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Review

Blood flow control in the brain: possible biphasic mechanism of functional hyperemia

Minoru Tomita
Department of Neurology, School of Medicine, Keio University, Tokyo 160-8582, Japan

Background: About 120 years ago, Roy and Sherrington hypothesized that the cerebral blood flow (CBF) is closely coupled to metabolism, and metabolism is closely coupled to function. This concept has colored all subsequent inquiries. However, recent studies have revealed a temporal and spatial mismatch between changes in CBF and metabolism.
Objective: This article aims to reappraise the nature of functional hyperemia in response to somatosensory stimuli.
Method: Firstly, the author discusses what is known and unknown about the control of CBF, reviewing the traditional concepts of autoregulation, neurogenic control, metabolic control and the role of endothelial cells. Secondly, recent papers showing a mismatch or uncoupling between function, metabolism and flow are considered. Thirdly, the reviewer uses his own published and unpublished data to point out the intrinsic and largely unrecognized limitations of spectroscopic techniques for evaluation of oxygen metabolism. Finally, a novel hypothesis is presented concerning the nature of functional hyperemia.
Results and conclusion: The reviewer deduces that the initial flow increase in functional hyperemia is elicited by central neural systems, since it is reported that the central cholinergic pathway increases CBF immediately after the onset of somatosensory stimuli. The flow increase occurs concurrently with neuronal activation, but is much faster than the increase of neuronal metabolism. The novel hypothesis is proposed that functional hyperemia is biphasic: an initial flow increase under central neural control and a delayed increase is under traditional metabolic control. The metabolic phase may supply more blood than is needed, and may last even after discontinuation of the stimulation (overcompensation). These two phases of hyperemia are suggested to be well mixed, presumably in glial processes, which coordinate blood redistribution in the surrounding microvascular network. Many stimuli from the environment might be managed simply by the neurogenic control of functional hyperemia, without the metabolic change.

Keywords: Autoregulation, central nervous system, cerebral blood flow, cholinergic fibers, endothelium, functional hyperemia, metabolic control, mismatch of flow and metabolism, neurogenic control, oxygen tension.

Control of blood flow in the brain is important, since uninterrupted delivery of oxygen and nutrition is essential for proper neuronal metabolism/function. Many mechanisms, both known and unknown, are likely to be involved, including autoregulation to maintain constant cerebral blood flow despite arterial blood pressure changes and to increase blood flow when required for functional activity. The most intriguing mechanism is the neural control of cerebral microvasculature, especially intraparenchymal innervation by nerves directly originating from basal neurons. The reviewer considers that these nerves are involved in the rapid response phase of functional hyperemia following somatosensory stimuli, since the metabolic control alone cannot account for the rapidity of the response. In general, the amount of blood flow depends on the interplay of two factors: the perfusion pressure and the resistance offered to the blood flow by the vessels. Under physiological conditions, flow resistance changes have been thought be mostly produced through arterial/arteriolar dilatation and constriction, playing a key role in the regulation of tissue blood flow. Many substances and mechanisms have been suggested to be involved in control of the vascular resistance. However, the vascular tone,

Correspondence to: Dr. Minoru Tomita, Department of Neurology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan; E-mail: mtomita@sc.itc.keio.ac.jp

vascular resistance and blood flow in the brain are regulated by neural and humoral factors in a quite different way from those of peripheral organs, although the anatomical structure of cerebral vessels resembles that of the vessels in the latter organs. The historical background of studies on cerebral blood flow regulation has been the subject of many excellent reviews (for example, see [1-7]). It is unanimously agreed that changes in arteriolar resistance regulate cerebral blood flow throughout the tissue, including capillaries and veins, as in other organs. That is, capillary and venous flow are considered to be compliant and passively responsive to the arteriolar flow. Mchedlishvili [6] and Johnson [8] in particular suggested that the arterioles resemble stopcocks of an aqueduct, with flow being mainly adjusted by vasoactive messengers, released from activated neurons. However, recent studies [9-11] have cast doubt on this simple paradigm. Firstly, the flow response to somatosensory stimulus is rapid: the capillary flow increases in a matter of less than 1 second. This time seems to be too short for messengers (potassium ions in this case) released from activated neurons to reach the target arterioles by diffusion in the tissue, to dilate the arterioles, and thereby to supply blood to the pertinent neurons [12]. Secondly, the stopcock concept cannot explain why the territory of functional hyperemia does not merely extend along the vascular tree; instead, the vascular responses in the remaining part of the vasculature peripheral to the arteriole, especially in the capillary network, appear to be coordinated. Poiseuille's law, which has often been used to explain the relationship between vascular diametric changes and flow changes, is only approximately valid when uniform diametric changes occur throughout the vascular tree. The site of flow resistance in the arterioles may sometimes shift to the capillary side.

The aim of this article is two-fold: firstly, to review the conventional concepts of mechanisms for the regulation of cerebral blood flow (CBF), and then to discuss functional hyperemia due to the activation of neurons occurring immediately after somatosensory stimuli. The discussion is mostly based on the author's own data obtained from experiments on more than 1000 cats and more than 200 rats conducted at the Department of Neurology, School of Medicine, Keio University, Tokyo. For abbreviations, see the last section in the text.

Conventional concepts of CBF control

Arterial blood pressure vs CBF

The contribution of arterial blood pressure as a driving force of blood perfusion through the cerebral microvasculature is enormous. However, unlike inanimate systems, where flow increases linearly with perfusion pressure, the brain has an amazing capability to maintain a constant CBF throughout the brain, and therefore throughout the microvasculature, despite changes in mean systemic arterial blood pressure (SABP) over a wide range of at least 60-150 mmHg (a much wider range, from 40 to 180 mmHg, was reported by Harper et al. [13]. This is the so-called autoregulation of the brain, as reviewed by Lassen [3]. To achieve autoregulation, the resistance of the cerebral vessels must vary with SABP. In fact, when the SABP increases, the arterial vessels constrict to prevent a CBF increase, and when SABP decreases they dilate to maintain a constant CBF. Many mechanisms have been postulated to explain autoregulation: neurogenic control [14], myogenic control [15], metabolic control [16], and tissue pressure control [8]. The author believes that all these theories are correct in certain respects, sometimes independently and sometimes cooperatively. When SABP increases above the threshold of autoregulation, the CBF starts to increase. When the blood pressure increase is very large and abrupt, a marked increase in blood flow occurs, which is called breakthrough. However, this reviewer has never seen breakthrough in either humans or animals in which the sympathetic nervous system remained intact, even at systolic blood pressures as high as 300 mmHg. Fujishima et al. [17] observed that the cerebral autoregulation range was shifted to a higher level in spontaneously hypertensive rats, presumably due to long-lasting hypertension, which would have led to elevation of the sympathetic tone and therefore the vascular tone. However, when the vessel tone is somehow reduced with hypotension, under the influence of vasodilators, or as a result of tissue damage following ischemia or trauma, a rapid increase in SABP may cause breakthrough. Tomita et al. [18] observed that a rapid rise in SABP induced by intravenous administration of noradrenaline to papaverine-pretreated cats resulted in apparent breakthrough with flow increase, marked dilatation of pial arteries, and mushroom-like brain swelling, which in some cases led to protrusion of brain tissue through the skull window [18]. Post-mortem examination of the cut surface of the swollen

brain revealed heterogeneous microvascular dilation (visualized by prefilling the vessels with carbon black). The swollen brain looked red (red swelling), in contrast with "white" brain swelling owing to ischemic edema of cytotoxic type [19]. When the SABP falls below the threshold of 60 mmHg, the CBF starts to decrease even though the cerebral vessels are fully dilated. The flow/pressure relationship becomes unpredictable because of an involvement of hemorheological factors (enhancement of non-Newtonian behavior: an unusual increase of blood viscosity with decrease in perfusion pressure) inherent in the blood *per se*. Tomita et al. [20] found that when the SABP was decreased in a stepwise manner to 20 mmHg by exsanguination from the femoral artery of a cat, the CBF decreased steeply. The CBF decrease was far larger than would be expected from the flow/pressure relationship. At the lowest SABP that could be practically achieved, the blood circulation appeared to persist only between the brain and the heart, which was termed by us "cardio-cephalic circulation". Extrapolation of the regression line of CBF and SABP suggested that CBF would reach zero at an SABP of 15.5 mmHg. Whether such a value of SABP corresponds to a yield point of the viscoelastic behavior of the blood, or a critical closing pressure of the cerebral vessels, remains to be determined [20]. In summary, the vasomotor tone of the cerebral vessels is adjusted by the sympathetic systems to maintain a constant CBF over a wide range of SABP.

Neurogenic control

In 1664, Thomas Willis showed that brain vessels are accompanied with dense accumulations of nerve fibers, but more than 300 years after that discovery, the question of neurogenic vasomotor control of cerebral vessels remains controversial [14]. In regard to adrenergic vasoconstrictive nerves, Forbes and Wolff [21] reported that stimulation of cervical sympathetic nerves produced constriction of the pial arteries on the ipsilateral side. In the case of cholinergic vasodilatory nerves, Chorobski and Penfield [22], and Cobb and Finesinger [23] reported that dilatation of pial vessels occurred when the central cut end of the ipsilateral or contralateral vagus was stimulated, but this dilatation was abolished if the VIIth cranial nerve was cut. They concluded that the unique source of dilator fibers for cerebral vessels is the VIIth cranial nerve (greater superficial petrosal nerve). However, numerous subsequent researchers have failed to achieve a consensus. This reviewer considers that the parasympathetic nerves are very sensitive to surgical trauma and air exposure of the brain surface, which are inevitable in animal experiments. Further, electrical stimulation, which has been used by various investigators, is very different from the physiological impulses conducted in normal nerves. Consequently, the variety of results in the literature is hardly surprising. A noninvasive human study was attempted by Harmel et al. [24] with Kety's N_2O method, which was believed to be the most suitable for CBF quantification measurement. When satellite ganglions were blocked bilaterally in human subjects, no significant change in CBF was observed. Scheinberg [26] confirmed that there was no significant change in CBF following unilateral stellate ganglion block by using a modified N_2O method. Based on these negative observations, it seems to be unfortunate that many authorities on CBF have dismissed the action of cerebral vasomotor nerves as negligible: the nerves invested in the cerebral vessels were assumed to be developmental vestiges that were no longer functional after birth. Against this general trend of negativity, a noteworthy observation of adrenergic nerves was made by Falck and Hillarp [26], using a histofluorescence technique: the cerebrovascular bed is supplied with a well-developed plexus of adrenaline-containing nerves. The sympathetic nerves were by no means vestigial, but were actively releasing adrenaline to cerebral vessels. Purves [27] pointed out that the post-ganglionic fibers supplying cerebral blood vessels derive from the superior cervical rather than the stellate ganglia, and that, in consequence, blockade of the stellate ganglia might be expected to be ineffective in denervating cerebral vessels. It is accepted that sympathetic nerves release noradrenaline together with neuropeptide Y from the nerve terminal and cholinergic nerves release acetylcholine into the empty cleft between the nerve terminal and muscle surface. The pharmacological action of noradrenaline (NA) administered intravenously (10 μg/kg/min of Levophed bitartrate) seemed to be vary depending on the basal tone of cerebral arteries. Tomita et al. [19] found that NA caused three different responses in pial arteries in cats depending on the initial SABP level, i.e., constrictive, unchanged and dilative, partly confirming Fog's observation [28]. When NA was given in hypertensive cats, the pial arteries constricted; when

it was given in normotensive cats, the diameter of the pial arteries remained unchanged; when it was given in hypotensive cats, the pial arteries dilated markedly, while peripheral arteries almost completely constricted. These different sensitivities of cerebral vessels to NA depending on the level of SABP (vascular tone) even changed depending upon the vessel types (i.e. carotid artery vs. femoral artery). When NA was administered to papaverine-pretreated cats, almost maximal dilatation of the cerebral vessels was induced, while simultaneously vasoconstriction was seen in the external carotid artery. This resulted in an uneven redistribution of blood between the brain and the extracranial tissues. Such redistribution between the internal and external carotid arterial systems seems to be occurring even when the SABP changes within the autoregulatory range, depending on the level of induced hypotension [29]. This seems to be due to a difference in vascular reactivity to NA between the external and internal carotid arteries, as shown by Kawai et al. [30] in isolated blood-perfused canine internal and external carotid arteries. Interestingly enough, 5-hydroxytryptamine (5-HT) caused a much more potent vasoconstriction than NA in the isolated internal carotid artery, while in the isolated external carotid artery, 5-HT caused only slight vasoconstriction, though NA produced a marked vasoconstriction [31]. When the SABP is at around the lower limit of autoregulation, almost all the common carotid blood flow is mobilized to the internal carotid artery at the expense of the external blood flow. This is the reason why the face of subjects with hypotensive shock looks pale. A marked redistribution of blood flow occurs with hypotension between the brain and other parts of the body [18]. In addition, redistribution of blood flow occurred even within the brain tissue. Tomita et al. [32] found that the vertebral arterial system in rhesus monkeys was less efficient in autoregulation of blood flow than the internal carotid arterial system. This could be explained by the fact that a rich supply of acetylcholinesterase-containing fibers was found in the anterior and middle cerebral, basilar and internal carotid arteries of the monkey, while the posterior and vertebral arteries were sparsely innervated [33]. The parasympathetic cholinergic nervous system may thus contribute to the neurogenic control of cerebral vessels. Mchedlishvili and Nikolaishvili [34] observed that the pial arteries dilate when the SABP is lowered, and the dilatation was abolished after intravenous injection of atropine, a cholinergic inhibitor. James et al. [35] provided evidence that the maintenance of CBF as the SABP is reduced depends on intact cholinergic vasodilator nerves. Strangely enough, the routes and substances (other than acetylcholine) acting in the nerve terminals of parasympathetic nerves have not been clarified until recently. Suzuki et al. [36] elaborated these issues. They traced the origins and pathways of the parasympathetic nerves in rats, monkeys and men, and concluded that the parasympathetic systems also play a role in tone regulation of the cerebral vessels via acetylcholine [37], vasoactive intestinal polypeptide (VIP) [38], which is co-localized in the nerves acting as a vasodilative transmitter, and neuropeptide Y [39], which is a vasoconstrictor. Thus, both sympathetic adrenergic and parasympathetic cholinergic nerves contribute to the resting tone of vascular smooth muscle within the physiological ranges of gas tensions and SABP. The nerves could interact with and act as controllers for other factors that affect vascular smooth muscles, which would further complicate flow regulation. For example, in response to CO_2, presumably the strongest vasodilator of the cerebral vessels, following section of the sympathetic nerves, the action of vasodilator nerves is unopposed and the response is enhanced. Contrary to this, if the dilator pathway is interrupted by sectioning of the facial nerves, vasoconstrictor activity is unopposed and vascular response is reduced [27]. The response of vascular smooth muscle is thus regulated via both vasoconstrictor and vasodilator pathways. The most important function is to integrate the response of the cerebral vascular bed with those of other peripheral beds, facilitating a preferential redistribution of blood flow to the cerebral circulation. For example, in response to the emergency of severe hypotension, redistribution of cardiac output occurs as the cerebral vessels dilate and the peripheral vessels constrict, and the autonomic nervous systems thus ensure that sufficient blood is diverted from other vascular beds to fill the cerebral vessels. The body circulation is strongly influenced by the integration of neural regulation with cardiovascular responses. Such fine adjustments in blood redistribution are presumably controlled at the vasomotor center in the brain stem. If one were to look only at cerebral blood flow, it would seem as if autoregulation were locally achieved by the sympathetic nervous system (neurogenic control). In addition, it is important to differentiate between

arterial diametric change and flow change, as discussed later. Therefore, "autoregulation" of CBF has to be confined to a mechanism inherent in the brain per se, and in a strict sense, autoregulation of cerebral blood flow should be reappraised under conditions where the effects of the sympathetic nervous systems are completely eliminated.

Other perivascular nerves also deserve comment, i.e., perivascular nitrergic nerves and peptidergic nerves. Acetylcholine released from parasympathetic nerves to the vessels requires intact endothelial cells to be converted to NO. Otherwise, acetylcholine per se is a vasoconstrictor. For vasodilation, acetylcholine has to diffuse through the muscle layer wall to reach the endothelial cells (see below). Lee [40] showed that NO-synthesizing enzyme-containing nerves (cholinergic nitric oxidergic nerves) exist in cerebral vessels, and they might allow NO to act directly on muscles. It should be noted that NO is also released from some neurons that contain neuronal nitric oxide synthase (nNOS) and even from astrocytes. Active peptides are present in some perivascular fibers. Sensory fibers, for example, contain substance P (SP), calcitonin-gene-related peptide (CGRP) [41], neurokinin A (NKA) and so forth, and release them. The roles of these peptides in relation to cerebral vessels are largely unknown. The sensory fibers originate mainly from the trigeminal ganglion and release vasodilating peptides. The peptides released from the trigemino-vascular fibers were reported by Sakas [42] to increase blood flow in the cortical gray matter via axon reflex-like mechanisms during acute severe hypertension or seizure. The fibers were suggested to be involved in the mediation of vascular headache, but little is known about this.

Metabolic control

In 1890, Roy and Sherrington [16] employed an oncometer to measure arterial wall expansion, and reported that sciatic nerve stimulation always produced expansion of the brain in laboratory animals. According to them, the expasion began almost immediately after the commencement of the stimulation and lasted for several seconds after the stimulation had ceased. They carefully excluded a blood pressure effect by concomitant measurements of arterial blood pressure and venous blood pressure, and the increase of flow was attributed by them to lactic acid formation. Based on these observations, they formulated the hypothesis of close coupling between neuronal function and blood flow, which has colored all subsequent inquiries into cerebral blood flow.

However, since functional hyperemia of the local brain tissue is usually accompanied with very little change in arterial blood pressure, it has not yet been established what is the key messenger, and, if it is a metabolite, what is the route of the messenger from activated neurons to the target arteriole. Meyer and Gotoh [43, 44] observed an increase in carbon acid gas pressure (PCO_2) and a biphasic change of slight decrease and increase in oxygen gas pressure (PO_2) with a certain delay in response to photic stimulation in cats and monkeys. They concluded that there are mechanisms of metabolic circulatory homeostasis involving these gases. Namely, when tissue becomes hypoxic (a decrease in PO_2), flow increases to supply oxygen, and vice versa. When tissue accumulates carbon dioxide (an increase in PCO_2) after an increase in function, flow increases to wash the gas away, and vice versa. However, these responses were rather slow (of the order of minutes). Wahl [45] considered that H^+, K^+ Ca^{2+} ions, and adenosine released from the tissue are possible candidates for the messenger to dilate the arterioles during cortical activation. These substances must affect the most resistant site of the vessels, that is, the arteriole. The scenario after neuronal activation is thus hypothesized to be as follows: activated neurons release messenger(s) - the messengers reach the arteriole by diffusion and dilate the arteriole-increase blood flow-supply blood to the territory in which the activated neurons are included. However, this process would take more than a few seconds and cannot explain the rapidity of the flow response, which occurs within a matter of milliseconds after stimulation. Segal and Duling [46, 47] postulated a transepithelial propagation by which arteriolar dilatation induced by ATP propagates upstream along an arteriole from the site of stimulation in arterioles of muscles. In the penetrating arterioles in the rat cerebral cortex, similar conduction of vasomotor responses was reported by Dietrich et al. [48], who considered that the triggering factor was ATP released from red blood cells in the hypoxic and acidic environment. Osada et al. [49] observed with a dual (epi-, and trans-) illumination technique that the intraparenchymal arterioles of the cat cerebral cortex formed a spindle-shape, comprising segmental constriction and dilatation, during passage of K^+-induced cortical spreading depression (CSD).

The dilatation propagated like an egg swallowed by a snake, sometimes bidirectionally, and finally resulted in full-length dilation of the arteriole. However, the time scale of the arteriolar propagation is of the order of minutes, and is too slow to explain the rapid occurrence of functional hyperemia. Sandor et al. [50] made a very interesting comment under the title of "The Roy-Sherrington hypothesis: facts and surmises" at the international meeting on *Brain Activation and CBF Control* held in Tokyo, 2002 [51]. Their abstract notes"The 110-year-old metabolic hypothesis of Roy and Sherrington cannot fully explain the increase of CBF during increased functional activity of the central neurons. CBF may increase (a) much faster than the accumulation of the metabolic end products, (b) out of proportion to metabolic demands, (c) without significant change in local metabolism. The tight coupling of neuronal activity and blood flow in the brain is demonstrated by a large amount of data. Perivascular nerve endings were identified in the outer smooth muscle layer of the pial and intraparenchymal vessels. Their axon terminals contain a large variety of neurotransmitters, often co-localized in synaptic vesicles. Stimulation of the nerves results in a release of transmitters into the 80-100 nm neuromuscular synaptic clefts, and their specific receptors were identified in the vessel wall. There is ample evidence to suggest that neurogenic stimuli via perivascular nerve endings may act as rapid initiators, inducing a moment-to-moment dynamic adjustment of CBF to the metabolic demands, and further maintenance of these adjusted parameters is ensured by the metabolic and chemical factors. The significance of the perivascular nerves in the regulation of the CBF, however, is either underestimated or completely neglected in the majority of textbooks for both medical students and clinicians. Since the regulatory role of the nervous system in the cerebrovascular bed has been fully appreciated among investigators in the last decades, revision of this antiquated view of the common medical knowledge is urgent". This reviewer completely agrees with the remarks of S ndor et al. [50]. Functional hyperemia clearly involves multifaceted phenomena for which multiple regulatory mechanisms operate serially or in parallel.

Role of endothelium in CBF control

In 1980, Furchgott and Zawadzki [52] presented an epoch-making report on the role of the endothelium, which had been thought to be a silent inner lining of vessels existing between blood and tissue. They found that the endothelial cells were not silent, but active: when endothelial cells of isolated vessels were removed by rubbing of the intimal surface, acetylcholine caused constriction of the vascular smooth muscle, but when the endothelial cells remained intact, acetylcholine dilated the vessels. They hypothesized that acetylcholine stimulated the release from endothelial cells of a substance(s) that causes relaxation of the vascular smooth muscle by acting on muscarinic receptors. The substance was called endothelium-derived relaxing factor (EDRF), which was later discovered to be nitric oxide (NO). NO is released by the vascular endothelium in response to a variety of chemical and physical stimuli. NO is a short-lived gas, which rapidly permeates through cell membranes, whereas ionic products of NO produced by oxidation can not pass through the membrane. The NO diffuses into the cells in the vessel wall and causes the smooth muscle to relax by activating soluble guanylate cyclases, increasing the cyclic guanosine monophosphate (cGMP) concentration and activating protein kinase G, resulting in vasodilation. It should be noted that NO is produced not only by endothelial nitric oxide synthase (eNOS) but also by neuronal nitric oxide synthase (nNOS) or NOS of other cells, which complicates the vascular reaction to control CBF. Endothelium produces prostacyclin (PGI_2), which can be released by physical stimuli such as shear stress or ischemia [53], as well as endothelium-derived hyperpolarizing factor (EDHF) [54], which is involved in ATP-sensitive K^+ channel opening on vascular smooth muscle cells, and carbon monoxide (CO), which is generated by heme oxygenase (HO) [55]. These mediators are distinct from EDRF by a number of physicochemical and pharmacological criteria, but all have a vasodilating action.

To counteract the vasodilating factors, endothelial cells have to release vasoconstrictive substances to regulate cerebral blood flow. The peptide endothelin (ET), which was found by Yanagisawa et al. [56], is one of the strongest vasoconstrictors currently known. Thromboxane A_2 (TxA_2) and endothelium-derived constrictor factor (EDCF) are also vasoconstrictors. Autacoids such as histamine from mast cells, bradykinin from brain parenchyma, eicosanoids (such as leukotrienes and TxA_2, derived from arachidonic acid) and free radicals are released into the tissue, and affect endothelial cells, causing flow changes,

blood volume changes, and changes in the permeability of the blood brain barrier. However, these substances appear only in pathological conditions, such as head trauma, ischemia, seizure and inflammation.

One very important point to make here is that endothelial cells cover not only the interior of vessels having muscles, but also form capillaries. Capillaries without muscles can contract because of the presence of contracting filaments, which form an endothelial cytoskeleton consisting of actin and vimentin. Employing a video enhanced contrast-differential interference contrast (VEC-DIC) microscope, Inoue et al. [57] observed that cultured human brain microvascular endothelial cells contract upon exposure to pure oxygen, with the appearance of stress fibers. Capillary vasomotion has been a matter of controversy since Krogh [58]. Employing an oxygen microelectrode with a sharp tip of μm order, Ozanne et al. [59] observed oscillation in a continuous record of oxygen tension at capillaries. Capillaries are usually quiescent under physiological conditions, but become active in pathological states, such as hypoxia, ischemia, etc. In hypoxia and ischemia, endothelins (ETs) can affect the activities of ion and water transport in cultured endothelial cells [60-62]. The morphological changes due to the loss of ionic homeostasis and water shift result in cell swelling [63, 64], disruption of the blood brain barrier and capillary blood flow changes due to water leakage and viscosity change. The surface of the endothelial cells changes to procoagulant from anticoagulant, with upregulation of adhesive molecules [65]. Using cultured endothelial cells derived from capillaries of human brain, McCarron et al. [66] demonstrated that functional interaction between ET-1 and NO involves changes in Ca^{2+} mobilization and the cytoskeleton in the capillaries of human brain. Interestingly, alterations in the endothelial cytoskeleton (actin and vimentin) were collocated with cGMP-dependent protein kinase, being accompanied with altered levels of phosphorylated vasodilator-stimulated phosphoprotein [67]. Thus, the functional interrelationship plays a role in regulating capillary tone, microcirculation, and blood-brain barrier function.

In general, capillary flow is unique, and unpredictable based on conventional hemodynamic concepts. It takes place in an extremely low Reynolds number world, where viscosity changes in time or in space (viscosity gradient), in addition to pressure gradient, play an important role. Capillary flow looks stationary to the naked eye, but is not. High-speed laser scanning confocal fluorescence microscopy [68] revealed that the flow of fluorescein isothiocyanate (FITC)-labeled red blood cells (RBCs) in capillaries fluctuates under physiological conditions, and that RBC capillary flow becomes stop-and-go, stagnant or sometimes even reversed under pathological conditions. Another factor influencing such a capillary flow irregularity, in addition to the enhanced non-Newtonian property of blood, could be hydration changes of the glycocalix [69, 70] or buffy coat [71-73] on the capillary surface. The endothelial lining of blood vessels presents a large surface area for exchange of materials between blood and tissues, and is critically involved in many processes, including regulation of blood flow, inflammatory responses and blood coagulation. Pries et al [70] commented that the luminal surface of the endothelium is lined with a glycocalyx, a layer of membrane-bound macro-molecules, which has been determined by electron microscopy to be several tens of nanometers thick. However, investigations *in vivo* have indicated the presence of a much thicker endothelial surface layer, with an estimated thickness ranging from 0.5 mm to over 1 μm, that restricts the flow of plasma and can exclude RBCs and some macromolecular solutes. Details of the layer composition and biophysical properties, its relevance to physiological processes, and its possible clinical implications remain to be studied. Other factors that may cause coordinated capillary flow also need further investigation, including individual capillary sphincters influenced by, for example, potassium concentration changes via an astrocyte network with intercellular tight junctions [74], and the structural interface between astroglia and vessels, including capillaries [75].

Functional hyperemia

Mismatch between function, metabolism and flow

The tight coupling between increased neuronal activity and local cerebral blood flow, known as functional hyperemia, is essential for normal brain function. However, uncoupling of CBF and oxidative metabolism during somatosensory stimulation has been observed [76], a finding that shocked neuroscientists. To discuss the mismatch between function, metabolism and flow, an international symposium on *Brain Activation and CBF Control* was held in Tokyo [51]. As one of organizers, the author summarized key reports presented at the

Symposium as follows. Buxton [77] stated, after presenting his modified oxygen limitation model, "it is likely that the mechanism that controls CBF does not depend on tissue oxygen tension at all. The reported initial increase of oxygen extraction at stimulus onset could reflect a brief uncoupling of CBF and metabolism". Hoge et al. [78] comparing BOLD (blood oxygen level dependent) signals and perfusion, concluding that their "results are suggestive of multiple independent control mechanisms for the CBF regulation that do not interact significantly". Kim et al. [79], employing BOLD and CBF measurements, showed that stimulus-evoked CBF response is spatially localized to individual sub-milimeter cortical orientation columns, which contradicted previous findings based on optical imaging and delayed positive BOLD measurements. Schwindt et al. [80] concluded that BOLD based-functional MRI (fMRI) gives misleading results for the exact localization of areas of increased neuronal activity, and the perfusion-weighted image better represents the area. Kanno et al. [81] reported that the relative CBF response was closely linked to the strength of neuronal activity, and his colleague Matsuura [82] added that the linearity was independent of metabolism. All the above reports imply that flow changes are concurrent with functional changes in space and time, but are not matched with metabolism. In the Roy and Sherrington hypothesis, the metabolic change must occur before flow change. In practice, an early decrease in tissue oxygenation in response to somatosensory stimulation was observed by Vanzetta et al. [83], employing spectroscopic analysis, and by Ances et al. [84], employing an oxygen electrode. However, the decrease in oxygen tension was not statistically significant in their reports. The author considers that oxygen tension is a very important parameter in rather slow regulation of flow, but not in the extremely early phase. One speculation concerning the role of increased oxygen consumption, and therefore hypoxia, is that neuronal activation would lead to an early deoxygenation (termed the "initial dip") preceding the CBF response, which is followed by hyperoxygenation. Lindauer et al. [85] discussed this complex issue in their paper entitled "Neuronal activation induced changes in microcirculatory haemoglobin oxygenation: to dip or not to dip". After presenting their own data using optical imaging spectroscopy with a modified Lambert-Beer law algorithm, microfiber spectroscopy, and oxygen-dependent phosphorescence quenching, they concluded that there was no evidence for the initial dip and that the apparent dip might be correlated to the kinetics of the CBF response. The author agrees with their conclusion. Leniger-Follert and L bbers [86] carefully studied the role of oxygen in microflow change. They found that microflow increased at all sites measured, in most cases within 1-2 s after the beginning of direct electrical stimulation of the brain, and maximum hyperemia was reached after the end of stimulation. The reaction pattern of microflow was uniform. As local PO_2 normally did not decrease and did not even show an initial decrease after the onset of stimulation, the hyperemia could not have been caused by local hypoxia.

However, we have to resolve the discrepancy of why several optical imaging studies showed the initial dip, while fMRI studies were unable to detect the initial dip even at high field strength. Malonek and Grinvald [87], Malonek et al. [88], and Nemoto et al. [89] used optical imaging techniques to visualize the response of the cerebral cortex to peripheral sensory stimulation on a millisecond timescale. Although the optical signals obtained with the spectroscopic method showed the best temporal and spatial resolution, the optical signal changes are likely to be contaminated with concurrent flow changes, because this reviewer has shown that changes in optical signals are a function of changes in both hemoglobin concentration and flow [90, 91]. The optical signals obtained in *in vivo* experiments from the animal brain can not be exclusively interpreted as changes in (oxy)hemoglobin concentration. **Figure 1** shows records of reflected light intensities at wavelengths of 540, 570, and 620 nm from the surface of a blood in a transparent tube, obtained with the same method as that used by Malonek and Grinvald [87]. When the blood was made to flow with an infusion pump, the intensities of reflected light changed almost linearly with the flow changes, though actually there was no change in either blood oxygenation or hemoglobin concentration, which of which were confirmed before and after the flow. The records resemble those of oxy- and deoxyhemoglobin at the onset of functional activation, but they are simply due to the flow effect of the blood per se, i.e., they are flow-dependent light scattering changes. In practice, optical changes in the brain could be highly localized to the area of activation in the early phase, while in the latter phase, with the occurrence of real functional hyperemia, the flow increase would became larger,

spreading to a more peripheral zone. In short, optical imaging spectroscopy may detect oxyhemoglobin and deoxyhemoglobin concentration, but the data analysis is compromised by the flow effect (light scattering changes) occurring during functional activation. The fact that the optical properties (optical density, light scattering, light reflection, and light transmission) of blood change with flow, has been ignored in the theoretical analysis of spectroscopical signals. These changes were shown by us to be related to the flow-dependent changes in red blood cell aggregation and flow-dependent changes in red blood cell shape [90]. In addition, red blood cells become spherical with oxygenation and flattened with deoxygenation [92]. Optical imaging spectroscopy thus detects not only signals due to oxyhemoglobin concentration (metabolism), but also signals due to light scattering (flow), and can not separate the changes due to metabolism and flow. Further, the source of the BOLD signal is said to be deoxyhemoglobin, but collocation of deoxyhemoglobin with the site of metabolism is blurred because of dilution by arterial blood or deoxyhemoglobin spreading into the venous blood. Again, signals due to metabolism and flow are mixed. To separate the components, the independent measurement of blood flow alone is indispensable. Although concomitant flow studies with optical signal detection have been carried out, arterio-venous (A-V) shunting of blood represents a further complication, arterializing blood which is actually draining from the tissue. Although the amount of shunted blood is elusive, it is thought to vary with brain activation [92], perhaps in the range of approximately 10 % to 30 %. A laser Doppler flow probe may detect a rather delayed response to somatosensory stimuli, because of mislocation and poor spatial resolution, as follows. Schiszler et al. [11] measured changes in microvascular flow using a new optical method [93] which yields 2-D flow maps with 500 times higher spatial resolution [94] than that of laser Doppler flowmetry. They compared the two dimensional (2-D) map of intrinsic optical signals and the 2-D flow map obtained simultaneously from exactly the same location. They found that optical reflection changes and flow changes in the somatosensory area of the rat brain occurred concurrently after the onset of hind paw electrical stimulation. They also found that a laser Doppler probe of 1 mm in diameter smeared the small local initial flow change, and that blind positioning of the probe missed the appropriate location, delaying the apparent flow response. For these reasons, apparent blood oxygenation changes evaluated from initial optical signal changes reported by spectroscopists [87-89] might be predominantly due to flow effects occurring immediately after the stimulus, as shown in **Fig. 1**. If such a rapid flow

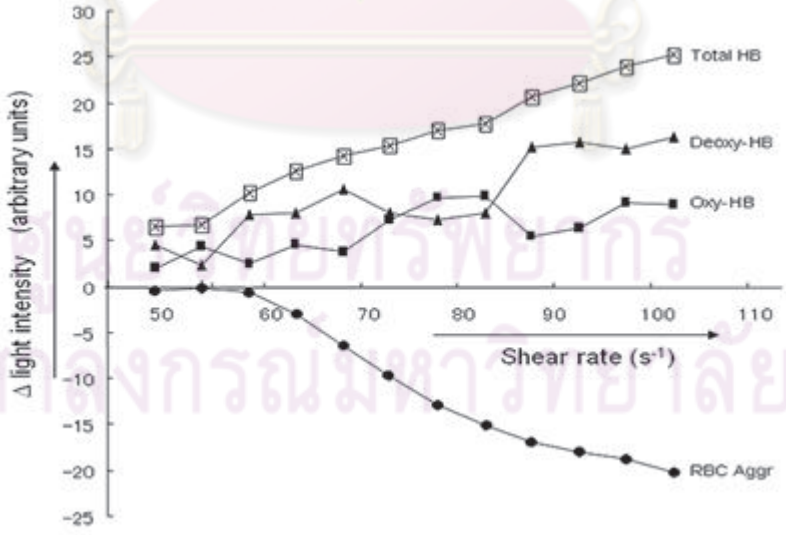

Fig. 1 False apparent increases in oxyhemoglobin, deoxyhemoglobin, and total hemoglobin of blood during gradual flow increase in a tube. The data may be interpreted as indicating an increase of blood volume with flow (functional hyperemia), although in fact there was essentially no change in blood hemoglobin or blood oxygenation in the tube. When such response curves are obtained from the in vivo brain surface at various wavelengths, their interpretation in terms of blood oxygenation changes is highly questionable. (Reprinted from [91] with permission from Elsevier).

increase occurs, actual PO_2 changes could be observed as apparently delayed. The initial part of the PO_2 record could be unchanged because a decrease in PO_2 due to oxygen consumption is counterbalanced by an increase in PO_2 due to arterial inflow of blood.

S ndor's comments [50] were timely, since they implied that the initial flow increase responding to somatosensory stimuli occurs earlier than the metabolic change. However, this might puzzle readers because the flow increase of functional hyperemia is considered to be elicited by messengers released from activated neurons, so that it should occur after metabolic change. This issue is elaborated in the following section.

Central nervous system

The sympathetic noradrenergic/cholinergic systems innervate mainly the large extraparenchymal pial vessels. It is observed that the innervation becomes less dense, with only a few nerve fibers accompanying the branched-off arteries penetrating into the parenchyma. The innervation of microvessels and capillaries by an entirely different system derived from central neurons has been found by Rennels and Nelson [95]. The central innervation was confirmed by Raichle et al. [96] who observed central noradrenergic nerve fibers on small intraparenchymal blood vessels, including capillaries. They showed that when the noradrenergic cell bodies in the locus coeruleus were stimulated with carbachol, a prompt reduction in hemispheric cerebral blood flow was produced. The intraventricular administration of the alpha-adrenergic blocker phentolamine had the opposite effect. On the other hand, vasodilating cholinergic fibers originating from the basal forebrain were reported by Adachi et al. [97]. Upon stimulation of a forepaw of rats by pinching, they found that CBF started to rise immediately. The rise was accompanied with significant increases of systemic blood pressure. Following spinal transection at the first thoracic level, the blood pressure response to forepaw pinching was suppressed, whereas the increase in cortical blood flow still took place. Kimura et al. [98] found no change in glucose metabolism when the CBF increased markedly upon electrical stimulation of the unilateral nucleus basalis of Meynert. Lacombe et al. [99] also reported the possible implication of projections from the substantia innominata (SI) to the cerebral cortex in the control of local cortical blood flow. SI stimulation simultaneously increased cortical PO_2 and decreased cortical PCO_2, significantly more in the frontal than in the parietal cortex, and ipsilaterally rather than contralaterally. Both the CBF and the tissue gas changes induced by SI stimulation were strongly potentiated by infusion of physostigmine. The evidence presented favors a role for the cholinergic projections of the SI in CBF increase without changes in aerobic metabolism. Iadecola et al. [100] reported that the vasodilation elicited in cerebral cortex by stimulation of the cerebellar fastigial nucleus (FN) is mediated by input pathways coming from the basal forebrain. Hamel et al. [101] also noted that acetylcholine afferents project to intracortical microvessels and to interneurons that primarily synthesize NO, somatostatin and neuropeptide Y, but also VIP. These neurons correspond to distinct sub-populations of GABA (γ-aminobutyric acid) interneurons that were found to send numerous projections to local microvessels. Cortical GABA interneurons may thus play a role in neurovascular coupling as relays for subcortical vasoactive pathways [102]. As one possibility to explain the very rapid response of CBF to somatosensory stimuli, Hamel (personal communication) suggested that if the stimulated thalamic afferents reach cortical microvessels, then the release of glutamate from them could directly activate perivascular astrocytic endfeet and induce a rapid vasodilatory effect. The route is not transsynaptic, but rather involves spilled-over or ectopically released glutamate that would induce postsynaptic excitatory activity in astrocytes [103]. This process could be independent of the concurrent activation of pyramidal cells and interneurons by the same afferents that results in increased neuronal activity and, hence, increased local perfusion.

The flow increase occurs concurrently with neuronal activation, but much faster than the increase of metabolic activity of the neurons, as if the flow increase is preparing the environment for an on-going enhancement of neuronal metabolism. Taking these findings together, the author is led to the novel hypothesis that functional hyperemia takes place in two steps: an initial flow increase under central neural control and a delayed increase under well-known metabolic control. The second step under traditional metabolic control sometimes supplies more blood than is needed and lasts even after the stimulation is discontinued (overcompensation). The two phases

of hyperemia are mixed well, presumably in glial processes, which coordinate blood redistribution in the surrounding microvascular network. It seems likely that, when awake, individuals perceive numerous stimuli from their environment, the majority of which could be managed simply by the first phase of functional hyperemia without the second phase.

Conclusion

1) Redistribution of blood between the brain and the non-brain organs occurs as an emergency response to a decrease of blood pressure. The cerebral arteries dilate, while other peripheral arteries are constricted by the sympathetic nervous system. The mechanism acting on the cerebral vessels is often called autoregulation under neurogenic control, but in a strict sense, it is redistribution.

2) Metabolic control occurs when neural activity increases. Vasodilatory metabolic products have been considered to be the mediators of functional hyperemia in response to peripheral somatosensory stimuli. However, analysis of mismatch in timings indicates that the early increase in CBF is probably under neurogenic control, e.g., from the cholinergic fibers originating in the basal forebrain.

3) There are two neurogenic (neural) control systems for the cerebral vessels. One involves extrinsic neurogenic control by the sympathetic noradrenergic/cholinergic system, and the other is the intrinsic (central) autonomic nervous system.

4) It is proposed that functional hyperemia is biphasic, with the early phase being neurogenic and the later phase metabolic. However, the two phases are well mixed, and their contributions to functional hyperemia are not fully separable.

List of abbreviations

A-V=arterio-venous,
BOLD= blood oxygen level dependent, a specific term in functional MRI,
CBF=cerebral blood flow,
CBV=cerebral blood volume,
cGMP=cyclic guanosine monophosphate,
CGRP=calcitonin-gene-related peptide,
CO=carbon monoxide,
CSD=cortical spreading depression,
EDCF =endothelium-derived constrictor factor,
EDHF =endothelium-derived hyperpolarizing factor,
EDRF=endothelium-derived relaxing factor,
eNOS=endothelial nitric oxide synthase,
ET=endothelin,
FITC=fluorescein isothiocyanate,
fMRI =functional MRI,
FN=fastigial nucleus.
GABA=γ-aminobutyric acid,
HO=heme oxygenase,
MRI=magnetic resonance imaging,
NA=noradrenaline,
NKA=neurokinin A,
NO=nitric oxide,
nNOS=neuronal nitric oxide synthase,
PO_2, PCO_2 =partial pressure of oxgen gas or carbon acid gas, respectively,
nNOS= neuronal nitric oxide synthase,
PGI_2=prostacyclin,
Reynolds number=nondimensional parameter indicating the ratio of viscous-to-inertia effect,
RBC=red blood cell,
SABP=systemic arterial blood pressure,
SI=substantia innominata,
SP=substance P,
TxA_2=Thromboxane A_2,
VEC-DIC=video enhanced contrast-differential interference contrast,
VIP =vasoactive intestinal polypeptide,
2-D=two-dimensional,
5-HT =5-hydroxytryptamine.

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Appendix

The terminology of hyperemia

There seems to be some semantic confusion in the terminology of “hyperemia”. August Beer [104] defined “postisch mic Hyper mia” after arterial occlusion as ‘arterial’ or ‘reactive’ hyperemia and “prim re passive Hyper mia” after venous

compression as 'venous' or 'congestive (Stauungs) hyperemia. The original concept was an 'excess' of blood in the tissue, with no emphasis on increased flow. Based on his experimental measurements of both cerebral blood volume (CBV) and CBF, the author classified tissue hemodynamic status into four types: Firstly, a combination of increased CBV and increased CBF constitutes hyperpefusion hyperemia, including post-ischemic or reactive hyperemia, hyperemia produced by hypercapnia, part of the luxury perfusion syndrome, and functional hyperemia. Secondly, a combination of increased CBV and decreased CBF constitutes low perfusion hyperemia or hypoperfusion hyperemia, in which congestion (Stauungs) is involved. Thirdly, hyperperfusion oligemia, involving a combination of decreased CBV and increased CBF; this is rare, but may be seen after NA administration to hypertensive animals. Fourthly, hypoperfusion ischemia involves a decreased CBV and decreased CBF, as is commonly encountered in ischemic regions of the brain. It was recommended that the term "hyperemia" be restored to the original concept of an excess of blood in the tissue 20 years ago, but it seems to be very difficult to change the well-established conventional usage of the term hyperemia to refer to increased blood flow with or without CBV increase. In the literature, hyperemia and CBF increase are used interchangeably.

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