Journal of Cardiology and Cardiovascular Sciences

Mini Review Article Open Access

Genetic targeting of astrocytes in the gliovascular unit of the adult brain

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Article Info

Article Notes
Received: April 16, 2018
Accepted: June 07, 2018

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ABSTRACT

The acute nature of neurological stroke disorders highlights the massive dependence of the brain on oxygen and energy supplies. The mechanisms of neural blood flow regulation have been intensely studied but are still unclear. Astrocytes are glial cells that communicate with both neurons and blood vessels, making them a cellular candidate for mediating neuronal blood flow. Evidence suggests that perivascular astrocytes of the brain do in part mediate blood flow corresponding to neuronal activity. However, the role of astrocytes in this respect is still to be defined and characterized. Fortunately, many recent technologies have emerged that allow us to investigate how astrocytes may accomplish this task. Gene expression strategies with rodent lines and viral transduction have allowed us to investigate astrocytes in a more targeted manner. Additionally, a variety of tools used previously to study neurons are now being applied to astrocytes. Studying how astrocytes may orchestrate brain blood flow is important for our ability to understand and treat neurovascular disease. We review current methods used to experimentally target, monitor, and manipulate astrocytes in the context of mediating neuronal blood flow.

Introduction

The brain consumes massive amounts of energy in order to carry out complex actions and behaviors. Failure to meet this high metabolic demand can result in neural injury and death—as occurs in ischemic stroke, and other brain pathologies. To ensure normal brain function, neuronal metabolic demand is dynamically coupled to local vascular responses ("neurovascular coupling" or "functional hyperemia")[1, 2]. While there are several different glial cell types that compose the blood brain-coupling unit, astrocytes are ideally situated to mediate such coupling as astrocyte processes closely contact both neurons and blood vessels. Recent methodological advances have allowed us to dissect the relative contributions of astrocytes to the neurovascular unit in both health and disease, providing greater insight into the mechanisms underlying the coupling response. Here, we review current methods of genetic targeting, monitoring, and manipulation of astrocytes in the adult brain.

Genetically Targeting Astrocytes

Astrocytes express a particular gene signature that allows us to selectively target them in the midst of other cell types of the brain. Currently the most common method to selectively target astrocytes is through transgenic mouse lines. For example, researchers have expressed Cre in astrocytes using astrocyte-specific promoters

(Glial fibrillary acid protein (Gfap), Glutamate-aspartate transporter (Glast), among others)[3, 4]. These astrocyte transgenic mice lines have given us insight into how astrocytes contribute to a variety of behaviors and neurovascular properties[5, 6]. It should be noted however that gene expression in astrocytes is heterogeneous between, and even within brain regions; therefore transgene expression in astrocytes can vary considerably[7]. It has thus become increasingly imperative to study properties of astrocytes in their particular circuits in order to understand their function. In any case, transgenic targeting has been and continues to be a powerful and convenient method for studies of brain astrocytes.

In comparison to utilizing transgenic mouse lines, viral targeting strategies can increase spatial specificity in addition to many experimental benefits. Using viruses, we can control developmental stage and timing at which we express genes in astrocytes. This is potentially important because it allows us to dissect the contributions of astrocytes to time-sensitive processes such as neurogenesis or memory formation[8]. Also, viral manipulations may potentially allow for investigation of astrocyte activity in non-murine species, without having to overcome the technical difficulties of creating non-murine transgenic animals.

Many viral vectors have been developed for targeting neurons. There are a growing variety of viruses that have tropism for astrocytes and other glial cells. Various groups, including our own, have used canine adenoviruses (CAV), adeno-associated viruses (AAV), and lentiviruses (LV) to target and manipulate astrocytes. In Table 1, we have summarized some of the major viral strategies to target astrocytes in specific brain regions. We briefly review these viral vectors below:

Lentiviral targeting of astrocytes

LV along with AAV, are the most commonly used viral vectors used to facilitate targeted gene expression in the central nervous system (CNS). Several groups have successfully used LV to selectively express transgenes in astrocytes within specific brain regions[9-11]. LV vectors incorporate into genomic DNA with high efficiency, and are able to maintain persistent transgene expression for prolonged periods[12], and are often better suited for expression of larger, and more difficult-to-express transgenes relative to other viral types[13]. However, LVs exhibit a relatively short range of diffusion, which prohibits wide application of this transgene delivery method for systemic studies.

Adeno-Associated Virus targeting of astrocytes

AAVs have serotype-specific tropism within the CNS[14]. Unlike LV, AAVs do not integrate into the genome in vivo; rather, AAVs form episomes that nevertheless mediate persistent, long-term gene expression in non-dividing cells[15]. The variety of serotypes available, combined with use of cell-type specific promoters, has allowed for highly specific targeting of various cell populations within the nervous system. While a variety of AAV serotypes have been found to transduce astrocytes, serotype 8 appears to do so with the highest efficiency for most brain regions[16]. However, transduction efficiency of various AAV serotypes

Table 1. Summary of viral targeting strategies used to target astrocytes in the brain.

Viral Serotype	Viral Introduction Method	Animal Type and Age	Brain Region of Interest	Reference(s)
AAV2/1	Neonatal Intracerebroventric-ular Injection	CB57BL/6J P0 Neonatal	Cortex	[19]
AAV2/5	Stereotactic Injection	FVB/NJ Mice (4-8 week old) Male CB57BL/6J 6-8 week old	Hippo. Cortex Striatum	[16, 20-22] [23]
AAV2/8	Stereotactic Injection	Male CB57BL/6J 6-8 week old	Cortex Hippo. Striatum Amygdala	[5, 16, 24]
AAV2/9	Intravenous Injection Stereotactic Injection	CB57BL/6J ~10 week old	Hippo. Cortex Striatum Midbrain Cerebellum	[25, 26]
CAV2	Intravenous Injection	CB57BL/6J 6-8 week old	Hippo. Cortex	[8]
Mokola SIN-W-PGK	Stereotactic Injection	Male CB57BL/6J 7 week old	Neuronal Cultures Hippo. Striatum Cerebellum	[9]

can vary depending on age/maturation status of the animal. While AAV and LV viral vectors are effective for targeting astrocytes in particular brain regions, they do not allow us to target astrocytes systemically, throughout the entire brain.

Canine Adenoviral astrocyte targeting

Genetic targeting of astrocytes that specifically modulate neurovascular coupling has been difficult, as it is not known whether these vascular-associated astrocytes have a unique gene expression pattern distinguishable from other, non-vascular associated astrocytes. Recently, we reported that intravenously delivered Canine Adenovirus 2 (CAV2) preferentially targets perivascular astrocytes throughout the adult brain[8]. We can therefore deliver transgenes to astrocytes systemically, rather to only regions closely surrounding our injection site. Additionally, delivering the virus via intravenous infusion overcomes a major limitation of intraparenchymal viral injection—that injection into the brain can trigger astrocytic reactive gliosis, potentially altering astrocyte behavior[17]. Although CAV2 is delivered systemically, astrocytes are preferentially infected, therefore using this approach, we are able to systemically deliver transgenes to CNS astrocytes, without sacrificing cell-type specificity of the viral targeting. Targeting cell populations at the blood-brain interface without parenchymal insult is highly attractive as a method, since it has clinical implications for patients with blood-brain barrier degeneration[18]. Further studies will need to be performed to examine intravenous CAV2 tropism in non-rodents.

In summary, while both transgenic rodent models, and viral targeting are highly useful methods to study astrocyte function, viral targeting has greatly expanded the practical application of molecular approaches to manipulating astrocyte physiology. Using CAV2 allowed us to specifically target perivascular astrocytes throughout the CNS. It will be interesting to further study this population through imaging and experimental manipulations.

Monitoring Astrocyte Activity

In the past few decades, Ca^{2+} imaging has emerged as a major technology to monitor neuronal activity and more recently, astrocyte activity in rodents. Ca^{2+} is of interest because it is a key intracellular messenger that supports enzymatic production of vasoactive molecules in both neurons and astrocytes[1]. A particularly well-supported theory proposes a mechanism and timescale in which astrocyte Ca^{2+} levels rise prior to hemodynamic responses[1]. Both early and recent studies used Ca^{2+} imaging of brain slices demonstrate that rises in astrocyte Ca^{2+} levels are linked to astrocyte function in mediating neuronal and vasculature communication[27-29]. We next review two technical ways to monitor astrocyte Ca^{2+} fluctuations in the context of monitoring astrocyte-mediated blood flow.

One of the earliest methods developed for monitoring Ca^{2+} in astrocytes is via bulk-loading them with Ca^{2+}-sensitive fluorescent dyes (such as Rhod-2 or Fluo-4) for imaging[30, 31]. Studies using this technique in cortical slices have allowed the field to demonstrate that astrocytic Ca^{2+} activity is indeed linked to blood flow responses[28, 29] in multiple cortical regions. However, using the same dye loading techniques coupled with in vivo Ca^{2+} imaging, researchers were not able to confirm whether or not astrocyte Ca^{2+} activity is indeed linked to hemodynamic responses[32, 33]. To explain these conflicting studies, it is thought that Ca^{2+}-mediated signaling in the distal, finer astrocytic processes are thought to be particularly important for neuro-glio-vascular signaling[34]. Importantly, one major caveat to previous dye-loading studies is that this technique is mostly effective for analyzing the astrocyte soma rather than its finer distal processes[35].

Genetically encoded Ca^{2+} indicators (GECIs) have enabled several groups to examine the dynamics of fine-process Ca^{2+} signaling during spontaneous and evoked activity both in vivo and in situ[4, 36]. Recent further optimization of GECIs has allowed for increased monitoring of Ca^{2+} signals in fine processes[37], increased temporal specificity and sensitivity[38], and simultaneous imaging of astrocyte Ca^{2+} signaling changes across multiple cellular compartments[39]. By using GECIs, Otsu and colleagues [6] recently observed that Ca^{2+} responses in astrocyte processes precede hemodynamic activity in the olfactory bulb. This corroborates studies that demonstrated that astrocyte process Ca^{2+} activity operates on a different timescale in comparison to the somata[34, 40]. In combination with the high resolution of two-photon microscopy, these GECIs have allowed researchers to analyze the distal processes of astrocytes at unprecedented detail, allowing for more accurate studying of neuro-glio-vascular communication.

Manipulation of Astrocytes

A wealth of in vitro slice physiology techniques has been developed to electrically and mechanically stimulate astrocytes to study signaling properties[41, 42]. In tandem with new advancements in astrocyte activity monitoring, increasingly sophisticated techniques are being developed to manipulate astrocytes to determine their roles in gliovascular coupling in vivo. Similar to monitoring, techniques of astrocyte manipulation are based upon our knowledge of astrocyte physiology. We briefly review two aspects of manipulations below:

Pharmacological Manipulations

To silence astrocytic Ca^{2+} activity in in vitro slice preparations[43] the fast Ca^{2+} chelator BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid) has been used to internally chelate astrocytes, effectively removing the ions from physiological activity. Using Ca^{2+}

chelators, multiple groups have demonstrated that Ca^{2+} is important for astrocyte regulation of blood flow[29, 44]. This research demonstrated that neurovascular responses are effectively abolished when chelating Ca^{2+} in slice culture. Several other drugs targeting astrocytic signaling have been based on the implication of the endocannabinoid-based[5] and ATP-based pathways in astrocytes[45]. Although these pharmacological approaches have been invaluable in uncovering mechanisms of astrocyte function, they can be limited in their specificity, and ability to be used in vivo. Once again, astrocyte promoter-driven expression has been helpful for selective targeting.

Genetic Manipulations

Along with the high demand of more specific methods in targeting astrocytes, many genetic techniques targeting different aspects of astrocyte signaling have been developed both in vitro and in vivo. Several pharmacological studies showed that astrocyte Ca^{2+} signaling is at least in part mediated by inositol triphosphate-dependent release (IP_3) from intracellular stores[46]. Thus, it is conceivable that knocking-down or knocking-out of the IP_3 receptors specifically in astrocytes may allow us to study astrocyte silencing. To genetically manipulate this signaling, researchers have used the astrocyte-specific IP_3 receptor-2 (ITPR2) gene knockout mice and found that the astrocyte Ca^{2+} responses to neuronal activity are somewhat diminished[47]. However, while Ca^{2+}responses in the astrocyte somata may be depend on ITPR2 signaling it appears that Ca^{2+} flux in distal fine processes appears to be at least partially ITPR2-independent, mediated by extracellular influx or release from mitochondrial stores[47, 48]. Taken together with studies that suggest astrocyte fine processes are responsible for neurovascular coupling, ITPR2 manipulations may not be appropriate for studying astrocyte-mediated functional hyperemic responses[27].

Many of the same techniques used to manipulate neuronal activity, including opsins and Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), have recently been used to manipulate the activity of astrocytes. One successful example is the use of AAV8-delivered Gfap-promoter restricted excitatory DREADD hM3D(Gq) to manipulate astrocytes in the basolateral amygdala, revealing the astrocyte's role in fear behavior[5]. However, astrocyte stimulation via a similar AAV8-delivered Gfap-promoter restricted hM3D(Gq) construct did not affect neurovascular coupling in the mouse visual cortex[49]. This suggests that there is variability in this approach that requires further optimization. Overall these genetic approaches provide another option for us to control astrocyte activity.

The variety of methods for expressing Cre in astrocytes allows researchers in the glial field to take advantage of ready availability of floxed transgenic chemogenetic, optogenetic, and diphtheria toxin receptor (iDTR) mouse lines to control astrocyte activity. We should note that in using the CAV method that we recently reported to target astrocytes, we found severe defects in hippocampus-dependent memory following diphtheria toxin mediated-ablation of astrocytes[8]. This provides us a novel noninvasive option to target astrocytes for behavior studies.

Conclusions and future perspectives

Astrocytes may play a vital role in the ability of neurons to communicate with vasculature. In order to study the mechanisms that govern the gliovascular communication, many techniques used to study neurons of the brain have been co-opted for astrocytes. The combination of current imaging, pharmacological, and transgenic methods provides enormous advantages to dissect out astrocytes to study their function and underlying mechanisms. Viral-based methods, especially when delivered through vascular system, provide unique advantages and opportunities for manipulating astrocytes responsible for neuro-glio-vascular signaling. More importantly, viral-based targeting strategies provide potential for translational targeting of astrocytes contributing to human neuro-psychiatric diseases.

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