Astrocytes Function in Matching Blood Flow to Metabolic Activity

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The brain possesses an intrinsic regulatory mechanism to maintain an adequate supply of O2 and nutrition despite local increases in neuronal activity. Brain astrocytes function as an intermediary cell type by responding to glutamate released from activated neurons, and they couple cerebral blood flow by producing cytochrome P-450-derived epoxyeicosatrienoic acids that induce vasodilation and increase capillary density.

The brain is unique with respect to substrate requirements to meet metabolic energy demands because it is solely dependent on aerobic metabolism. Glucose is the only substrate that is efficiently and completely metabolized as a source of cellular energy. Therefore, neural activity is sustained through adequate delivery of glucose and O2 via highly autoregulated blood flow. Metabolically driven increases in blood flow occur in discrete brain areas, supporting neuronal activity associated with specific functions, such as voluntary movement, sight, and so forth. This increased blood flow to discrete neural circuits occurs over a backdrop of “stable” perfusion to the entire brain. On activation of a group of neurons mediating specific functions, blood flow becomes “hyperemic” in that it increases beyond what is thought to be needed to support neuronal metabolic demand. The reason for such hyperemia or the mechanism of the discrete anatomic increase in blood flow associated with a specific function is not entirely clear.

Consistency of flow despite fluctuations in arterial pressure is not unique to the brain; however, the near-perfect autoregulatory index is. Nutritive blood flow is defined as flow within the microcirculation that is recorded by methods measuring red blood cell flux such as laser-Doppler flowmetry (5). Numerous reports have demonstrated that autoregulation of blood flow in response to increases in arterial pressure is an inherent property of the vessel wall. Cannulated, isolated cerebral arteries exhibit muscle cell activation on increasing pressure (10), leading to elevation of active wall tension and reuction in diameter. Diameter changes in small cerebral arteries in response to pressure is sufficient to maintain constant blood flow (10). Constant flow and, presumably, a relatively constant pressure head is requisite for distribution of blood flow to metabolically active regions of the parietal cortex. The second-to-second distribution of blood flow to the brain in response to initiation of neural activity is thought to be metabolically driven (19). There is a great deal of data demonstrating accumulation of dilatory metabolites, such as adenosine, K+, and so forth, from neurons on increased neuronal firing, leading to the hypothesis that paracrine metabolites from neurons dilate the microcirculation and increase cerebral blood flow (CBF) (19).

A second mechanism hypothesized to mediate hyperemia in the brain in response to neural activation is stimulation of intracranial ganglia that dilate arteries on release of specific transmitters (6, 16). Abundant adventitial nerve fibers exist in cerebral arteries as small as 30 μm in diameter (6). Good reviews exist discussing both the transmitters associated with these adventitial fibers and the identification of a specific ganglion responsible for this innervation (6). However, even with the abundant morphological data related to adventitial innervation and identification of specific dilatory transmitters, a functional role for these intracranial pathways in the regulation of normal functional hyperemia in the brain has been difficult to demonstrate. An exception to this may involve activation of nerve fibers that release nitric oxide (NO) as a neurotransmitter (8). Constitutive NO synthase plays an important role in the regulation of blood flow in most vascular beds, including the brain. NO is released from nerve fibers that are embedded within the adventitial layer of cerebral arteries (8). Although not thoroughly understood, it is thought that NO is released along with acetylcholine (8). The relationship between acetylcholine and NO in neurons is not well understood. NO released from vascular endothelium, astrocytes, and neural pathways modulates arterial tone and the action of other vasoactive metabolites on the brain. In this regard, neurally released NO may function as a controller/modulator of CBF under normal, nonpathological conditions (8). Despite the likelihood that neurally released NO functions to control minute-to-minute regulation of CBF, much work is left to be done in this area.

Astrocytes sense neuronal activity and match blood flow accordingly: a hypothesis

Existing data support the hypothesis that astrocytes function as an intermediary cell type functioning to sense neuronal metabolic activity and match blood flow accordingly (15, 16). Much of what was thought regarding the function of astrocytes is currently being questioned in light of new methodology that was not available years ago when the function of astrocytes was defined. For example, we now know that the blood-brain barrier is primarily a function of vascular endothelium, which exists as a tightly coupled syncytium of cells segregating blood from cerebrospinal fluid (CSF) (16). Although astrocytes send “foot processes” to the cerebral microcirculation, this cell type...
does not form a tight barrier against blood/CSF elements. Astrocytes are anatomically located between neurons and the cerebral microcirculation. Most of the current information on the anatomic relationship between astrocytes and neurons exists with respect to the parietal cortex. In the parietal cortex, there is direct contact between neurons and the microcirculation (14). Astrocytes form an anatomic and functional linkage between neurons and the cerebral microcirculation beginning with arterioles that are <30 μm (16), as seen in Fig. 1A. There is direct, “synaptic like” apposition between cells of the microvasculature and the astrocytes that surround them (Fig. 1B). Such structural orientation suggests a functional relationship between these two cell types. The rest of this short review will attempt to define a hypothesis regarding the functional relationship between these two cell types.

Glutamate stimulates release of arachidonic acid from astrocytes, which is metabolized by cytochrome P-450 enzymes to epoxyeicosatrienoic acids

Astrocytes cultured from the parietal cortex of neonatal rat pups release arachidonic acid (AA) from the plasma membrane phospholipid pool on exposure to glutamate (1). This AA is metabolized into epoxyeicosatrienoic acids (EETs), which can be measured in cells and the culture media (1). The only cytochrome P-450 cDNA identified in rat pup cortical astrocytes, which catalyze the formation of EETs from AA, is completely homologous with a previously sequenced P-450 2C11 cDNA (1). Release of EETs from astrocytes is inhibited pharmacologically by miconazole (1). Inhibition of P-450 epoxygenase activity in vivo results in 20–30% reduction in CBF (1, 2, 10). EETs potently dilate cerebral arterioles through activation of K+ channels (7). These data together suggest that AA, possibly released by glutamate, is metabolized by a P-450 2C11 epoxygenase into vasodilator metabolites. The apposition of neurons and microvessels discussed above provides evidence for the hypothesis that astrocytes could act to sense neural activity via “spillover” of the excitatory neurotransmitter glutamate, which then increases nutritive CBF. As proof of principle, it must be shown that this mechanism plays a role in one or more physiological control processes involving a functional increase in blood flow.

Inhibition of P-450 epoxygenase activity blocks functional hyperemia in response to stimulation of specific neural pathways

Stimulation of the whisker barrel cortex is accompanied by hyperemic flow, which can be blocked by inhibition of P-450 epoxygenase activity. Stimulation of this area of the parietal cortex results in a significant increase in epoxye in the CSF. Under normal conditions, there is substantial amount of EET formed in the CSF (Fig. 2), the level of which reaches 131.2 ng/ml of CSF. In addition, the rate of formation of EETs in the rat brain averages 4.14 ± 1.4 ng/min/mg protein, which is sufficient to account for the evoked hyperemic response. These findings lend support to the earlier finding that inhibition of P-450 epoxygenase activity reduces CBF by 20–30% in anesthetized rats (1).

Given that glutamate is the primary excitatory transmitter involved, combined with the data demonstrating that glutamate has been shown to release EETs from cultured astrocytes, a logical assumption is that the sequence of events leading to functional hyperemia on whisker barrel stimulation is the release of P-450-derived epoxides from astrocytes on stimulation by glutamate released from excitatory synapses in the barrel cortex. We proposed this hypothesis earlier, and it is now our intention to extend this hypothesis into one that incorporates specific mechanisms and broadens its implications.

Action of P-450-derived EETs on cerebral arterial muscle and capillary endothelium

Metabolism of AA by P-450 epoxygenases generates four regioisomers of EET, namely, 5,6-; 8,9-; 11,12-; and 14,15-EETs. The functional epoxide moiety is located across the...
unsaturated bond of the AA molecule (10). Of these, 11,12- and 14,15-EETs are the most stable and most potent with respect to their action on cerebral arterial muscle (7). EETs are formed in and released from astrocytes. The action of EETs on cerebral arterial muscle is to hyperpolarize the plasma membrane by enhancing outward $K^+$ conductance (7). The precise mechanism through which EETs act on $K^+$ channels is not fully understood; however, work by Campbell and colleagues (12) suggests that small-molecular-weight $G$ proteins are implicated. Apart from the action of EETs on arterial membrane ion channels, they are also potent mitogens in vascular and capillary endothelium (4, 13). It is not known if there is a link between the actions of EETs on ion channels and mitogenic activity or if these are two distinctly different mechanisms. Application of EETs to cultured endothelial cells stimulates large increases in thymidine incorporation (4) without formation of capillary-like tube structures. However, with respect to brain capillary endothelium, when plated on a monolayer of cultured astrocytes, formation of capillary-like tube structures occurs that is attenuated by inhibition of $P$-450-catalyzed EET production (13).

At this point we can only speculate about why capillary tube formation is dependent on physical apposition with astrocytes. Speculation includes growth factors present in astrocytes and specific extracellular matrix production forming three-dimensional scaffolding. Trophic factors may also be released by astrocytes similar to those found in motor neurons associated with skeletal muscle development, which may participate in capillary tube formation (11). Two striking findings have emerged from studies in our laboratory. First, fatty acid metabolism by cytochrome $P$-450 enzymes is a necessary component in that inhibition of cytochrome $P$-450 enzyme activity blocks capillary angiogenesis, and second, when cultured together the morphology of astrocytes and endothelial capillary cells changes dramatically. When cocultured, astrocytes project structures that form direct physical contacts with endothelial structures resembling capillaries. These capillary-like structures (Fig. 3) stemming from brain capillary endothelium are similar in morphology to endothelial tubes described in other endothelial cultures (3). Whether or not such structures in vitro correlate to in vivo capillaries remain to be determined, and healthy skepticism in this regard is appropriate.

A role for $P$-450-derived EETs in the process of capillary endothelial tube formation in culture is supported by substantial published and preliminary data. Briefly, cultured astrocytes produce EETs that are absent after inhibition of $P$-450 enzyme activity (1). Astrocytes express a $P$-450 2C11 cDNA coding for enzymes that catalyze formation of EETs from AA (1). Native EETs induce mitogenic activity in capillary endothelium and epithelium, including thymidine incorporation, elevation of intracellular $Ca^{2+}$, and activation of $G$ protein-mediated signaling (3, 9, 13). Gene microarray analysis related to induction of gene expression by cytochrome $P$-450-derived EETs on endothelial cells identified a number of targets that were upregulated, including those that control cellular matrix and adhesion molecules—necessary components in vascular remodeling and angiogenesis (unpublished observations). Recent reports have suggested that EETs, apart from any direct actions, also increase epidermal growth factor activity and potency of action (4). In at least one specific experimental paradigm, i.e., coculture of capillary endothelial cells and cortical astrocytes, it was evident that astrocytes are absolutely essential for tube formation. However, the EETs that are released from astrocytes induce mitogenic activity but are not by themselves sufficient for capillary tube formation. From the above findings we have formulated the hypothesis that astrocytes induce mitogenic activity via release of growth factors stimulated by $P$-450 epoxyenase metabolites. However, the matrix laid down by astrocytes, along with other signaling molecules, appears to be a requisite for formation of three-dimensional structures capable of directing the flow of blood in the brain. The existing view regarding functional hyperemia in the brain is somewhat paradoxical in that it is thought that the delivery of substrate in terms of glucose and oxygen is far in excess of what is needed to support neuronal activity (15). An important question is why neuronal activation elicits hyperemic flow. Although there has been a great deal of speculation in this regard, the hypothesis set forth below is new and will no doubt be controversial.

**Hyperemic blood flow to neurons increases capillary density**

As discussed above, specific neuronal activation results in an increase in blood flow to those metabolically active neurons that is hyperemic in nature; i.e., the increase in blood flow is in excess of metabolic demand (2, 15). Our increased understanding of the role that astrocytes play in brain function is advancing our knowledge of brain physiology and vascular biology. It is becoming evident that one of the primary functions of astrocytes, at least in the parietal cortex, is to act as an intermediary cell type anatomically located between neurons and arterioles to sense neural activity and elevate blood flow to meet neuronal metabolic demand (17). The physiological role of

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**FIGURE 2.** Chromatographic elution profiles of fluorescently derivatized cerebrospinal fluid (CSF, 0.1 ml) drawn from cisterna magna of Sprague-Dawley rats, using tridecanoic acid (TA) as internal standard. Peaks are identified by comparison with derivatized profiles of authentic standards. The regionosymmetric epoxyeicosatrienoic acids (EETs) eluted between 59 and 65 min. 

![Graph](http://physiologyonline.physiology.org/content/17/2/29/fig2)
for hyperemic flow is less evident. Our data support the idea that P-450-derived EETs from astrocytes initiate mitogenic activity within capillary endothelial cells. These EETs potently increase outward K+ current through large-conductance Ca2+-activated K+ channels, causing cell membrane hyperpolarization. In endothelial cell membranes, hyperpolarization increases intracellular Ca2+ (9), inducing release of vasoactive metabolites such as NO; in arteriolar smooth muscle, hyperpolarization dilates by reducing Ca2+ entry through L-type Ca2+ channels. Both of these mechanisms potently dilate cerebral microvessels and increase blood flow. In a normal environment in which astrocytes provide the necessary matrix, angiogenesis may occur in vivo as it does in coculture conditions in vitro. Repetitive stimulation of specific neuronal pathways would be expected to induce capillary formation due to chronic release of EETs, such that functional hyperemia in the brain may be one of the determining regulators of capillary density. Those areas with the highest density of capillaries would receive more blood per unit time, leading to augmented support of metabolic need, i.e., faster blood flow response and more efficient support of neuronal activity. Figure 4 is an attempt to depict the fundamental aspects of an inclusive hypothesis positioning a central role of astrocytes in functional hyperemia and optimization of capillary density.

FIGURE 3. Changes in morphology of capillary endothelial cells and astrocytes when cocultured. A: the morphology of capillary endothelial cells cultured alone. Endothelial cells were stained with Dil-Ac-LDL (red). B: formation of capillary-like structures (arrows) in coculture of capillary endothelial cells and astrocytes. Scale bar, 0.1 mm.

FIGURE 4. Schematic representation of the hypothesis for functional hyperemia and angiogenesis driven by astrocytes. Glutamate spillover from presynaptic neuronal terminals binds to receptors on astrocytes, which activate phospholipase C (PLC) and diacylglycerol (DAG) lipase to release arachidonic acid (AA) from astrocytes. Conversion of AA to EETs is catalyzed by cytochrome P-450 2C11 epoxygenase. The EETs potently increase an outward K+ current, which causes hyperpolarization and vessel dilation. EETs also induce elevation of intracellular Ca2+ and trigger sequential signaling events for mitogenesis and angiogenesis of endothelial cells. IP3, inositol 1,4,5-trisphosphate.
This hypothesis is indirectly supported by data demonstrating that in areas of chronically high neuronal activity the number of microvessels is increased compared with areas of lower activity (18). As discussed above, one of the poorly understood functions of the brain is the fact that neuronal activation is supported by an increase in blood flow that is markedly higher than that thought to provide adequate substrate (15). This over-perfusion of metabolically active neurons may be an indirect consequence of the mechanisms responsible for increasing flow to neural networks on activation. If astrocytes mediate functional increases in flow through stimulation by the excitatory neurotransmitter (glutamate) via synaptic “overflow,” it is unlikely that this overflow is regulated and it therefore may continue to activate astrocytes even after neural function is complete. Release of EETs or other vasoactive products from astrocytes may continue for some period, leading to sustained hyperemia of muscle cells and increased blood flow lasting beyond the initial stimuli. Finally, the amount of substance released from astrocytes may be in excess of that needed to support normal activity, leading to hyperemic flow. The hypothesis includes the notion that the same paracrine (transmitter) substance responsible for increased blood flow released from astrocytes on neural activation also stimulates mitogenic activity of capillary endothelial cells, suggesting that a secondary consequence of functional hyperemia is initiation of angiogenesis.

Chronic stimuli originating from a specific area will result in repetitive hyperemic flow. If the above-stated hypothesis is correct, repeated functional hyperemia will, in time, result in increased capillary density. As capillary density increases, the efficiency of functionally mediated flow will increase. An increase in capillary density to an area of the brain that is chronically activated might be expected to result in reduced time to increase nutritive flow on neuronal activation as well as enhanced performance of neuronal circuitry and function. Such a scenario implicates this process in learning on repetitive activation of a specific pathway. Such increased capillary density may be necessary to accommodate the metabolic demands of recruited circuitry as well as the time for functional increase in local blood flow. Such a process may be critical in learning motor skills. Similarly, loss of capillaries may result in inadequate metabolic support to active neurons, reduced functional capacity due to poor substrate delivery, and reduced performance of tasks supported by those neural pathways that have lost capillary density.

Conclusion

The hypothesis as stated in this communication is well supported by the literature. The supporting data are not as hypothetical as the underlying concepts. We still do not understand how hyperemic flow in response to a specific neural activation is required to support function, and therefore much work is still needed in this area. The increased oxygen and glucose delivery measured by imaging techniques such as functional magnetic resonance imaging and positron emission tomography scanning indicate neural activation. It is now necessary to determine if the functional hyperemia measured by more sensitive and localized methods such as laser-Doppler flowmetry is linked and necessary for neural activation. Additionally, further study is needed to determine if astrocytes mediate these responses, act as sensors of neural activity, and regulate capillary density.

References