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The Bimodal Nature of Neurovascular Coupling: Slow Tonic and Rapid Phasic Responses are Separately Controlled by Specific Astrocyte Metabotropic and Ionotropic Glutamate Receptors

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Abstract

Neurons, by virtue of their complex and continuously changing signaling roles in brain, must be able to regulate access to energy in order to maintain their ability to communicate meaningful frequency-encoded information. This is accomplished by release of neurotransmitters to astrocytes that in turn signal the vascular system to increase cerebral blood flow (CBF). This process has been termed "neurovascular coupling" (NVC). It has also been observed that NVC is bimodal in that there are two separate mechanisms for control of CBF. One type is rapid [phasic] in response to changes in glutamatergic synaptic activity and release of glutamate (Glu), K^+ and nitric oxide (NO). Uptake of Glu and K^+ by astrocytes induces Ca^{2+} waves activating regional astrocyte syncytium have to liberate prostaglandins which in turn dilate capillaries by relaxing surrounding pericytes. The NO dilates arterioles by relaxing surrounding smooth muscle cells. These agents acting in concert sharply increase CBF within 1-3 seconds. The other type is slow [tonic] reflecting ongoing neuronal metabolic activity of all neuron types independent of changes in synaptic activity or astrocyte Ca^{2+} waves and eliciting modest oscillations in CBF in 10's of seconds. In this review, we describe two neuronal signaling mechanisms that match the criteria for phasic and for tonic regulation of CBF. The difference is being the nature and source of the "Glu" released and of their targeted astrocyte receptors. Dependence on synaptic activity limits phasic responses to gray matter, but tonic responses can regulate CBF in both gray matter and white matter and may be the primary regulator of CBF in white matter.

Keywords: Brain energy metabolism; Cerebral blood flow; Glucose; Glutamate; N-acetylaspartylglutamate; Neurovascular coupling; Ionotropic; Metabotropic

Introduction

Neuronal signaling and metabolism

The function of neurons is communication and to do this efficiently, neurons must maintain a constant readiness. This entails two separate processes; housekeeping activities to maintain their structural and metabolic integrity, and second, maintaining an ability to spike as required. Much progress has been made in understanding the encoding of spike-generated neuronal languages. These sometimes very complicated and specific signal trains require adequate amounts of adenosine tri-phosphate (ATP) for neurons to perform at any level of required synaptic activity. Each spike and recovery period lasts about 1 ms and individual neurons may spike at up to 800-900 spikes/s (Hz). The spike is generated by depolarization of the plasma membrane with K^+ leaving the neuron and Na^+ entering the neuron making the interior somewhat less negative. The membrane is rapidly repolarized after each spike via Na^+/K^+ ATPase using ATP to restore the internal to external negative potential [1]. This produces adenosine di-phosphate (ADP) as a byproduct which must then be regenerated into ATP. To do this, neurons take up and oxidize D-glucose (Glc) using O_2, both of which are supplied by the vascular system. Since the total energy supply available to the brain is limited [2], it is vital for neurons to be able to divert scarce energy supplies to areas of high metabolic need and/or increased spiking activity. Neurons have sufficient supplies of stored ATP for repolarization to send meaningful messages for only several minutes. Therefore, it is important to understand how neurons communicate with the vascular system for supply of sufficient energy to maintain their complex, rapid, and continuously changing signaling roles. This activity to regulate and divert cerebral blood flow (CBF) as needed involves interaction between neurons, astrocytes and the vascular system and the process has been termed neurovascular coupling (NVC).

Literature Review

The bimodal nature of neurovascular coupling

In brain, it has been observed that there are two types of NVC that control changes in CBF [3]. One type is rapid [phasic] in response to increased glutamatergic neuron synaptic activity and characterized by release of nitric oxide (NO) generated by neuron nitric oxide synthase (nNOS) [4] and liberation of K^+ and free glutamate (Glu) to extracellular fluid (ECF). Astrocytes, a component of the "tripartite synapse", take up Glu and K^+ via specific channel transporters: the high affinity sodium-dependent ionotropic Glu AMPA transporter subunits 1-4 (iGluA1-4) [5,6] and the K^+ weakly rectifying (Kir4.1) transporter respectively [7], inducing astrocyte Ca^{2+} currents and then Ca^{2+} waves that activate regional astrocyte syncytium's. These Ca^{2+} activated astrocytes synthesize and release second messengers to the vascular system via cyclooxygenase-1 (COX-1) and the secondary action of terminal prostaglandin synthases [8]. Prostaglandin E_2 is reported to dilate capillaries by relaxing capillary endothelial-associated pericytes and capillary dilation appears to account for about 84% of the increase in CBF [9]. The neuronal NO (nNO) along with astrocyte NO (aNO) and vascular endothelial NO (eNO) relax smooth muscles and dilate

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arterioles [4]. Acting together, NO and prostaglandins generate a phasic response to increased synaptic firing, a response characterized by an increase in CBF and a rapid positive blood oxygenation level dependent (BOLD) magnetic resonance (MR) response. Increases in BOLD and in cerebral blood volume (CBV) are initiated in 1-3 s by arteriole dilation [10] which appear to precede astrocyte Ca^{2+} waves that occur in 3-6 s [11]. The second type is slow [tonic], independent of synaptic firing, without triggering astrocyte Ca^{2+} waves and characteristic of resting state brain activity operating over minutes [3]. These authors estimate that about 50% of brain vasodilation is controlled by the tonic system. Whereas several trigger molecules were known to control rapid phasic NVC, how the brain accomplished slow tonic NVC remained obscure. The observed characteristics of phasic and tonic NVC are shown in Table 1.

A candidate for control of slow tonic NVC

While a trigger for phasic NVC had been identified with the neurotransmitter Glu reaching the astrocyte ionotropic iGluA1-4 receptor, the nature of the tonic neurotransmitter and its astrocyte receptor was unknown. The physiological role of the neurotransmitter N-acetylaspartylglutamate (NAAG), with its bound Glu, and its targeted astrocyte metabotropic glutamate receptor 3 (mGluR3), was also unknown [12-15]. A hypothesis based on the independent findings that NAAG peptidase that cleaves NAAG into N-acetylaspartate (NAA) and Glu was highly expressed only in astrocytes [16] and that NAA acylase that cleaves NAA into aspartate and acetate for recycling was highly expressed only in oligodendrocytes [13] suggested that NAAG might play a role in neuron-glial signaling for the purpose of regulating CBF. Neurons produce approximately 1 molecule of NAAG for every 400 molecules of glucose (Glc) oxidized [17]. Based on the specific characteristics of the slow tonic trigger [3] and listed in Table 1, it was recently proposed that NAAG was the astrocyte-targeted neurotransmitter for regulation of tonic control of CBF [18]. NAAG fits the description closely in that it is directly tied to the rate of Glc oxidation rather than to synaptic events, and can be liberated to ECF via a non-synaptic mechanism, perhaps associated with the neuron membrane ATP-binding cassette subfamily C, member 5 (ABCC5) NAAG efflux transporter [19]. Also, its dedicated metabotropic receptor, mGluR3 is a G-protein Gi/Go bound receptor negatively coupled to adenylate cyclase that does not trigger Ca^{2+} increases in astrocytes, thus excluding its involvement in rapid synaptic events that trigger astrocyte Ca^{2+} waves and release of other NVC agents [20]. In addition, evidence of a connection between NAAG and CBF was previously obtained by inhibiting astrocyte mGluR3-associated NAAG peptidase activity in mice with 2-(phosphonomethyl) pentanedioic acid (2-PMPA) and observing that there was a prolonged global drop in the BOLD signal of about 3% [21].

The nature of the BOLD signal

The BOLD signal is an MR water signal that is diminished by an increase in red blood cell (RBC) paramagnetic deoxyhemoglobin (Hb) resulting from the drawdown of O_2 from RBC oxyhemoglobin (HbO_2) by activated neurons [17]. Thus, the BOLD signal varies inversely with RBC Hb levels, and the signal increases as CBF increases bringing a fresh supply of HbO_2 and reducing Hb levels. Therefore, the decrease in the BOLD signal in the case of inhibiting the action of NAAG peptidase was interpreted as a lack of increase in CBF and a sign that a normal NVC mechanism had been uncoupled to some degree by blocking the release of Glu at the astrocyte surface [21].

Characteristics	Rapid phasic NVC	Slow tonic NVC
Synaptic firing	Dependent	Independent
Astrocyte Ca^{2+} waves	Yes	No
Timeframe	1-3 Seconds	10's of seconds
BOLD response	Rapid large increases	Slow small oscillations
Capillary dilation	Yes	Yes
Arteriole dilation	Yes	No

Table 1: Characteristics of phasic and tonic NVC in brain.

Uncovering the multicellular genesis for obtaining sufficient energy and oxygen during rest and any level of spiking activity

The brain is the most complex organ in the body and the physiological function of neurons is to transmit meaningful information in the form of encoded spike frequencies. In order to do this neuron must maintain a state of constant readiness. The brain while only about 2% of body weight uses approximately 25% of its daily energy intake [22]. In addition, the heterogeneity of neuronal cells and regions that comprise the brain is such that the needs of even very small regions of brain may change quickly over time and in a highly variable temporal fashion. To deal with such a complex organization both locally and regionally, it is vital that neurons which have limited energy stores are able to continuously signal their needs to the vascular system. As described, they do this by liberating specific neurotransmitters to astrocytes whose end feet are in close contact with both neurons and the vascular system endothelial cells. The mechanism for rapid "phasic" changes in focal CBF has been identified with glutamatergic synaptic release of Glu and K^+ to astrocytes. A second method "tonic" has also been identified (Table 1) that does not depend on spiking, and is associated with housekeeping activities such as synthesis of proteins and the myriad metabolites that sustain their ability to carry out their signaling functions [3]. In this short review, evidence is presented that phasic changes in brain CBF are a function of glutamatergic synaptic release of K^+ and of Glu that is targeted to an astrocyte ionotropic Glu receptor, and that tonic changes in CBF are a function of non-synaptic release of peptide-bound Glu by many neuron types in the form of NAAG targeted to an astrocyte metabotropic Glu receptor where the Glu is liberated by the action of NAAG peptidase. This process is highly complex and involves the coordinated activities of neurons, astrocytes, pericytes, smooth muscle cells, vascular endothelial cells, and oligodendrocytes. The multicellular genesis of these two NVC control mechanisms is presented in Table 2.

Discussion

In this review, we present evidence of two separate mechanisms used by neurons to communicate their needs for increased energy. One is phasic in response to rapid changes in signaling activity that results in increases in CBF in 1-3 s. The other is tonic that results in increased CBF in 10's of seconds to minutes. Both appear to use neuronal "Glu" transmitted to juxtaposed astrocyte endfeet that in turn signal a neuron's metabolic requirements to the vascular system. Phasic NVC uses Glu leaked from synapses and activates the astrocyte ionotropic iGluA1-4 receptor, initiating astrocyte Ca^{2+} waves and release of prostaglandins and NO that rapidly increase CBF in a region of increased spiking. While the nature of the tonic transmitter is still open, we proposed that the non-synaptic release of NAAG, a non-excitatory form of Glu targeted to the astrocyte metabotropic mGluR3 receptor, matches the criteria for the tonic transmitter as shown in Table 1. After docking with the mGluR3 receptor, NAAG is cleaved by astrocyte NAAG peptidase forming Glu which then can activate astrocytes without initiating Ca^{2+} waves, to release prostaglandins that increase CBF. This bimodal mechanism is unusual in that it appears to use two distinct forms of

Component	**Slow tonic NVC (50%)**	**Rapid phasic NVC (50%)**	
Control mechanism	***Metabotropic Glu receptor (mGluR3)***	***Ionotropic Glu receptor (iGluA1-4)***	
Sites of action	***Capillaries (100%)***	***Capillaries (84%)***	***Arterioles (16%)***
Neurons			
Trigger	rate of Glc oxidation	rate of firing	rate of firing
Timeframe	10's of seconds	3-6 seconds	1-3 seconds
Number of neurons	each as individual	2 or more synapsed	2 or more synapsed
Neurotransmitters	NAAG**	Glu***, K^+	nNO
Source	NAAG non-synaptic efflux (ABCC5)	synaptic leakage (tripartite synapse)	neuron NOS
Target cells	Astrocytes	Astrocytes	Smooth muscle
Astrocytes			
Receptors	mGluR3	iGluA1-4, Kir4.1	
Enzymes	NAAG peptidase		astrocyte NOS
Products	NAA, Glu		NO
Ca^{2+} waves	no	yes	
Activators	Cox-1	Cox-1	
Messengers	prostaglandins	prostaglandin E2	aNO
Target cells	Pericytes	Pericytes	Smooth muscle
NVC			
Response	slow change in HbO_2	rapid change in HbO_2	rapid change in HbO_2
Measure	BOLD	BOLD	BOLD
Inhibitors	2-PMPA Cox-1 inhibitors	firing inhibitors Cox-1 inhibitors	firing inhibitors NOS inhibitors
Brain regions served	Gray and white matter	Gray matter	Gray matter

* Table is generated from literature cited in this review
** All neuron types can synthesize NAA. NAA is the only precursor of NAAG
*** Glutamatergic neurons

Table 2: Multicellular genesis of slow tonic and fast phasic NVC and their respective metabotropic and ionotropic "Glu" receptor control mechanisms.*

the neurotransmitter "Glu", two different release mechanisms and two types of Glu receptors in order to signal astrocytes to increase CBF. In gray matter, the actions of these two systems cannot be separated in time or space and both systems may interact with astrocytes at all times. However, in white matter, the dearth of synapses precludes strong phasic responses to signaling and it is likely that only the tonic system is responsible for maintaining substantial neuron axon metabolic requirements. Failure of either the phasic or the tonic system to supply adequate levels of energy to neurons and their axons in a timely manner could lead to a chronic lack of energy and inability to transmit a full range of meaningful frequency-encoded information. The functions of the mGluR3 receptor and NAAG peptidase have recently been associated with several human brain disorders including Alzheimer's disease, Parkinson's disease. Huntington's disease, cognitive loss, and neuropsychiatric disorders, and are current targets for therapeutic drug interventions [23]. Availability of adequate energy in both gray and white matter is the critical factor for normal neuron function.

Conclusion

We hope that this review is helpful in understanding the many facets of this developing story and that it leads to new approaches to understand the etiology of brain disorders. In summary, we postulate:

1. There are two mechanisms controlling NVC, one rapid [phasic] and one slow [tonic].

2. Phasic NVC is associated with the rate of synaptic spiking and tonic NVC is associated with the rate of neuron Glc oxidation.

3. Both mechanisms use the neurotransmitter "Glu"; phasic in the form of free Glu, and tonic as NAAG bound Glu.

4. Both neurotransmitters target astrocytes, the key component in NVC.

5. They are targeted to different Glu receptors on astrocytes, phasic to an ionotropic receptor and tonic to a metabotropic receptor.

6. Both mechanisms can operate in gray matter, but only tonic in white matter.

7. The NVC neurotransmitter in white matter is likely NAAG which is present in highest concentrations in axons and can be released to astrocytes non-synaptically at nodes of Ranvier.

8. Failure of either mechanism to supply adequate energy as needed may be reflected in a variety of brain signaling and metabolic disorders.

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