola, 1995). More recently, evidence has also emerged for direct actions of acetylcholine on pericytes in retinal microvessels (Wu et al., 2003).

Additionally, several types of neuropeptide-containing terminals have been found to make perivascular associations. For example, the brainstem parabrachial nuclei contain substance P and neurotensin in perivascular processes (Milner & Pickel, 1986a, 1986b). Vasoactive intestinal peptide (VIP) is in perivascular terminals in cortex (Papasalas & Papadopoulos, 1998), and application of VIP dilates cortical
vessels (Yaksh, Wang, & Go, 1987). Moreover, stimulation of individual cortical GABAergic neurons containing VIP and NOS produces local vasodilation, and vasodilation can be evoked by application of VIP to slices (Cauli et al., 2004). Somatostatin and neuropeptide Y (NPY) appear to play the opposite role: vasocostriction is produced following exogenous application of either peptide (Cauli et al., 2004; Long, Rigamonti, Dosaka, Kraimer, & Martinez-Arizala, 1992) or by stimulation of individual GABAergic somatostatin-containing neurons (Cauli et al., 2004). In addition to microvessels, some major cerebral arteries are surrounded by perivascular NPY-containing terminals, and constrict in response to NPY application (Tuor, Kelly, Edvinsson, & McCulloch, 1990). However, it must be noted that the vasoactive effects of neurotransmitters and neuropeptides vary in different animal species (Faraci & Heistad, 1998). Therefore, the species needs to be taken into account in the interpretation of their physiological effect.

The function of the innervation of intracerebral microvessels has been debated extensively (Edvinsson & Hamel, 2002). One possible function of neuromodulatory innervation of blood vessels may be to focus the vascular response to regions experiencing neural activity. That is, when local flow increases, more distal pial arteries that supply the activated region must also increase flow. This raises the possibility of increasing flow in other microvessels supplied by the arteries. In order to restrict the increase in flow to the area of increased activity, microvascular adjustments involving both the activated area and surrounding areas must be made. Although there is certainly a role for intravascular control of upstream blood supply (see below), it is possible that perivascular neuromodulators also play a role. Additionally, as has been suggested (Krimer et al., 1998; Raichle et al., 1975), innervation of endothelial cells could modulate the transfer of substances across the blood–brain barrier.

2.3. Energy deficit as the stimulus for functional hyperemia

Increases in CBF have been correlated with local energy use during activity, although the extent to which energy deficit drives the increase in CBF is still a subject of active inquiry (see reviews by Attwell & Laughlin, 2001; Shulman, Rothman, Behar, & Hyder, 2004). Certainly, understanding functional hyperemia requires addressing how the brain uses energy, and which processes are most energy demanding. This question has been recently addressed by several studies. Attwell and Laughlin (Attwell & Laughlin, 2001) used a “bottom-up” approach in which specific cellular functions were given an energy cost based on published data, and then summed up to construct an “energy budget” for the rodent and primate brain. The results of this analysis (Fig. 3) indicate that over 80% of the energy in the primate brain is used for processes related to glutamate signaling at synapses, a conclusion also reached by Ames (Ames, 2000) and Shulman and colleagues (Sibson et al., 1998).

The energy used by inhibition has remained controversial. Some reports suggest that inhibitory synapses use less energy than excitatory synapses (Waldvogel et al., 2000), while others have noted that increased glucose usage was associated with inhibition of hippocampal pyramidal and auditory cells (Ackerman, Finch, Babb, & Engel, 1984; Nudo & Masterton, 1986). Although the ion fluxes that generate resting and action potentials are the same in inhibitory neurons and excitatory neurons, the electrochemical gradient down which Cl− moves postsynaptically at inhibitory synapses is less than that down which Na+ moves at excitatory synapses, implying less energy expenditure to pump the ions back. Furthermore, cortical inhibitory neurons and synapses are only one tenth as numerous as excitatory neurons (Abeles, 1991; Brainten & Schuz, 1998).

Most of the energy used by the brain derives from oxidative metabolism. Energy can also be produced, albeit less efficiently, by the anaerobic metabolism of glucose or glycolysis. The telltale sign of glycolysis is lactate production, which increases with activation (Frahm, Kruger, Merboldt, & Kleinschmidt, 1996; Prichard et al., 1991; Ueki, Linn, & Hossmann, 1988). Why does the brain need to use glycolysis? Gusnard and Raichle (Gusnard & Raichle, 2001) have recently examined this issue. One possibility is that, because glycolysis generates energy faster than glucose oxidation, the rapid increase in energy demands at the onset of activation is best met by anaerobic metabolism of glucose released from glycogen. With sustained activation, the increase in CBF has time to develop fully, resulting in an increase in the tissue delivery of oxygen and glucose. At this point the energy metabolism switches from anaerobic to aerobic glucose metabolism. This view is supported by the following observations. First, brain activation increases the utilization of glycogen, which in brain is present mainly in glial cells (Brown, Tekkot, & Ransom, 2003; Swanson, Morton, Sagar, & Sharp, 1992). Second, during sustained activation lactate production is maximal at the onset of activation and then decreases with time (Prichard et al., 1991). Third, oxygen consumption increases only minimally at the onset of activation and then rises progressively as the activation continues (Fox & Raichle, 1986; Fox, Raichle, Mintun, & Dence, 1988; Mintun, Vlassenko, Shulman, & Snyder, 2002). Thus, it would seem that the brain uses anaerobic glycolysis at the onset of activation and, if energy demands are sustained, oxidative metabolism.

Magistretti and colleagues have proposed a model that fits well with the idea of a shift between anaerobic and aerobic glucose metabolism during activation (Magistretti, Pellerin, Rothman, & Shulman, 1999; Pellerin & Magistretti, 2003). Glutamate released during synaptic activity is taken up by astrocytes through specific glutamate transporters (GT), GLAST and GLT-1, coupled to Na+, so that for each glutamate transported 3 Na+ enter the cell (Fig. 3). To re-establish the ionic gradient, Na+ is pumped out of the cell by activation of the Na/K ATPase, which is fueled by glycolytically derived ATP. The lactate so produced is passed on to neurons that metabolize it aerobically, generating ATP. ATP is then used to fuel the ion pumps that reestablish ionic gradients after depolarization. Therefore, it is conceivable that the
increase in lactate observed at the onset of activation is derived from astroglial glycolysis. The subsequent reduction in lactate and rise in oxygen consumption reflects the aerobic metabolism of lactate by neurons. This hypothesis, which has generated considerable interest and controversy (Chih & Roberts, 2003; Pellerin & Magistretti, 2003), is supported by a growing body of evidence indicating that: (1) glutamate stimulates anaerobic glycolysis in astrocyte cultures leading to lactate release into the medium (Pellerin & Magistretti, 1994; Takahashi, Driscoll, Law, & Sokoloff, 1995); (2) neurons and astrocytes in culture have the potential of metabolizing both glucose and lactate, but neurons prefer extracellular lactate and astrocytes favor glucose (Bouzier-Sore, Voisin, Canioni, Magistretti, & Pellerin, 2003; Itoh et al., 2003); (3) MCT2, the monocarboxylic acid transporter that carries lactate, is preferentially located in neurons (Pierre, Magistretti, & Pellerin, 2002); (4) downregulation of glial glutamate transporters attenuates cerebral glucose utilization (Cholet et al., 2001; Voutsinos-Porche et al., 2003). Because the critical evidence supporting the astrocytes-to-neurons lactate shuttle hypothesis is based on in vitro experiments, the biological relevance of the model has been questioned (Chih & Roberts, 2003). Therefore, there is a need for in vivo evidence supporting the cellular compartmentalization of these metabolic processes. In the meantime, the “lactate shuttle” idea remains a useful working hypothesis that fits well with the available data on glucose-oxygen coupling in the activated brain.

A recent study indicates that NADH, produced during energy metabolism by transfer of electrons and protons from glucose to cytosolic free NAD(+), augments blood flow (Ido, Chang, & Williamson, 2004). In retina and visual cortex of rats, visual stimulation produces an elevated blood flow that is enhanced by the injection of lactate and diminished by injection of pyruvate. This photostimulation-induced flow is prevented by inhibition of NOS. The authors suggest that lactate provided additional electrons and protons for transfer to NAD(+), converting it to NADH, while pyruvate received transferred electrons and protons from NADH, converting it to NAD(+). These data led to the suggestion that cytosolic free NADH fuels a signaling cascade that increases nitric oxide production, which in turn augments blood flow in photostimulated retina and visual cortex. The significance and generality of this finding is still unclear.

2.4. Propagation of responses along cerebral blood vessels

During functional hyperemia, local vasodilation increases flow most effectively if the upstream arterioles also dilate (Duling et al., 1987). This has been observed in...
several brain areas, for example, activation of whisker barrel cortex increases vascular diameter in pial arterioles that are several hundred micrometers away from the site of activation (Cox, Woolsey, & Rovainen, 1993; Erinjeri & Woolsey, 2002; Ngai, Ko, Morii, & Winn, 1988). In cerebellum, direct electrical stimulation of parallel fibers increases vascular diameter of local arterioles and of the upstream branches from which these arterioles originate (Iadecola, Yang, Ebner, & Cheng, 1997).

Several possible mechanisms have been proposed for controlling upstream vasodilation, including widespread neurovascular innervation and “intramural” signaling within the vascular wall. The former possibility seems increasingly unlikely: the main innervation of larger pial arterioles originates from outside the brain (Fig. 1), and is not well linked to the localized neural pathways involved in activation. Moreover, cutting the nerves innervating pial arterioles does not attenuate their dilation in response to somatosensory stimulation (Ibayashi et al., 1991). Intramural vascular signaling, in contrast, is supported by several lines of evidence. Endothelial cells and smooth muscle cells in the brain are connected by homocellular gap junctions, and can propagate vasodilation in a retrograde fashion (Dietrich, Kajita, & Dacey, 1996; Sokoya et al., 2006). Flow-mediated vasodilation also could contribute to intramural vascular signaling (Fujii, Faraci, & Heistad, 1991). Local vasodilation increases flow velocity in upstream branches, which, due to increased shear stress, leads to the local release of endothelium-dependent vasodilators (Busse & Fleming, 2003). These vasodilators relax the larger arteries and amplify the increase in flow. However, it is unclear to what extent shear stress induces vasodilation in cerebral vessels (Bryan, Marrelli, Steenberg, Schildmeyer, & Johnson, 2001a; Bryan, Steenberg, & Marrelli, 2001b; Madden & Christman, 1999; Ngai & Winn, 1996; Wilkerson et al., 2005) and the mechanisms of retrograde propagation of vasodilation need to be explored further.

3. Functional brain imaging

Changes in CBF are measured as a proxy for neural activity in several modern imaging techniques, including positron-emission tomography (PET), single photon emission computed tomography (SPECT), and fMRI. One of the most widely utilized is fMRI, and the most common technique used in fMRI is the blood oxygen level-dependent (BOLD) contrast signal. When hemoglobin (Hb) in red blood cells loses oxygen, its iron becomes paramagnetic, which generates magnetic field gradients between the deoxyHb-containing compartments and protons of neighboring water molecules (Ogawa, Lee, Kay, & Tank, 1990). This oxygen-dependent field contrast can be detected with magnetic resonance imaging, and confers the dual advantages of non-invasive imaging and high spatiotemporal resolution. The BOLD contrast signal depends on CBF, cerebral blood volume, and blood oxygenation (Ogawa et al., 1993).

Several uncertainties remain as to the mechanisms underlying the BOLD signal. For example, as detailed above, both neurotransmitter-related signaling and energy demand are correlated with increased CBF, but controversy remains over the relative contribution of each process to the BOLD signal (reviewed in (Attwell & Laughlin, 2001; Shulman et al., 2004)). Another issue is the extent to which the BOLD signal accurately reflects the location and magnitude of the neural events that produce it. Clearly, this is important in determining the degree to which fMRI data yields quantitative information about particular brain regions, and the spatial precision of this information.

3.1. Correlation between the BOLD signal and neural activity

Accurate interpretation of the BOLD signal requires understanding what sort of neural activity produces it (Logothetis & Wandell, 2004). Increasing evidence supports a linkage between CBF changes and local somatic and dendritic activity, rather than output spikes (synchronized action potentials). In the cerebellar cortex, stimulation of the excitatory parallel fibers increases CBF, but inhibits Purkinje cell spiking (Lauritzen, 2001; Mathiesen et al., 1998). In cerebral cortex, the BOLD signal correlates well with local field potentials, which reflect inputs, local-circuit activity, and somatodendritic processing and integration of these inputs in cortical neurons (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). In contrast, cortical neuron output spikes are less well correlated with the BOLD signal (Logothetis et al., 2001).

An important question is whether the spatial extent of the BOLD signal exactly matches that of neuronal activity. As discussed in the preceding sections, local neural activity produces an increase in blood flow that propagates to a larger area (Iadecola et al., 1997; Malonk & Grinvald, 1996). Potentially, contributions from both upstream vasodilation and from downstream draining vessels carrying away deoxyHb could enlarge the BOLD signal beyond the area of neural activity. However, these factors can be minimized by knowing local vasoarchitecture and by optimizing the scanning conditions (Logothetis, 2003). Indeed, in paired physiological recordings and fMRI, there is a linear correlation between electrical activity and the BOLD signal using a voxel size of approximately 3–5 mm$^2$, suggesting good spatial accuracy at this level of resolution (Kim et al., 2004; Logothetis et al., 2001). At smaller voxel sizes, the correlation between BOLD contrast and physiological activity varies considerably, with false positives occurring outside the area of neural activity (Kim et al., 2004).

Interpretation of BOLD signals can also be confounded by regional variations in the BOLD response. It has been widely observed in visual cortex that an initial negative “dip” in the BOLD signal precedes the increase in CBF and the secondary increase in the BOLD signal (Logothetis & Wandell, 2004; Ugurbil, Toth, & Kim, 2003). This transient dip has been attributed to a brief local increase in deoxyHb.
before the increase in CBF brings an oversupply of oxyHb (Fox & Raichle, 1986; Fox et al., 1988). The initial dip has been observed across species and in awake as well as anesthetized animals. However, in the rat somatosensory cortex, the initial “dip” was not seen, rather CBF changes occur before the increase in CBF brings an oversupply of oxyHb (Silva, Lee, Iadecola, & Kim, 2000). This discrepancy suggests that regional and/or species differences may exist in the coupling between deoxyHb levels and CBF.

In conclusion, neurotransmission alters blood flow through several processes working in concert. Synaptic activity triggers neurons and astrocytes to release vasoactive mediators. These mediators act on local blood vessels to produce vasodilation of arterioles, and perhaps capillaries, at the site of activation. Concurrent activation of neurovascular projections and local interneurons can restrict the flow response to the activated area through the coordinated release of vasodilator and vasoconstrictor agents. Vascular endothelial cells and smooth muscle cells further communicate among themselves to propagate vasodilation upstream and fine tune the local distribution of blood flow. These factors together contribute to the neural activity-induced alterations in CBF that form the basis of functional brain mapping techniques. There is still much debate concerning the specific sources of the BOLD signal used in fMRI studies. Although the neurovascular mechanisms of BOLD are not completely understood, this technique remains a powerful tool for exploring the function of the behaving human brain.

References


