

ROLE OF GLIAL CELLS IN SEIZURES AND EPILEPSY: INTRACELLULAR CALCIUM OSCILLATIONS AND SPATIAL BUFFERING

Spatial Buffering During Slow and Paroxysmal Sleep Oscillations in Cortical Networks of Glial Cells In Vivo

Amzica F, Massimini M, Manfredi A

J Neurosci 2002;22:1042–1053

The ability of neuroglia to buffer local increases of extracellular K^+ has been known from in vitro studies. This property may confer on these cells an active role in the modulation and spreading of cortical oscillatory activities. We addressed the question of the spatial buffering in vivo by performing single and double intraglial recordings, together with measures of the extracellular K^+ and Ca^{2+} concentrations ($[K^+]_{out}$ and $[Ca^{2+}]_{out}$) in the cerebral cortex of cats under ketamine and xylazine anesthesia during patterns of slow sleep oscillations and spike-wave seizures. In addition, we estimated the fluctuations of intraglial K^+ concentrations ($[K^+]_{in}$). Measurements obtained during the slow oscillation indicated that glial cells physically take up part of the extracellular K^+ extruded by neurons during the depolarizing phase of the slow oscillation. During this condition, the redistribution of K^+ appeared to be local. Large steady increases of $[K^+]_{out}$ and phasic potassium accumulations were measured during spike-wave seizures. In this condition, $[K^+]_{in}$ rose before $[K^+]_{out}$ if the glial cells were located at some distance from the epileptic focus, suggesting faster K^+ diffusion through the interglial syncytium. The simultaneously recorded $[Ca^{2+}]_{out}$ dropped steadily during the seizures to levels incompatible with efficient synaptic transmission, but also displayed periodic oscillations, in phase with the intraseizure spike-wave complexes. In view of this fact, and considering the capability of K^+ to modulate neuronal excitability both at the presynaptic and postsynaptic levels, we suggest that the K^+ long-range spatial buffering operated by glia is a parallel synchronizing and/or spreading mechanism during paroxysmal oscillations.

Calcium Oscillations in Neocortical Astrocytes under Epileptiform Conditions

Tashiro A, Goldberg J, Yuste R

J Neurobiol 2002;50:45–55

Morphological and functional alterations in astrocytic glia are often found in epileptic syndromes, although the exact role of astrocytes in epilepsy is poorly understood. During calcium imaging of epileptiform events in juvenile neocortical slices we previously discovered cells with spontaneous oscillations in their intracellular free calcium concentration ($[Ca^{2+}]_i$). We have now characterized these oscillations using two in vitro models of epilepsy and find that they are produced by astrocytes. Astrocytic oscillations are widespread throughout the imaged territories, are remarkably regular and have long periods, averaging 100 s, which become shorter during development. Astrocytic oscillations are uncorrelated among themselves and with epileptiform events, are blocked by internal release antagonists and are stimulated by caffeine. Astrocytic calcium oscillations could mediate reactive astrogliosis, contribute to the pathogenesis of chronic epileptic syndromes, and be used as a diagnostic test for epileptic tissue.

COMMENTARY

Studies seeking to understand the basis of neuronal hyperactivity and synchronization during epileptic seizures have almost exclusively focused on the intrinsic properties of neurons and the behavior of neuronal circuits. Although glial cells far outnumber neurons, their contributions have been largely ignored. Indeed, the role of glial cells in seizures and epilepsy is probably no better understood than their role in normal brain function. The articles discussed below address two phenomena or physiological mechanisms that have been known for many years and are likely linked to seizure activity. The

classic experiments of Orkand, Kuffler, and Nicholls (1) on glial cells in the amphibian nervous system led to the concept that glia play an important role in ion homeostasis, particularly the regulation of potassium during periods of high activity. These two articles address specific components of this general hypothetical mechanism in quite different ways.

One of the more interesting phenomena of the glial network is their potential to propagate waves of increased intracellular calcium from one glial cell to another. For technical reasons, this phenomenon has mostly been studied in cultures of astrocytes (2). In the work of Tashiro et al. (3), slices from normal rats were treated with bicuculline or magnesium-free medium with an extracellular potassium concentration of 6 mM, which generated epileptiform burst discharges in the neurons. After these treatments, the glia often displayed "calcium waves" (i.e., oscillations in the intracellular concentration of calcium). Although seizure-like activity appeared to cause calcium waves, these two forms of activity were not well correlated temporally. Previous research has suggested glutamate (2) and neuronal activity (4) can trigger calcium waves, but the cause, function and effect of the seizure-induced calcium waves is not clear. The important question is: Do calcium waves represent a form of communication over long distances in brain tissue? This issue has long been the basis of discussion (5). The authors speculate that calcium waves could be responsible for reactive astrogliosis and contribute to the pathogenesis of chronic epilepsy. Little or no evidence is presented to support these speculations, but nonetheless, the article highlights the idea that seizure-like activity can lead to changes in the intracellular concentration of calcium in glia. This mechanism may contribute to the ability of electrographic seizure activity to progressively spread along unconventional pathways (i.e., independent of traditional chemical synaptic pathways). Although these data suggest that calcium waves represent propagation of information across substantial distances in the cerebral cortex, whether this mechanism is actually involved in the spread of epileptic seizures and epileptogenesis remains to be determined.

The article by Amzica et al. (6) examines changes in the membrane potential of glial cells and in the extracellular concentration of potassium at different sites during sleep-associated oscillations and spike-wave seizure-like events. The authors provide evidence that extracellular potassium is taken up by glial cells during the depolarizing phase of the slow oscillation that is associated with sleep. During spike-wave seizures, the concentration of intracellular potassium increased before the concentration of extracellular potassium when the glial cells were remote from the epileptic focus. This suggests that potassium diffuses faster through the intercellular glial syncytium than through the extracellular space. The authors also corroborated the previously established idea that the concentration of extracellular calcium declines during seizure activity to a level that

is incompatible with chemical synaptic transmission (e.g., see references 7 and 8). These two observations lead to the hypothesis that the glial syncytium plays a role as a mechanism for the synchronization and/or propagation of seizure-like activity.

One strength of the work of Amzica et al. (6) is the development of experimental evidence concerning the spatial buffer hypothesis using multiple intragial and ion-specific extracellular recordings. This ability to evaluate intracellular glial potentials and extracellular potassium concentration at different sites allowed the authors to address this question. What remains unclear is the degree to which the calcium decrease is actually associated physiologically with depression of chemical synaptic transmission. The authors invoke calcium-mediated glutamate release from glia (9-11), and glutamate-induced depolarization of neurons as a mechanism of seizure propagation. Additional research is needed to understand how transmission of increased intracellular potassium concentration would actually serve as a long-distance form of communication to propagate electrographic seizures. The authors propose that the local increase in extracellular potassium that is associated with an epileptic seizure causes a "persistent intragial depolarization" that "travels along the glial syncytium." This mechanism, and its hypothetical importance to the spread of epileptic seizures, should also be the basis for further experiments using other seizure models.

The observations in these two articles indicate that seizure-like activity has substantial if not profound effects on the physiological functions of glial cells. How these phenomena contribute to the chronic process of epileptogenesis is a field that will have to be developed further in the future.

References

1. Orkand RK, Nicholls JG, Kuffler SW. Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J Neurophysiol* 1966;29:788-806.
2. Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ. Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science* 1990;26:470-473.
3. Tashiro A, Goldberg J, Yuste R. Calcium oscillations in neocortical astrocytes under epileptiform conditions. *J Neurobiol* 2002;50:45-55.
4. Dani JW, Chernjavsky A, Smith SJ. Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron* 1992;8:429-440.
5. Smith SJ. Do astrocytes process neural information? *Prog Brain Res* 1992;94:119-136.
6. Amzica F, Massimini M, Manfridi A. Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. *J Neurosci* 2002;22:1042-1053.
7. Heinemann U, Lux HD, Gutnick MJ. Extracellular free calcium and potassium during paroxysmal activity in the cerebral cortex of the cat. *Exp Brain Res* 1977;27:237-243.

8. Pumain R, Kurcewicz I, Louvel J. Fast extracellular calcium transients: Involvement in epileptic processes. *Science* 1983;222:177–179.
9. Parpura V, Basarsky TA, Liu F, Jefinija K, Jefinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature* 1994; 369: 744–747.
10. Hassinger TD, Atkinson PB, Strecker GJ, Whalen LR, Dudek FE, Kossel AH, Kater SB. Evidence for glutamate-mediated activation of hippocampal neurons by glial calcium waves. *J Neurobiol* 1995; 28: 159–170.
11. Innocenti B, Parpura V, Haydon PG. Imaging extracellular waves of glutamate during calcium signaling in cultured astrocytes. *J Neurosci* 2000;20:1800–1808.

by F. Edward Dudek, Ph. D.