Functional Hyperemia in the Brain
Hypothesis for Astrocyte-Derived Vasodilator Metabolites

David R. Harder, PhD; Nabil J. Alkayed, MD, PhD; Andrew R. Lange, PhD; Debebe Gebremedhin, PhD; Richard J. Roman, PhD

Background—Cerebral blood flow is tightly coupled to neuronal metabolic activity, a phenomenon referred to as functional hyperemia. The mechanisms underlying functional hyperemia in the brain have been extensively studied, but the link between neuronal activation and nutritive blood flow has yet to be defined. Recent investigations by our laboratory and others have identified a potential role for astrocytes as an intermediary cell type in this process.

Summary of Review—This short review will develop the hypothesis that cytochrome P450 epoxygenase activity in astrocytes catalyzes formation of epoxyeicosatrienoic acids (EETs), which act as potent dilators of cerebral vessels and are released in response to glutamate receptor activation within astrocytes. Neuronal activity stimulates release of arachidonic acid from the phospholipid pool of astrocytic membranes. We provide evidence that the arachidonic acid released on stimulation of glutamate receptors within astrocytes is metabolized by cytochrome P450 2C11 cDNA enzymes into EETs.

Conclusions—The EETs thus formed will be released and activate K<sup>+</sup> channels, increase outward K<sup>+</sup> current, and hyperpolarize the plasma membrane. The resulting membrane hyperpolarization inhibits voltage-gated Ca<sup>2+</sup> channels and leads to arteriolar dilation, thereby increasing regional nutritive blood flow in response to neuronal activity. (Stroke. 1998;28:229-234.)

Key Words: astrocytes n blood flow n vasodilation

The regulation of CBF in response to neuronal metabolic demand is a dynamic and tightly regulated process. Given that neuronal metabolism relies almost exclusively on oxidative metabolism, all normal function, from control of motor activity to cognitive ability and memory acquisition, requires adequate delivery of oxygenated blood. The cellular and molecular mechanisms underlying this coupling of neuronal metabolism to CBF remain poorly understood. Metabolic regulation of CBF is thought to involve release of vasoactive metabolites in response to neuronal activity. Numerous studies over the past decade have implicated modulation of ion channels by several diverse stimuli as one of the principal transduction processes responsible for defining the activation state of cerebral arteriolar muscle and thereby adjusting blood flow to meet metabolic demand. Very recently, studies from a number of laboratories, including our own, have defined a predominant role for metabolites of AA in controlling ion channel activity in excitable cells, including vascular muscle. Astrocytes may be the principal cells in the CNS through which neuronal signals are transduced to cerebral vessels by means of these AA metabolites. Anatomic studies have revealed that astrocytes communicate with neurons through intimate membrane contacts<sup>1</sup> and envelope cerebral blood vessels with foot processes.<sup>1</sup> This anatomic configuration combined with recent findings concerning the capacity of astrocytes to release vasoactive fatty metabolites of AA implies a role for astrocytes in the coupling of neuronal activity to CBF. Our laboratory, as well as others, has found several lines of evidence that support this hypothesis. Astrocytes contain RNA and protein for several enzymes capable of metabolizing AA to vasoactive eicosanoids. In addition to cyclooxygenase, astrocytes express a cytochrome P450 (P450) epoxygenase,<sup>2</sup> which catalyzes the formation of EETs from AA. EETs have been previously found to be potent dilators of cerebral vessels.<sup>3,4</sup> This review will summarize the current knowledge concerning the role of EETs in the cerebral circulation, as well as recent work by our laboratory demonstrating that the activity and expression levels of a P450 epoxygenase in astrocytes can be modulated by glutamate, the major excitatory neurotransmitter in the CNS. Further in vivo studies will be discussed that demonstrate a role for this P450 epoxygenase in the CBF responses to glutamate. These topics will be discussed in the context of the hypothesis that astrocytes act as an intermediary cell type between neurons and the cerebral microvasculature to redistribute local CBF to meet metabolic demand under normal conditions.

Anatomic Location of Astrocytes Between Cortical Neurons and Microvasculature

Astrocytes are the most predominant type of glial cell in the CNS and constitute over 50% of the cell mass in the brain.<sup>1</sup>
Astrocytes extend projections referred to as “astrocytic foot processes” to other cell types in the CNS, including microvascular capillaries and arterioles, as depicted in Fig 1. Also depicted in Fig 1 is the close proximity of astrocytic and neuronal membranes; electrical coupling at such sites may facilitate the propagation of calcium waves from neurons to astrocytes.5,6 Such close association of astrocytes and neurons may also lead to signals being transmitted to astrocytes by neurotransmitters released from presynaptic neurons during increases in neuronal activity. This anatomic juxtaposition of astrocytes between neurons and blood vessels implicates astrocytes as integrators of neuronal signals that can be transduced to astrocytes.5,6 Such close association of astrocytes and neurons may also lead to signals being transmitted to astrocytes by neurotransmitters released from presynaptic neurons during increases in neuronal activity. This anatomic juxtaposition of astrocytes between neurons and blood vessels implicates astrocytes as integrators of neuronal signals that can be transduced to cerebral vessels. Such integration of neuronal signals would lead to modulation of vascular tone and thus changes in local cerebral vessels. Such integration of neuronal signals would lead to modulation of vascular tone and thus changes in local cerebral perfusion. Signals may be transmitted to astrocytes from neurons by direct electrical coupling5,6 or by binding of released neurotransmitters, such as glutamate, to receptors on astrocytes.7 Either mechanism, as will be discussed, can lead to increases in the release of vasoactive metabolites of AA from astrocytes.

Astrocytes express functional metabotropic and ionotropic glutamate receptors that mediate a number of cellular signaling events including elevation of intracellular Ca2+8–11 activation of phospholipase C,9 activation of cell-cell signaling,12,13 and release of AA into the cytosol.14,15 Elevation of intracellular calcium due to electrically propagated calcium waves from neurons may also activate several of these signaling processes in astrocytes, including activation of calcium-dependent phospholipases and the subsequent release of AA. Once released from the membrane phospholipid pool, free AA can be metabolized by a number of enzymatic metabolic pathways in astrocytes. Primary cultures of glial fibrillary acidic protein–positive hippocampal astrocytes contain message and protein for both constitutive (COX-1) and inducible (COX-2) cyclooxygenase, as depicted in Fig 2.

The expression of COX-2 is often seen in cultured astrocytes. While we have seen expression of COX-2 message in cortical tissue similar to that found in normal intact brain, we do not know under which conditions it is normally expressed. Other investigators have found the expression of mitogen- and glucocorticoid-inducible COX-2 in brain neurons but not in the glia.16 Since the discrepancy between ours and this previous observation may be due to the impact of cell culture conditions, the reader needs to interpret these findings with caution. Fig 2 shows that cultured astrocytes retain the COX-2 isoform. It is unlikely that the strong COX-2 message is due to neuronal contamination, in that at least 90% of cells stain positive for glial fibrillary acidic protein. It should also be noted that the presence of molecular message through reverse-transcription polymerase chain reaction does not always mean that there is translation of sufficient protein for product formation. Another (and a potentially more important) enzyme system present in astrocytes capable of metabolizing AA into potent vasodilator metabolites is the P450 monooxygenase system.

**Astrocytic P450 Enzymes**

The P450 gene family constitutes one of the oldest families of cDNAs yet defined. At the present time, over 240 cDNAs have been reported that encode for P450 enzymes.17 While many of these genes are nonmammalian, the diversity and number of P450 genes underscore the complexity of a system that oxidizes a plethora of substrates, including fatty acids. There are many differences between P450 enzymes and the other major enzymatic pathways generating AA metabolites such as cyclooxygenase and lipoxygenase, which have been described in detail in other reviews.18

We have cloned and sequenced a P450 gene from cortical astrocytes that has nearly complete sequence homology with a P450 2C11 cDNA previously sequenced in rat liver.19 This

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**Selected Abbreviations and Acronyms**

AA = arachidonic acid
CBF = cerebral blood flow
CNS = central nervous system
COX-1 = constitutive cyclooxygenase
COX-2 = inducible cyclooxygenase
DAG = diacylglycerol
EETs = epoxyeicosatrienoic acids
P450 = cytochrome P450

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**Figure 1.** Astrocytes (3) are juxtaposed between neurons (4) and cerebral arterioles (2). Astrocytic foot processes (1) contact and completely envelope arterioles in the cerebral circulation. From Kandel et al.1

**Figure 2.** Astrocytes express mRNA and protein for COX-1 and COX-2. Left, Western blot of cell lines transfected with COX-1 or COX-2 (control lanes) and cell lysate of primary cultures of rat hippocampal astrocytes (astrocyte lanes). Right, Reverse-transcription polymerase chain reaction (RT-PCR) of rat hippocampal astrocyte mRNA using primers specific for internal regions of rat COX-1 or COX-2.

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2C11 cDNA encodes an enzyme that, in the presence of NADPH and molecular oxygen, catalyzes the epoxidation of AA into EETs. There are four regioisomers of EETs, namely, 5,6-, 8,9-, 11,12-, and 14,15-EETs (Fig 3).

All four regioisomers dilate cerebral vessels. However, 11,12- and 8,9-EETs appear to be the most potent in vitro, in that they induce cerebral vasodilation at relatively low concentrations. Gebremedhin et al. reported that exogenously applied 5,6-, 8,9-, 11,12-EETs dilate isolated pressurized cat cerebral arteries and that this effect was time dependent. All these EETs induced peak dilation of cerebral arteries at 2 to 3 minutes after application, with 8,9- and 11,12-EETs being more potent at each concentration tested. However, although similar in potency to 8,9-EET in the same study, 11,12-EET induced vasodilation that was sustained over 5 minutes after application. This previous study also demonstrated that activation of large-conductance Ca\textsuperscript{2+}-activated K\textsubscript{+} channel mediates the EET-induced cerebral arterial dilation. Here we also provide evidence showing that 11,12-EET, at a concentration of 1 nmol/L, markedly increases the open state probability of a large-conductance Ca\textsuperscript{2+}-activated single-channel K\textsubscript{+} current recorded from vascular smooth muscle cells of cat cerebral microvessels (50 to 100 μm) (Fig 4). This increase in K\textsuperscript{+} channel activity hyperpolarizes the cell membrane and inactivates voltage-gated Ca\textsuperscript{2+} channels, thereby causing dilation of the cerebral microvessels. Consistent with this later observation, we have recently reported that the various EETs induce stereospecific dilation of renal arterioles (<50 μm) in the nanomolar concentration range, which is mediated through activation of a large-conductance Ca\textsuperscript{2+}-activated K\textsubscript{+} channels in rat renal arteriolar smooth muscle cells. These findings appear to indicate that the EETs are more potent in dilating resistance-sized vessels than large arteries.

The in vivo responses and potencies of the regioisomers appear to differ from those observed in vitro. Ellis et al. observed that only 5,6-EET dilated pial arteries when superfused over the brain in vivo; the other regioisomers were without effect. Such differences between in vivo and in vitro responses and in the time course of responses may reflect differences in the relative susceptibility of particular regioisomer to degradation and/or metabolism by other enzyme systems such as epoxide hydrolase and cyclooxygenase.

Recent publications have demonstrated that EETs are released from astrocytes on exposure to glutamate and that P450 2C11 protein in astrocytes is markedly upregulated by prolonged exposure to glutamate. Inhibition of P450 epoxygenases, and thus formation of EETs, results in a 30% reduction in baseline CBF and prevents the increase in CBF observed in response to exogenous application of glutamate.

Glutamate-Induced Release of EETs May Mediate Functional Hyperemia in the Brain

The purpose of this section is to develop a hypothesis defining the mechanisms of second-to-second functional hyperemia in the brain. For the purpose of this review, functional hyperemia will be defined as a local increase in CBF to match and support neuronal metabolic demand with respect to a specific neuronal function, ie, movement of a limb or performance of a mathematical problem. As discussed above, astrocytes possess functional glutamate receptors, both within their cell bodies and projecting foot processes. Glutamate activates phospholipase C and releases AA from the phospholipid pool through formation of arachidonyl DAG and the subsequent action on DAG monoacylglycerol lipases. Glutamate also activates...
P450 2C11 enzyme activity with subsequent formation of and release of EETs.\textsuperscript{22} EETs released from astrocytes potently dilate cerebral microvessels by enhancing outward $K_\text{1}$ current and hyperpolarizing cerebral and other arterial muscle cells.\textsuperscript{2,4,21,24} Thus, a hypothesis emerges which states that release of glutamate during normal neuronal activity can bind to glutamate receptors on astrocytes to increase AA turnover and release EETs from astrocytes that quickly and potently dilate the cerebral microcirculation to “shunt” flow to those areas surrounding metabolically active neurons. This anatomic-metabolic arrangement of neurons, astrocytes, and cerebral arterioles may be the basis for coupling of neuronal activity to increases in blood flow. PIP\textsubscript{2} indicates phosphatidylinositol 4,5-biphosphate; IP\textsubscript{3}, inositol triphosphate.

The hypothesis for a predominant role of P450-derived EETs in the control of CBF by astrocytes comes from evidence from our laboratory and that of Dr Earl Ellis (unpublished data, 1996). Reports from the Ellis group, which have been confirmed in our laboratory, demonstrate that EETs are the predominant AA metabolite formed by astrocytes.\textsuperscript{2,22,26,27} There are no consistent reports that glutamate stimulates the formation of cyclooxygenase products, whereas EET formation is significantly enhanced in the presence of glutamate.\textsuperscript{22,27} Pharmacological inhibition of P450 epoxygenase significantly blunts the transient increase in nutritive laser-Doppler blood flow in response to glutamate infusion (Fig 6). Similarly, intraventricular (lateral ventricle) application of antisense oligonucleotides against P450 2C11 24 hours earlier blocks the transient increase in blood flow to exogenous glutamate (Fig 7). The inhibitory effect of the antisense 2C11 oligonucleotides on the increase in CBF to glutamate superfusion was specific since animals treated with the sense oligonucleotide
and molecular mechanisms by which functional hyperemia is controlled under normal conditions will lead to a greater understanding of how dysregulation of these mechanisms may lead to pathological states. Such pathways may present important targets for pharmacological intervention in several disease states, including stroke, Alzheimer’s disease, and other cerebrovascular disorders.

References


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