The Molecular Mechanisms of Neural Flow Coupling: A New Concept

Tsutomu Nakada

From the Center for Integrated Human Brain Science, Brain Research Institute, University of Niigata, Niigata, Japan.

ABSTRACT

The phenomenon known as neural flow coupling (NFC) occurs at the capillary level where there are no known pressure controlling structures. Recent developments in advanced magnetic resonance imaging technologies have made possible in vivo direct investigations of water physiology that have shed new insight on the water dynamics of the cortical pericapillary space and their complex functionality in relation to NFC. Neural activities initiate a chain of events that ultimately affect NFC. First, neural activities generate extracellular acidification. Extracellular acidosis in turn produces inhibition of aquaporin-4 (AQP-4) located at the end feet of pericapillary astrocytes, the water channel which regulates water influx into the pericapillary space and, hence, interstitial flow. Reduction of pericapillary water pressure results in a negative balance between pericapillary and intraluminal capillary pressure, allowing for capillary caliber expansion. Proton permeability through the tight junctions of the blood brain barrier is significantly high owing to the Grotthuss proton "tunneling" mechanism and, therefore, carbonic anhydrase (CA) type IV (CA-IV) anchored to the luminal surface of brain capillaries functions as scavenger of extracellular protons. CA-IV inhibition by acetazolamide or carbon dioxide results in the accumulation of extracellular protons, causing AQP-4 inhibition and a secondary increase in rCBF.

Keywords: neuro flow coupling, aquaporin 4, interstitial flow, protons, neural activation.

Acceptance: Received December 10, 2014. Accepted for publication December 10, 2014.

Correspondence: Address correspondence to Tsutomu Nakada, MD, PhD, FAAN, FANA, Center for Integrated Human Brain Science, Brain Research Institute, University of Niigata, 1-757 Asahimachi, Niigata, 951-8585, Japan. E-mail: tnakada@bri.niigata-u.ac.jp.

J Neuroimaging 2015;25:861-865.
DOI: 10.1111/jon.12219

Introduction

Recent studies have shown that the classic circulation model of cerebrospinal fluid (CSF) is incomplete.[1] Production of CSF is not only dependent on choroid plexus but also on water flux in the pericapillary (Virchow Robin) space.[2,3] Historically, CSF flow through the pericapillary space is known as interstitial flow, and is considered to play a role equivalent to the systemic lymphatic system.[4–10] Advancements in modern magnetic resonance imaging (MRI) technologies have allowed for the *noninvasive* investigation of water flow in vivo. These studies revealed that water dynamics of the pericapillary space, ie, interstitial flow, is controlled by aquaporin-4 (AQP-4), the main subset of the aquaporin water channel family in the brain.[11–13] It has also been demonstrated that inhibition of AQP-4 is strongly coupled with an increase in regional cerebral blood flow (rCBF).[14] These observations have led to a better understanding of the architectural significance and functionality of the cerebrovascular system.[15,16] This article is a concise review of the modern concept of neural flow coupling (NFC) and its relationship to water dynamics in the pericapillary space.[5–10,16]

Cerebral Autoregulation: Upstream Control

Cerebral autoregulation signifies an intrinsic ability of the cerebral vasculature to maintain cerebral blood flow at a relatively constant rate of approximately 50 ml per 100 g brain tissue per minute in the face of blood pressure changes.[17–22] Autoregulation generally functions between mean blood pressures of 60 to 150 mmHg. It is maintained in parasympathetically and/or sympathetically denervated animals,[21] and the system is independent from extrinsic neural control. Instead, intrinsic neural nitric oxide (NO) control[22] and release of vasoactive substrates by the brain are believed to play essential roles in maintaining constant cerebral perfusion.[19,20] Perfusion is held constant by means of the cerebral vasculature smooth muscle that constricts and dilates in response to elevated and decreased systemic pressure, respectively.[17–22] Although this "upstream" control of inflow pressure appears to be rather straightforward, the physiologic mechanisms underlying NFC, neural activity-associated rCBF increase, were until now poorly understood.

Virchow Robin Space and Interstitial Flow: Cerebral Lymphatic Equivalent

Fluid-filled canals surrounding perforating arteries and veins in the parenchyma of the brain were recognized in early modern medicine and described in detail by Rudolph Virchow and Charles Philippe Robin.[23,24] The space is commonly referred to as the Virchow Robin space. It is now clearly understood that the fluid in the Virchow Robin space constitutes interstitial flow that drains into the CSF system (Fig 1). Virchow Robin interstitial flow is believed to play a role similar to systemic lymphatics.[2–10]

The basic function of systemic lymphatics is drainage of cellular debris subjected to molecular scrutiny before returning

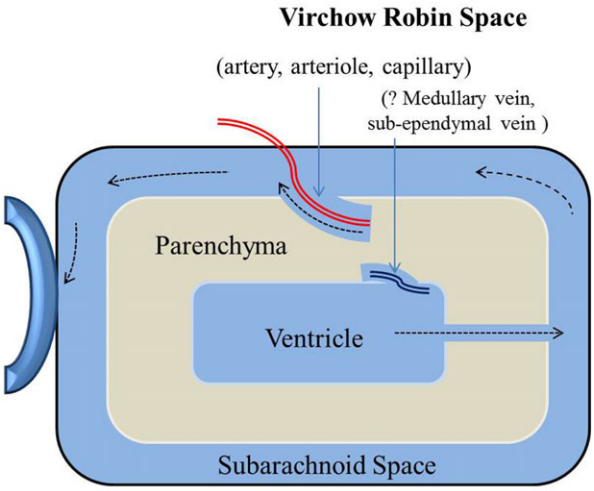

Fig 1. Virchow Robin space and interstitial flow. The ventricles and subarachnoid space represent the cerebrospinal fluid (CSF) space in the brain. The Virchow Robin space is a continuous canal surrounding penetrating vessels. Interstitial flow runs within the Virchow Robin space and drains into the subarachnoid space. Contrary to the classical concept of CSF flow, water CSF within the subarachnoid space is now believed to be dependent on interstitial flow in the Virchow Robin space. Although not as yet mainstream, the Virchow Robin space likely surrounds the medullary veins and subependymal veins as well. As shown in Figure 3, water influx from the systemic circulation into CSF is strongly dependent on interstitial flow in the Virchow Robin space through aquaporin-4 (AQP-4).

to the venous circulation. Although there is no conventional lymphatic system in the brain, interstitial flow of the Virchow Robin space constitutes its equivalent, and plays an essential role in clearing toxic proteins from brain parenchyma. Interstitial flow, and, hence, CSF circulation, eventually drains into the venous system through the cerebral dural sinuses (Pacchionian bodies), the latter playing a role similar to the thoracic duct of the systemic lymphatic system.

An important and intriguing example of interstitial flow protein clearance is β-amyloid clearance.[3,5,7,8,14–16] β-amyloid is essential for synaptic formation.[25] Nonetheless, excess β-amyloid can result in aggregation of the protein and senile plaque formation. Drainage of β-amyloid by interstitial flow through the Virchow Robin space into CSF is likely critical for maintaining proper homeostasis of β-amyloid production and clearance. The role of prealbumin, abundant in CSF, as β-amyloid chaperon and in preventing β-amyloid's natural tendency to plaque formation, further supports the β-amyloid clearance hypothesis.[26]

Pericapillary Water Dynamics and AQP-4

The aquaporin family is a large collection of integral membrane proteins that enable the movement of water across biological membranes. Three isoforms, namely AQP-1, AQP-4, and AQP-9, have been identified in brain in vivo. Expression of AQP-1 within CNS capillaries is actively suppressed, and AQP-1 in the brain is uniquely found in the choroid plexus epithelium. AQP-9 is only scarcely expressed in the CNS and is considered to have no significant role.[12,27] By contrast, AQP-4 is expressed abundantly in the brain and has a specific distribution: the subpial and perivascular endfeet of astrocytes.[2,10–14]

Active suppression of AQP-1 expression in brain capillaries is essential for proper maintenance of the blood brain barrier, preventing excessive movement of water across capillary walls.[27,28] Active water influx into the CSF system from the blood stream has been shown to be regulated by AQP-4, not AQP-1, indicating that interstitial flow plays an important role in CSF dynamics.[2]

As cerebral equivalent of the systemic lymphatic system, interstitial flow dysfunction can be expected to result in reduction of β-amyloid clearance. Indeed, senile plaque bearing transgenic mice showed significant decline of water influx into the CSF system, to the extent similar to that found in AQP-4 knockout mice.[29] Positron emission tomography studies in AD patients have shown virtually identical results.[14]

NFC and rCBF

Increased rCBF associated with brain activation is a well-recognized phenomenon that is known as NFC. Since this is a micro-, rather than macroenvironmental event occurring within an area limited to 250 μm around the site of neural activity, the regulatory mechanism for NFC should be within the capillaries.[30]

Considering blood flow to be steady, laminar flow within a long cylindrical pipe (Fig 2), the Hagen-Poiseuille equation gives volumetric blood flow, Φ, as

$$\Phi = \frac{\pi}{8\eta}\frac{\Delta P}{L}R^4$$

where ΔP is pressure loss (differences in inflow and outflow pressure), L is the length of the vessel tube, η is blood viscosity, and R is the radius of the vessel.[31]

Given that steady inflow pressure is rigorously controlled by upstream arterial autoregulation[17,18] and constant venous pressure, under physiological conditions, cerebral blood flow is virtually determined by the radius of the vessel and increases parallel to its fourth power

$$\Phi \sim R^4$$

The relationship implies that even small changes in capillary caliber have significant effects on rCBF.

Capillaries are devoid of muscle and, hence, are not under neural control. The perforating vessels of the cerebral cortex are surrounded by a fluid-filled perivascular (Virchow Robin) space. At the capillary level, fluid pressure within the vessel lumen is directly opposed by pericapillary fluid pressure. Therefore, the parameter that determines capillary caliber is the pressure balance between luminal (intracapillary) and outer (pericapillary) fluid pressures. Since intracapillary pressure reflects the inner pressure of arterioles, and is therefore a function of upstream arterial autoregulation,[17,18] it is the fluid pressure of the pericapillary space that inversely determines cerebral capillary caliber changes and, hence, rCBF, as follows:

$$\Phi \sim R^4 \sim {}^1/_{P_{peri-capillary}}$$

AQP-4 controls the water dynamics of the pericapillary space in the brain.[2,10,14] Therefore, AQP-4 activities play a role in controlling rCBF. Simply put, under physiological conditions, rCBF correlates inversely to AQP-4 activities.

$$rCBF \sim {}^1/_{AQP4\,activities}$$

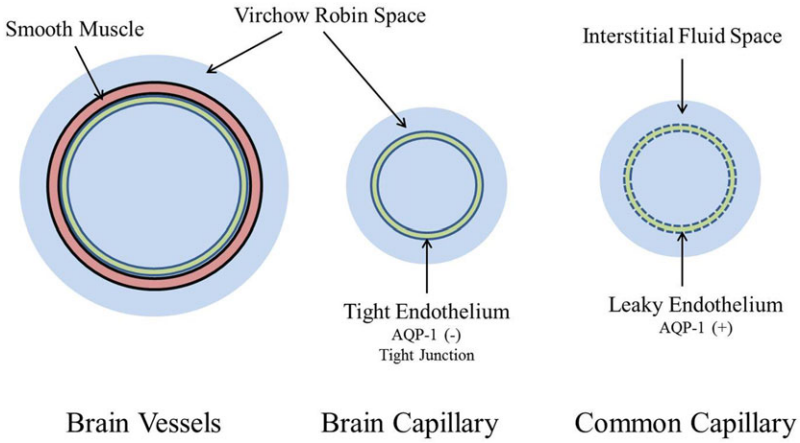

Fig 2. Vessel diameter is determined by tension of smooth muscle in artery, arteriole, venule, and vein (Brain Vessels). Capillaries are devoid of muscle and in capillaries with tight endothelium such as brain capillaries, capillary caliber is determined by the pressure balance between luminal and outer fluid pressures (Brain Capillary). For capillaries with leaky endothelium (Common Capillary), pressure balance is quickly equalized without capillary caliber changes.

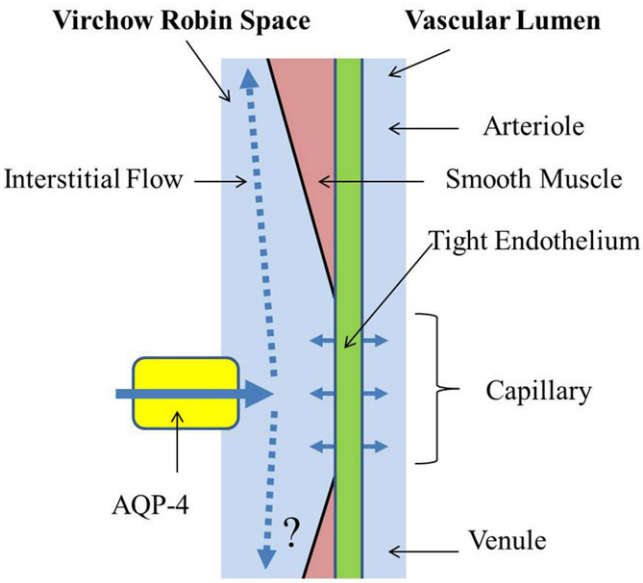

Fig 3. Pericapillary water dynamics. Water permeability of brain capillaries is restricted due to the tight endothelium, presence of tight junctions and active suppression of AQP-1. By contrast, significant water flow is present in the Virchow Robin space (interstitial flow) and is supported by active water inflow through AQP-4. Although it has not been clearly confirmed (?), interstitial flow may similarly be present along the medullary and subependymal veins.

Indeed, reduced AQP-4 activities by its inhibitor TGN-020 effectively increased rCBF in mice.[13] Under physiological conditions, AQP-4 is believed to be inhibited by extracellular protons similar to other AQP isoforms.[32] Therefore, rCBF is predicted to correlate with extracellular proton density (Figs 3 and 4).

$$rCBF \sim [H^+]_{extra}$$

The steps outlined above provide a fresh understanding of the underpinnings of NFC.

Neural Activities and Extracellular Acidosis

Since the original description by Urbanics et al[33] extracellular (interstitial) acidification associated with neural activities has been extensively studied by various investigators.[34–36] Modern MRI technologies demonstrated unequivocally that regional neural activities in humans are accompanied by extracellular acidosis found in a virtually identical distribution as the neural activity-induced increase in rCBF detected by blood oxygenation level-dependent contrast.[37] Therefore, at least from a phenomenological stand point, neural activity-induced extracellular acidification plays a role in NFC. Although the precise underlying mechanisms remain to be elucidated, it appears clear that neural activity-induced interstitial acidification, and the resultant inhibition of AQP-4, is indeed a main mediator of neural activity-associated rCBF increase. Further support for this concept comes from the "Diamox effect." Acetazolamide (Diamox) is a carbonic anhydrase (CA) inhibitor and a powerful agent for increasing rCBF. This "Diamox effect" is well known to be accompanied by interstitial acidosis in the brain.[38]

Within the large CA family, CA type IV (CA-IV) represents the dominant CA in the cerebral cortex and is anchored to the luminal surface of cerebral capillaries.[39] It has been shown that interstitial CA activity in the brain is attributable to CA-IV.[40] The human NBC1 sodium bicarbonate cotransporter directly interacts with CA-IV. The tethering of intracellular CA type II (CA-II) and extracellular CA-IV in proximity to the NBC1 HCO_3^- transport site maximizes the transmembrane HCO_3^- gradient local to NBC1 and thereby activates the transport rate.[41]

Since proton permeability through the tight junctions is significantly higher than for other small molecules, owing to the Grotthuss proton tunneling mechanism,[42] capillary CA-IV with NBC1 and CA-II effectively function as scavenger of extracellular protons generated by neural activation (Fig 5). CA inhibition by acetazolamide or excess of carbon dioxide (CO_2) in capillary blood results in accumulation of extracellular protons which in turn inhibit water flux through AQP-4. The resultant negative pressure relation with respect to intraluminal capillary pressure affects capillary dilatation and an increase in rCBF.

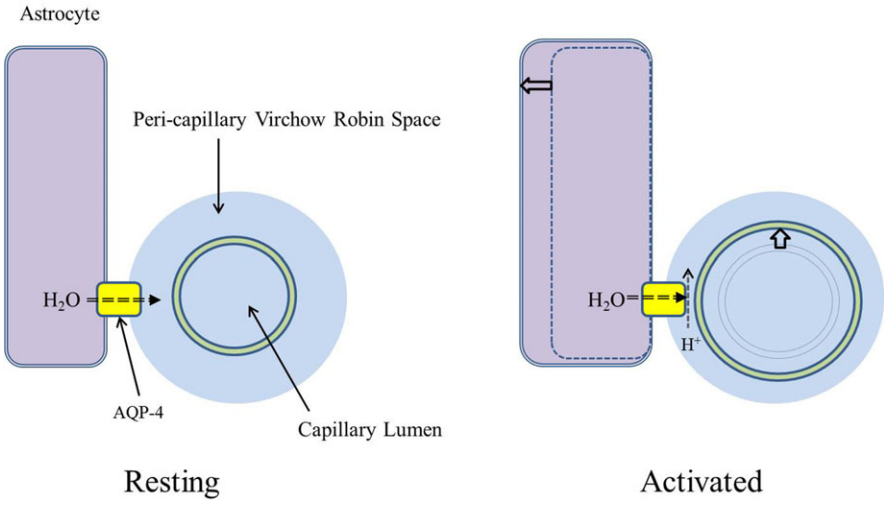

Fig 4. Neural activation. Neural activation produces extracellular acidification accompanied by increase in rCBF and astrocyte swelling. Proton inhibition of AQP-4 results in a reduction of water flow from astrocytes into the pericapillary Virchow Robin space, astrocyte swelling and capillary expansion due to reduction of pericapillary fluid pressure.

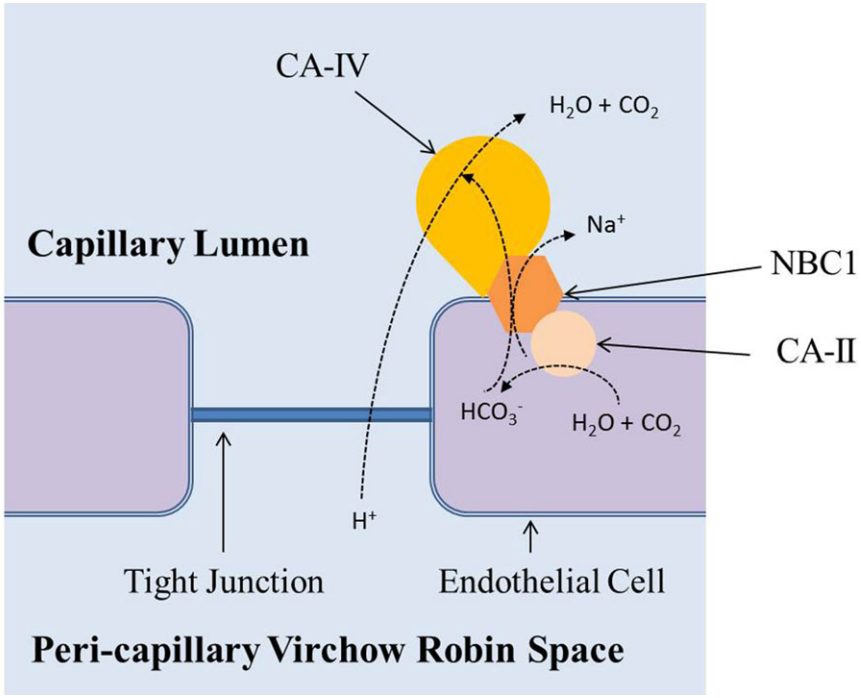

Fig 5. CA-IV system. Complex of CA-IV anchored to luminal surface of cerebral capillary, human NBC1 sodium bicarbonate cotransporter and intracellular CA-II. Their proximity maximizes the transmembrane HCO_3^- gradient local to NBC1 and thereby activates the transport rate. Because of the high proton permeability through tight junctions, capillary CA-IV with NBC1 and CA-II effectively function as scavenger of extracellular protons generated by neural activation. CA inhibition by acetazolamide or excess of CO_2 in capillary blood results in accumulation of extracellular protons.

Summary: A New Concept

Technological advancements, especially those in the field of MRI, have led to a new level of understanding of the physiologic underpinnings of neural activation-induced rCBF increase, a phenomenon known as NFC. The main player mediating NFC is the proton. Neural activities produce interstitial acidification. Excess protons inhibit AQP-4 activities in the pericapillary Virchow Robin space, resulting in a reduction in the pericapillary pressure. The negative balance between pericapillary and intraluminal capillary pressure induces dilatation of capillaries and an increase in rCBF. Acetazolamide or excess CO_2 blocks active clearance of interstitial protons which are ordinarily highly permeable through the tight junctions and, similar to neural activities, causes interstitial acidification and an increase in rCBF.

The precise molecular mechanism of extracellular acidification associated with neural activities remains to be elucidated. Such acidification has been shown to be associated with

intracellular alkalinization of astrocytes.[36] Active proton extrusion by astrocytes appears to be the most attractive explanation.[14,36] The functional significance of NFC has been linked to elimination of heat production brought about by neural activities. A heat-sensitive voltage-gated proton channel similar to neutrophil Hv1 may play a role, although much remains to be investigated.[14,43]

The work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology (Japan).

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