Perivascular nerves and the regulation of cerebrovascular tone

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HIGHLIGHTED TOPIC | Regulation of the Cerebral Circulation

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Hamel, Edith. Perivascular nerves and the regulation of cerebrovascular tone. J Appl Physiol 100: 1059–1064, 2006; doi:10.1152/japplphysiol.00954.2005.—Brain perfusion is tightly coupled to neuronal activity, is commonly used to monitor normal or pathological brain function, and is a direct reflection of the interactions that occur between neuronal signals and blood vessels. Cerebral blood vessels at the surface and within the brain are surrounded by nerve fibers that originate, respectively, from peripheral nerve ganglia and intrinsic brain neurons. Although of different origin and targeting distinct vascular beds, these “perivascular nerves” fulfill similar roles related to cerebrovascular functions, a major one being to regulate their tone and, therein, brain perfusion. This utmost function, which underlies the signals used in functional neuroimaging techniques and which can be jeopardized in pathologies such as Alzheimer’s disease, stroke, and migraine headache, is thus regulated at several levels. Recently, new insights into our understanding of how neural input regulate cerebrovascular tone resulted in the rediscovery of the functional “neurovascular unit.” These remarkable advances suggest that neuron-driven changes in vascular tone result from interactions that involve all components of the neurovascular unit, transcoding neuronal signals into vasomotor responses not only through direct interaction between neurons and vessels but also indirectly via the perivascular astrocytes. Neurovascular coupling is thus determined by chemical signals released from activated perivascular nerves and astrocytes that alter vascular tone to locally adjust perfusion to the spatial and temporal changes in brain activity.

cerebral blood vessel; vasomotion; vascular tone; regulation; cerebral blood flow; neurovascular coupling; astrocytes

NERVES FIBERS IN BRAIN VESSELS were identified originally by Thomas Willis in the late 1600s and, since then, several investigators have documented the innervation of extracerebral vessels located at the base and on the surface of the brain and identified the ganglia of origin of these perivascular nerves as well as the nature of the vasoactive substances they release on vessel walls (for review, see Ref. 9). A general consensus is that the “extrinsic innervation” of extracerebral blood vessels finds its origin either in the superior cervical ganglion [sympathetic innervation containing norepinephrine and neuropeptide Y (NPY)], the sphenopalatine and otic ganglia [parasympathetic nerves containing vasoactive intestinal peptide (VIP), acetylcholine (ACh), nitric oxide (NO) synthase (NOS) and, in human, peptide histidine isoleucine or methionine], and the trigeminal ganglion [sensory nerves containing calcitonin gene-related peptide (CGRP), substance P (SP), neurokinin A, and pituitary adenylate-cyclase activating polypeptide (1)]. However, upon their entry into the brain parenchyma, cerebral arteries loose their peripheral nerve supply and, once the Virchow–Robin space has vanished, receive neural input from neurons located within the brain itself, hence the appellation of “intrinsic innervation” of the brain microcirculation. The neural regulation of the microcirculation has been most extensively studied in the cerebral cortex, where it receives afferents from subcortical pathways (6, 18, 20) as well as from local cortical interneurons (29) (Fig. 1). Key features of perivascular nerves, whether associated with vessels located outside or inside the brain, are their lack of classical synaptic junctions at the site of contact with the blood vessels, general enrichment within less than 1 μm from the vessel wall, and ability to directly modulate the tone of the vessels upon stimulation (6, 9).

ROLES OF THE EXTRINSIC INNERVATION

The main role of the sympathetic system, independent from its direct contractile or trophic effects on brain vessels, probably relates to its capacity to shift the upper limit of the autoregulation curve toward higher pressures, a response mediated in part by NPY and aimed at protecting the brain against blood pressure increases due to sympathetic activation (5, 15). In contrast, the parasympathetic system, a potent dilator of brain vessels upon stimulation, does not appear to play a significant role in either autoregulation or other physiological cerebrovascular responses, but its implication in pathological situations such as ischemia or migraine headache has been advanced (Ref. 15, see also below). The trigeminovascular
pathway, which provides the unique sensory innervation to brain vessel, appears as a “protective” system that is able to restore vessel tone after vasocontractile stimuli, a response mediated by the potent vasodilator CGRP released from trigeminovascular afferents. Antimigraine drugs like “triptans” act as agonists at prejunctional 5-HT\textsubscript{1} receptors on trigeminovascular afferents where they inhibit the release of CGRP and other peptides. Blood vessels located within the brain parenchyma, or the microcirculation, are innervated by “intrinsic” nerve pathways that find their origin in the central nervous system (CNS). For cortical microvessels, anatomical and/or functional evidence indicate that they receive NA, 5-HT, ACh, or GABAergic afferents from either subcortical neurons from the locus coeruleus, raphe nucleus, basal forebrain, or local cortical interneurons. Inset: schematic representation of the “neurovascular unit” as seen at the electron microscopic level with the vascular [endothelium (medium gray) and smooth muscle or pericyte (dark gray)], astroglial (light gray), and neuronal (axon varicosities are highlighted) compartments (modified from Ref. 6, with permission). ACh, acetylcholine; CGRP, calcitonin gene-related peptide; GABA, \(\gamma\)-aminobutyric acid; NA, norepinephrine; NKA, neuropeptide A; NOS, nitric oxide synthase; NPY, neuropeptide Y; PACAP, pituitary adenylate-cyclase activating polypeptide; SOM, somatostatin; SP, substance P; VIP, vasoactive intestinal polypeptide; 5-HT, serotonin.

Fig. 1. Schematic representation of the different types of perivascular nerves. The “extrinsic” nerves to cerebral blood vessels at the surface of the brain come from the peripheral nervous system (PNS) and originate either in the superior cervical (SCG), sphenopalatine (SPG), or otic (OG) or trigeminal (TG) ganglion. Antimigraine drugs like “triptans” act as agonists at prejunctional 5-HT\textsubscript{1} receptors on trigeminovascular afferents where they inhibit the release of CGRP and other peptides. Blood vessels located within the brain parenchyma, or the microcirculation, are innervated by “intrinsic” nerve pathways that find their origin in the central nervous system (CNS). For cortical microvessels, anatomical and/or functional evidence indicate that they receive NA, 5-HT, ACh, or GABAergic afferents from either subcortical neurons from the locus coeruleus, raphe nucleus, basal forebrain, or local cortical interneurons. Inset: schematic representation of the “neurovascular unit” as seen at the electron microscopic level with the vascular [endothelium (medium gray) and smooth muscle or pericyte (dark gray)], astroglial (light gray), and neuronal (axon varicosities are highlighted) compartments (modified from Ref. 6, with permission). ACh, acetylcholine; CGRP, calcitonin gene-related peptide; GABA, \(\gamma\)-aminobutyric acid; NA, norepinephrine; NKA, neuropeptide A; NOS, nitric oxide synthase; NPY, neuropeptide Y; PACAP, pituitary adenylate-cyclase activating polypeptide; SOM, somatostatin; SP, substance P; VIP, vasoactive intestinal polypeptide; 5-HT, serotonin.

pathway, which provides the unique sensory innervation to brain vessel, appears as a “protective” system that is able to restore vessel tone after vasocontractile stimuli, a response mediated by the potent vasodilator CGRP released from trigeminovascular afferents. Most recent research on the trigeminovascular system has focused on its role in migraine headache (30). Indeed, it was recently shown in human or animal models that cortical spreading depression, a wave of cortical depolarization that underlies migraine aura (17) and, possibly, also migraine without aura (31), activates trigeminovascular afferents and initiates a cascade of events that culminate into CGRP (SP and neurokinin A) release, blood flow increase, and inflammation within the meningeal dura (2). Such a scheme permits the reconciliation of disturbed cortical brain activity and activation of vascular sensory and parasympathetic nerves, the latter via the brain stem trigeminoautonomic reflex activation of the superior salivatory nucleus and, consequently, the sphenopalatine ganglion, which leads to perivascular release of dilators such as VIP, ACh, and NO (2). In light of this pathogenic process, “triptans,” the most recently developed symptomatic anti-migraine drugs target, among other 5-HT\textsubscript{1} receptors, those located prejunctionally on trigeminovascular sensory afferents. They inhibit CGRP release (16, 30) (Fig. 1) and thus prevent changes in meningeal blood vessel tone, throbbing...
pain, and the overall manifestation of head pain associated with migraine.

**INTRINSIC PATHWAYS**

The neuromediators present in perivascular nerves around cortical microvessels, the changes induced in cortical perfusion after activation of their neurons of origin, as well as the distribution of specific populations of receptors within the different cellular compartments of the “neurovascular unit” and their ability to regulate microvascular tone have been quite well described. Detailed reviews on these aspects have appeared (6, 18, 20), and only the most salient and recent findings will be highlighted here. However, before doing so, it is important to revisit the basic concept of the functional neurovascular unit. The latter is anatomically best described as a “neuronal-astrocytic-vascular” tripartite unit (6) (Fig. 1, inset). Indeed, perivascular neuronal varicosities, irrespective of the neuromediators they contain, abut primarily on astrocytic end-feet surrounding blood vessel walls, with a smaller proportion directly contacting the vessel basal lamina. Such an arrangement implies that perivascularly released neurotransmitters and mediators can activate receptors on both vascular and astroglial cells to alter the tone of brain microvessels.

**Subcortical Vasoactive Pathways**

The best-studied intrinsic neural pathways that project to cortical microvessels are those originating in the nucleus basalis, locus coeruleus, or raphé nucleus, and respectively containing ACh, norepinephrine, or 5-HT. Upon electrical or chemical stimulation, these subcortical areas elicit increases or decreases in cortical cerebral blood flow (CBF). Anatomical, molecular, and pharmacological studies have provided unequivocal evidence that shows that 1) these neurons send projection fibers to cortical microvessels and surrounding astrocytes, 2) specific receptors for the vasoactive mediators they release exist on microvascular endothelial and/or smooth muscle cells that can either dilate or constrict cortical microvessels upon activation, and 3) receptors are also found on astrocytes, thereby providing an additional means for modulation of microvascular tone following changes in neuronal activity. Specifically, projections from basal forebrain neurons to cortical microvessels and associated astrocytes, hereafter referred to as “perivascular” afferents, contain primarily ACh but also NOS, the synthesizing enzyme for the gaseous dilator NO (18). Coincidently, the increase in cortical perfusion elicited by stimulation of the basal forebrain is decreased after blockade of ACh receptors or inhibition of NOS activity. Muscarinic M5 receptors have been identified as those mediating cerebral vasodilatation (10, 33). However, multiple muscarinic ACh receptors also exist on astrocytes and, although not yet demonstrated, it cannot be excluded that these cells also contribute to the perfusion response through the release of vasoactive mediators (19, 25a), as will be described below for the norepinephrine- and glutamate-mediated changes in cortical perfusion. A similar scenario has been reported for serotoninergic afferents to cortical microvessels, which, depending on the rostrocaudal level of stimulation of their cells of origin within the brain stem raphé nucleus, increase or decrease cortical CBF (for review, see Ref. 6). Correspondingly, cortical microvessels are endowed with several 5-HT receptors including 5-HT$_1_B$ receptors that mediate contraction of cortical microvessels (11). A role for astrocytes in the 5-HT-mediated changes in cerebral microvascular tone and, hence, cortical perfusion has not yet been demonstrated despite the presence of several 5-HT receptor subtypes in astrocytes (6).

In contrast, a role for astrocytes in mediating the decrease in cortical CBF observed following stimulation of the locus coeruleus has recently been highlighted. In fact, it is known that stimulation of noradrenergic neurons in the locus coeruleus leads to a reduction in cortical CBF and that perivascular noradrenergic afferents in the cerebral cortex target mainly perivascular astrocytes rather than microvessel walls (for references, see Ref. 23). Recently, in cortical and hippocampal brain slices, it was evidenced that application of norepinephrine triggers increases in intracellular Ca$^{2+}$ concentrations ([Ca$^{2+}$]$_i$) in astocytes and perivascular astrocytic end-feet and that this response elicited constriction of the microarterioles on which the end-feet abutted (23). Furthermore, the authors were able to show that the contraction was mediated by 20-HETE, a cytochrome P450A derivative of arachidonic acid. However, other studies in cortical brain slices showed that a rise in astrocytic [Ca$^{2+}$]$_i$ after increased neuronal activity by electrical stimulation (13) or synaptically released glutamate (36) induced dilatations of cortical arterioles. In the latter study, the vasoactive signaling molecule corresponded to a cyclooxygenase product of arachidonic acid, likely PGE$_2$, but could not be unequivocally demonstrated. Furthermore, it was suggested by Filosa and colleagues (13) that suppression of [Ca$^{2+}$]$_i$, oscillations and accompanying vasomotion in microarterioles, possibly due to smooth muscle hyperpolarization, was involved in coupling local perfusion to increased neuronal activity. Despite apparent discrepancies between findings of microvascular contraction and dilatation mediated by changes in astrocytic Ca$^{2+}$, likely because of different experimental paradigms and the use or not of preconstricted vessels in the slices, these studies emphasize the importance of further assessing this newly identified intermediary role of astrocytes in transducing neuronal signals into vasomotor responses (19, 27) and whether or not the endothelium is required for their vasomotor effects (24). Furthermore, as can be appreciated, several recent studies have used brain slices to investigate the role of astrocytes or neurons (see **Local Interneurons** in the regulation of microvascular tone. Although limited by the fact that brain slices are maintained in artificial conditions in which vessels are not pressurized and do not have intraluminal flow, it is unarguable that such preparations, in which neuronal-glial-vascular interactions are preserved and can be assessed in a controlled manner, offer an additional means to isolated microvessels and whole animal experiments for investigating the microcirculation.

**Local Interneurons**

A role for interneurons in the regulation of cerebrovascular tone has been proposed in the cerebral and cerebellar cortices, based on both anatomical and functional studies. Owing to the possibility of simultaneously visualizing neurons (or astrocytes) and microvessels in isolated brain slices, and assessing changes in vascular tone upon single-cell depolarization or activation (Fig. 2), a resurgence of interest in the regulation of microvascular tone has occurred (4, 13, 23, 27, 36). In this...
In respect, it was recently shown that the evoked firing of specific subsets of cortical GABA interneurons can induce either dilation or constriction of local microvessels, some of these being contacted by the stimulated interneurons (4). Although the nature of “vasomotor” interneurons could not be identified in all cases, those that colocalized VIP or NOS elicited dilation while those colocalizing somatostatin (SOM) induced contraction. The interneuron-driven vasomotor responses could be

![Fig. 2. “In vitro” investigation of the changes in microvascular tone in rat cortical slices after activation of single neurons (for details, see Ref. 4). A: visualization of a microvessel, the “patch” micropipette, and a recorded “vasomotor” interneuron, as seen online in a slice under infrared videomicroscopy. B: electrophysiological response of a recorded vasomotor neuron. C: the same section as in A after immunostaining of the recorded neurons (the right one being “vasomotor”) with biocytin (brown) and the blood vessel with laminin (gray). D: example of a vasocontractile response obtained in microvessels after depolarization of single interneurons. E: confocal image of a recorded interneuron immunodetected with biocytin (green) and NPY (blue; merge is turquoise indicating that the interneuron contains NPY), of the blood vessel on which it projects (laminin, red), and of its ACh afferents (red, immunodetected with choline acetyltransferase; arrows point to ACh varicosities contacting the cell soma of the interneuron). F: single-cell RT-PCR on the cytoplasm of a recorded GABA interneuron (expressing both GAD65 and GAD75 isozymes) that coexpressed mRNAs for neuronal NOS and NPY, and thus corresponded to a typical NO cell that is a subset of cortical NPY interneurons.

![Fig. 3. Summary of the regulation of cortical microvessels from cells located in subcortical areas and within the cerebral cortex. The possibility that interneurons also induce the release of vasoactive molecules from astrocytes is not included for clarity purposes. The known or suggested vasoactive mediators and the vascular receptors on which neuronal or astroglial (PGE2 and 20-HETE) signaling molecules are believed to act to induce dilatation or constriction are illustrated. Note that GABA has been shown to dilate, via GABA<sub>A</sub> receptors, pial vessels but not intracortical microvessels (12). M5, muscarinic receptors that mediate dilatation of cerebral microvessels; VAPC1, dilatory receptor for VIP in brain vessels; NPY1, NPY receptor mediating cerebral vasoconstriction; SSR2/4, somatostatin receptors on smooth muscle cells of cortical microvessels that can mediate contraction (4); 5-HT<sub>1B</sub>, contractile receptor for 5-HT, but note that a dilatory response mediated by the same receptor has also been reported (11); EP4, dilatory receptors for PGE2 in brain vessels (7); ?, the cerebrovascular receptor for 20-HETE is still unknown (35).]
mimicked by bath application of the vasodilatory (VIP) or vaso-contractile (SOM) agents colocalized with GABA within the identified interneurons and for which receptors, able to either dilate or constrict microvessels, are expressed by endothelial or smooth muscle cells of cortical microvessels (4). Astrocytes also expressed different receptor subtypes for either VIP or SOM, but their contribution, if any, in the dilation and constriction elicited by stimulation of these distinct subpopulations of GABA interneurons will require further investigation.

Additionally, the subpopulations of GABA interneurons that elicited changes in local microvessel diameter represented distinct targets for subcortical basal forebrain ACh and brain stem 5-HT afferents. Although the serotonergic input to these cortical interneurons was comparable (~40% of them being contacted on their cell soma or dendrites by 5-HT varicosities), the ACh afferents privileged the NOS/NPY and SOM cells (up to 70% of them being contacted by ACh terminals, about half from the basal forebrain) compared with 30–40% for the other types of interneurons (4) (Fig. 2E). These data suggest that specific subsets of cortical interneurons could act as relays to adapt perfusion to local changes in activity following afferent signals from subcortical pathways, such as basal forebrain ACh and brain stem 5-HT pathways (Fig. 3). This would be compatible with the earlier observation that the integrity of local interneurons is necessary for the cortical perfusion increase evoked by stimulation of vasoactive pathways remote from the cerebral cortex (21).

A similar role for interneurons was recently confirmed in the cerebellum (26), a brain area where functional hyperemia is dissociated from the spiking of Purkinje cells (28) but depends almost exclusively on the release of NO from cerebellar interneurons, more specifically the stellate cells (34). This recent study performed in cerebellar slices showed that the evoked firing of single stellate cells was sufficient to induce both NO release, as measured by amperometry, and dilatation of intraparenchymal and upstream pial microvessels. In contrast, Purkinje cell stimulation did not result in either NO flux nor microvascular dilatation. Together, these recent studies suggest that specific subsets of interneurons in the cerebral and cerebellar cortex can, upon depolarization, alter the tone of neighboring intraparenchymal microvessels and, as shown in the cerebellum, pial vessels. Because interneurons act as integrators of incoming afferent signals, these findings further suggest that they could also be involved in neurovascular coupling by precisely adapting perfusion to local changes in neuronal activity (Figs. 2 and 3). However, they also raise an important issue that still remains to be fully understood: namely, how changes in the tone of brain microvessels that result in increase or decrease in local perfusion deep in the brain parenchyma, mediated directly by neuronal and/or indirectly by astroglial signaling molecules, are transmitted to upstream resistance vessels to maintain blood volume and intracranial pressure constant. In this respect, flow-mediated and propagated dilatations have been reported in brain vessels (8, 22, 25), but shear stress-induced contraction of cerebral resistance arteries and arterioles also occurs (3, 14), and this independent from the endothelium. The exact mechanisms are still a matter of debate, but endothelial factors, cytoskeletal matrix components, and gap junctions between vascular and/or astroglial cells appear to be involved. A better understanding of specific proteins such as connexins and integrins in these retrogradely transmitted vascular responses appears essential to fully appreciate their contribution in this particularly important regulatory mechanism in brain (3, 20, 32).

FUTURE DIRECTIONS AND APPLICATIONS TO HUMANS

In addition to exploring how local changes in brain perfusion are transmitted to resistance vessels, the cellular and molecular mechanisms that regulate cerebrovascular tone and that are at the basis of the signals used in functional brain imaging need to be scrutinized. New tools have highlighted the contribution of neural and nonneuronal cells in these responses, but further investigation on the respective roles of neural, astroglial, and vascular cells and, mainly, the identification of the vasomotor signaling molecules is greatly needed. An improved knowledge of these may foster our ability to bypass neuronal and glial pathways to selectively target vascular cells with specific mediator(s) and therein remedy to dysfunctions related to inadequate regulation of brain perfusion. Indeed, it is undeniable that a better appreciation of these mechanisms should significantly help in the prevention, stabilization, or treatment of pathologies such as Alzheimer’s disease, migraine headache, and ischemic stroke (14a), in which signaling between neurons and brain vessels is threatened because of dysfunctions that affect the neuronal, astroglial, and/or vascular components of the neurovascular unit.

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