



Glowing Feet Control the Blood of Seizures

Ictal but Not Interictal Epileptic Discharges Activate Astrocyte Endfeet and Elicit Cerebral Arteriole Responses.

Gómez-Gonzalo M, Losi G, Brondi M, Uva L, Sato SS, de Curtis M, Ratto GM, Carmignoto G. *Front Cell Neurosci* 2011;5:1–12.

Activation of astrocytes by neuronal signals plays a central role in the control of neuronal activity-dependent blood flow changes in the normal brain. The cellular pathways that mediate neurovascular coupling in the epileptic brain remain, however, poorly defined. In a cortical slice model of epilepsy, we found that the ictal, seizure-like discharge, and only to a minor extent the interictal discharge, evokes both a Ca^{2+} increase in astrocyte endfeet and a vasomotor response. We also observed that rapid ictal discharge-induced arteriole responses were regularly preceded by Ca^{2+} elevations in endfeet and were abolished by pharmacological inhibition of Ca^{2+} signals in these astrocyte processes. Under these latter conditions, arterioles exhibited after the ictal discharge only slowly developing vasodilations. The poor efficacy of interictal discharges, compared with ictal discharges, to activate endfeet was confirmed also in the intact *in vitro* isolated guinea pig brain. Although the possibility of a direct contribution of neurons, in particular in the late response of cerebral blood vessels to epileptic discharges, should be taken into account, our study supports the view that astrocytes are central for neurovascular coupling also in the epileptic brain. The massive endfeet Ca^{2+} elevations evoked by ictal discharges and the poor response to interictal events represent new information potentially relevant to interpret data from diagnostic brain imaging techniques, such as functional magnetic resonance, utilized in the clinic to localize neural activity and to optimize neurosurgery of untreatable epilepsies.

Commentary

Astrocytes of the healthy brain fulfill an important homeostatic role to couple neuronal activity to cerebral blood flow, and thereby to adapt local cerebral blood flow to metabolic demands, a phenomenon termed neurovascular coupling (1, 2). Astrocytes react to neuronal activity by a rise in intracellular Ca^{2+} , and it is the increase in Ca^{2+} in the astrocytic endfeet that ensheath the microvasculature that triggers vascular responses in the arterioles. The physiological consequence is vasodilation exerted through a variety of mechanisms (1, 3). In epilepsy, the excessive discharge of neurons leads to an increase in metabolic demand that needs to be met by an increase in cerebral blood flow (4). If blood supply to an epileptogenic focus is insufficient, diffuse neuronal injury may result. Therefore, neurovascular coupling plays an important role in limiting damage following a seizure. However, the underlying mechanisms of neurovascular coupling within the context of epilepsy remain understudied. The study by Gómez-Gonzalo and colleagues was designed to assess whether neurovascular coupling, as extensively studied in the normal brain, is also operant during epileptiform discharges.

Two seizure models were studied: rat cortical slices exposed to picrotoxin under zero Mg^{2+} conditions, and an *in vitro* model of intact isolated guinea pig brain made epileptic by

arterial perfusion with bicuculline. Preparations were analyzed by a combination of patch clamp recordings and Ca^{2+} imaging, while arteriole responses (vessel diameter) were determined by differential interference contrast (DIC) microscopy.

In the slice preparation, the Ca^{2+} signal from cortical neurons could be used to determine the onset of the epileptiform discharge and to distinguish between ictal and interictal discharges. Of importance, the ictal, but not the interictal, discharges triggered a diffuse elevation of Ca^{2+} in astrocytic endfeet, as evidenced by the temporal profile of neuronal discharges and astrocytic Ca^{2+} responses, and by the colocalization of the Ca^{2+} -dye with an astrocyte-specific marker in perivascular structures. These findings, showing elevated Ca^{2+} in astrocytic endfeet in response to ictal, but not to interictal, discharges were validated in the isolated brain preparation.

How do arterioles respond to ictal and interictal discharges? If only ictal discharges elevate Ca^{2+} in astrocytic endfeet, then only ictal discharges should regulate the tone of the arteriole wall. By simultaneous Ca^{2+} and DIC imaging in cortical slices, Gómez-Gonzalo and colleagues studied changes in the intraluminal diameters of arterioles in response to ictal and interictal discharges, as well as the temporal relation between the Ca^{2+} elevation in the endfeet and the arteriole response. It is important to note that slice preparations generally lack an intraluminal blood flow, leading to an artificially dilated state of the arterioles (2). Under those conditions, the ictal discharge triggered vasoconstriction. To avoid this confound and recreate a more physiological constriction state, vessels were “pre-constricted” with the thromboxane A_2 analog U46619



before the induction of the seizures. Under those conditions, an ictal discharge triggered profound vasodilation in the majority of the pre-constricted arterioles. Of interest, ictal events triggered vasodilation with different delays. In the majority of arterioles, vasodilation occurred within approximately 3 seconds following the Ca^{2+} rise, and only those fast responses could be abrogated by depletion of intracellular calcium stores; under those conditions, arterioles were still responsive to the potent vasodilator adenosine. In contrast, delayed vasodilation occurred within approximately 20 seconds of the Ca^{2+} rise and was not affected by depletion of intracellular Ca^{2+} stores. Together, these findings indicate that only the fast dilating response is mediated by Ca^{2+} elevation in astrocytic endfeet.

These findings are not only of interest from a mechanistic perspective regarding the cellular pathways leading to hemodynamic changes in the epileptic brain, but they also have practical implications for commonly used diagnostic procedures. For example, blood oxygen level-dependent (BOLD) signals in fMRI depend in part on an increase in cerebral blood flow, and the assumption that the metabolic demands of neurons dictate the increase in cerebral blood flow. This somewhat simplistic view has recently been challenged based on the contribution of astrocytes to metabolic demands and to the regulation of cerebral blood flow (5). The findings by Gómez-Gonzalo and colleagues support the view that ictal discharges, activating astrocytes, lead to a significant increase in energy consumption of the brain and thereby affect the BOLD signals in fMRI. Furthermore, some diagnostic procedures such as combined fMRI-EEG studies rely on interictal discharges for the presurgical localization of the epileptogenic focus (6). However, the demonstration that interictal discharges do not affect astrocytic Ca^{2+} activation and cerebral blood flow in both experimental preparations warrants caution in the interpretation of interictal data sets from fMRI-EEG studies. The reduced neurovascular coupling during interictal events may also relate to the hypometabolism in the epileptogenic focus as observed by fluorodeoxyglucose PET imaging (7). Before more far-reaching conclusions regarding the interpretation of current imaging technologies can be reached, it needs to be ascertained that findings from in vitro studies such as those discussed here can also be replicated in more realistic animal models of epilepsy, which express spontaneous recurrent seizures and have normal brain perfusion.

Ictal discharges triggered vasodilation through at least two different mechanisms. The rapid response to the ictal discharge was directly related to the Ca^{2+} elevation in astrocytic endfeet, and it makes physiological sense to provide an immediate increase in blood supply to an epileptogenic brain area, likely an endogenous mechanism to limit hypoxic injury

to the brain. The delayed vasodilation was not linked to the acute rise in Ca^{2+} and is consistent with a postictal surge of the brain's endogenous anticonvulsant (8, 9) and vasodilator (10) adenosine. This dual activity of adenosine would allow the brain to reestablish energy homeostasis following a seizure by 1) a delayed and prolonged increase in cerebral blood flow, and 2) a reduction of metabolic demands during a state of postictal refractoriness.

The demonstration of functional neurovascular coupling during ictal discharges has exciting implications for our understanding of homeostatic mechanisms of the brain. In particular, the bioenergetic implications of increased astrocyte metabolic demands during ictal events have received little attention to date. Better understanding of the mechanisms underlying neurovascular coupling in epilepsy may lead to improved diagnostic approaches. The demonstration of these mechanisms in in vitro preparations is a first important step; however, it remains for future studies to validate these findings in in vivo models of epilepsy.

by Detlev Boison, PhD

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