CURRENT REVIEW IN BASIC SCIENCE

DO SINGLE SEIZURES CAUSE NEURONAL DEATH IN THE HUMAN HIPPOCAMPUS?

Luisa L. Rocha, MD, PhD,[1] Maria-Leonor Lopez-Meraz, PhD,[2,3] Jerome Niquet, PhD,[2,3] and Claude G. Wasterlain, MD, LSc[2,3,4]

[1]Pharmacobiology Department, Center for Research and Advanced Studies, Mexico City, Mexico; [2]Epilepsy Research Laboratory (151), Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA; [3]Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]Brain Research Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA

The question of whether repeated single seizures cause neuronal death in the adult human brain is of great clinical importance and might have broad therapeutic implications. Reviewed here are recent studies on the effects of repeated single seizures (in the absence of status epilepticus) on hippocampal volume and on neuronal death markers in blood and in surgically ablated hippocampi.

Based on the work of Meldrum (1) and others, it has generally been accepted that status epilepticus, even in the absence of systemic complications, causes neuronal death in vulnerable brain regions. Whether this outcome also applies to repeated single seizures (i.e., repetitive seizures that do not reach the severity of status epilepticus in patients with epilepsy) has been more controversial. Cavazos and collaborators (2) have used special methods to demonstrate neuronal loss in the hilus of the hippocampal dentate gyrus following a few kindled seizures. Their findings have been broadly accepted, confirmed (3), and extended by different techniques (4). If single seizures cause damage to the human brain that is sufficiently widespread to be of clinical significance, it raises questions that are difficult to answer: Which types of seizure put the brain at risk? Should each patient with uncontrolled epilepsy have serial MRIs to detect atrophy early? If a patient who is doing well clinically shows hippocampal atrophy, should treatment be dictated by the clinical picture or by the MRI? Should clinicians consider using one agent to control seizures and a different agent to protect the brain from their consequences? Until recently, however, repeated single seizures have not been shown to cause damage to the human brain or to animal brains in models of spontaneous recurrent seizures, which approximate human epilepsy much more closely than kindling.

The question of whether damage occurs from repeated single seizures has been reopened because of reports of progressive hippocampal atrophy seen on MRI and detection of enzymes usually associated with active cell death in surgically ablated human temporal lobes. In animals, a correlation between neuronal densities in the hilus and the lifetime number of spontaneous recurrent seizures has been reported (5). Fluoro-Jade positivity of CA1 (presumably, reflecting cell injury from spontaneous seizures) observed 40 days after status epilepticus, was briefly mentioned in an MRI study (5), and preliminary observations support that finding (Wasterlain CG, Niquet J, Suchomelova L, unpublished data, 2006). However, a systematic study of the extent and mechanism of neuronal injury are not available. In humans, several lines of evidence (e.g., biochemical markers, MRI, histology, immunocytochemical studies) raise the possibility that single seizures can cause neuronal death, but the interpretation of that evidence is not always straightforward and caution is highly recommended.

Indirect Markers of Neuronal Injury

One of the strongest pieces of evidence supporting seizure-induced neuronal injury in the human brain is probably the postictal rise in the serum level of neuron-specific enolase (NSE)—a marker of neuronal injury that is released from the brain after acute neurologic insults. However, changes in this marker depend on seizure type and on the size of the anatomic structures involved. For instance, patients with tonic–clonic seizures show NSE increases, whereas patients with single complex partial seizures present variable alterations (6–8). As expected, increases were very high in patients with complex partial status epilepticus (9). The reliability of elevations of serum NSE as a marker of neuronal injury is well established in animal models (10) and in human brain ischemia (11), but few studies involving mild insults are available.

Address correspondence to Maria Leonor Lopez-Meraz, PhD, Epilepsy Research Laboratory, VA Greater Los Angeles Healthcare System, 11301 Wilshire Boulevard, Building 114, Room 139, West Los Angeles, CA 90073. E-mail: lopezmerazml@ucla.edu

Brain Imaging Studies

A growing number of MRI-based studies report progressive hippocampal atrophy associated with uncontrolled temporal lobe epilepsy (TLE) in the absence of known episodes of status epilepticus. Some retrospective studies show a slow but significant loss of volume of the hippocampus with increasing seizure number (12,13) and increasing age (14,15); in addition, correlations between cessation of seizures and arrest of hippocampal atrophy have been demonstrated (13,16). Other studies show a negative correlation between the duration of epilepsy and *N*-acetyl-aspartate (NAA), a neuronal marker commonly used in MRI studies (17). Additionally, T2 relaxation time, a marker of gliosis, increases with seizure number and duration (18). However, some studies failed to demonstrate such correlations (19–21).

By its very nature, the finding of greater cortical atrophy than that seen in age-matched controls provides only indirect evidence of neuronal loss. Even a decline in the neuronal marker NAA is not direct proof of neuronal loss, since the marker is present in mitochondria and microsomes (22) and in rare circumstances, can change in the absence of cell death (23,24). None of the studies excluded patients with a history of status epilepticus or described seizure type. It is likely, however, that the bulk of seizures would be complex partial in the populations studied, because these are the most common kind of seizures occurring in TLE. Overall, the evidence is compatible with seizure-induced neuronal loss, but suggests that the amount of neuronal death with each seizure may be very small, as significant atrophy was seen only after many years of uncontrolled TLE.

Neuronal Counts

Mathern et al. (25) reported a negative correlation between carefully obtained neuronal counts and the duration of epilepsy. Although the occurrence of SE was not noted and the study was retrospective, this result might support an association between neuronal loss and uncontrolled single seizures.

Cell Death Markers in Surgical Samples

A number of studies report markers of active neuronal death found in temporal lobes removed surgically for intractable TLE. Most patients had no known history of status epilepticus or of other active brain disease, although in many cases these factors were not formally excluded. How certain is it that expression of cell death markers in those brains reflects ongoing neuronal death resulting from uncontrolled single seizures? The question is not easy to answer since none of those markers are totally specific for cell death, most reports involve a limited number of samples from a single laboratory, and there is a large bias in the literature favoring positive results.

A Brief Overview of Cell Death Mechanisms

Many cells initiate apoptosis when a cell death receptor, typically located on the cell surface, is activated and sends a signal to the nucleus, triggering the expression of various genes. In the extrinsic pathway, caspase-8 and eventually caspase-3 are then activated. In the intrinsic pathway, the eventual result is that an effector protein is sent to the mitochondria (e.g., to a pro-apoptotic member of the Bcl-2 family). Through a complex mechanism, cytochrome c is released from the space between the inner and outer mitochondrial membranes. In the cytoplasm, cytochrome c forms a complex with the apoptotic protease-activating factor 1 (Apaf-1) and that complex recruits procaspase-9, inducing its oligomerization and activation (26). Caspases form a cascade of proteases that activate one other in much the same way as occurs in the complement system. Caspase activation often starts with one of the initiator caspases (i.e., caspase-8 and caspase-9), which have long amino terminal prodomains that can be self-cleaved on oligomerization (27). These caspases then activate effector caspases via a cascade that often ends with the activation of caspase-3, which attacks structural proteins, activates DNA destruction, and inactivates DNA repair. Through its multiple proteolytic effects, caspase-3 is the main executioner of the cell. Caspases also may have other functions and contribute to cell survival, proliferation, and differentiation (28).

While these processes undoubtedly are present in apoptosis, recent reports indicate that caspase activation often is seen in cells with a necrotic morphology (29). Niquet et al. described a form of necrosis that depends on the mitochondrial release of cytochrome c and leads to activation of caspase-9, followed by activation of caspase-3 (30–32). Thus, this form of necrosis is not passive but depends on activation of a mitochondrial cell death program. The existence of this cell death pathway also indicates that activation of caspases is not specific for apoptosis; there are multiple amplification feedback loops along those pathways. Some cells, however, seem to induce apoptosis by a caspase-independent pathway (33) in which apoptosis-inducing factor (AIF), endonuclease G, and other proteins play an important role.

Caspases and Related Enzymes

The presence of immunoreactivity against the active form of caspase-3 and against cleaved caspase-1 in temporal lobes resected for intractable epilepsy, suggests active programmed cell death (34–37). However, about 10% of neurons in the hippocampus of epileptic patients with mesial temporal sclerosis show fractin immunostaining, which is an indicator of caspase-3 activation (38). Seizures associated with TLE also trigger pro-apoptotic–signaling pathways in the endoplasmic-reticulum–containing microsomal fraction, such as activation of caspases

6, 7, 8, and 9 (37,39). The nuclear localization of high levels of caspase-activated DNase, as reported by Schindler et al. (40), also supports the activation of caspase-dependent death mechanisms.

Markers of Caspase-Independent Death Pathways

AIF, a flavoprotein of unknown physiological function (41), like cytochrome *c*, is located between the inner and the outer mitochondrial membranes and sometimes is released when mitochondria are depolarized. It translocates to the nucleus (42) where it breaks down chromatin into large fragments and participates in caspase-independent neuronal death (43,44). AIF has been involved in cell death induced by oxidative stress (45), ischemia (46), and trauma (43). Results of preliminary studies show prominent translocation of AIF after status epilepticus, although its exact role has not yet been defined (Wasterlain CG, Lopez-Meraz M-L, Niquet J, unpublished data, 2006). High levels of AIF and of endonuclease G in surgical samples support a caspase-independent apoptosis signaling in human TLE. However, the fact that cells expressing cleaved caspase-3 do not express nuclear AIF, suggests separate signaling pathways in different neuronal populations (40).

Other Components of Cell Death Cascades

The most extensively studied cell death factors have been the Bax-Bcl2 family of mitochondrial-interacting proteins (47). These proteins are pro- or anti-apoptotic, depending on their ability to promote or suppress cytochrome *c* release. The balance between pro-apoptotic factors, (e.g., Bid, the BH3-interacting domain death agonist; Bim, the Bcl-2–interacting mediator of cell death; or Bax, the Bcl-2–associated X protein) and anti-apoptotic factors, such as Bcl-2, Bcl-x, and Bcl-w, determines the fate of the cell (48). Hippocampi from patients with epilepsy demonstrates increased expression of neuronal Bcl-2 and Bax (34,38). However, despite increased Bax expression and activation of caspases, there is no clear evidence of DNA fragmentation in neurons (38). Additionally, lowered Bim levels and higher binding of Bim to Bcl-w were observed in specimens of TLE patients (36), whereas Bad and Bid were not significantly different from control samples (36,37). The low Bim levels in the endoplasmic-reticulum–containing microsomal fraction and binding of caspase-7 to the X-linked inhibitor of apoptosis protein could be considered adaptive changes that counteract apoptotic signals and reduce the vulnerability to further neuronal loss (37). This interpretation is supported by the finding that Bim expression was inversely correlated to the number of maximal electroshock seizures in an animal model (36).

The presence of a high nuclear expression of cyclin B (an enzyme specific for the G2 phase of the cell cycle) accompanied by neuronal cytoplasmic expression of the death-related Bax protein in the hippocampus of patients with epilepsy has been interpreted as evidence of cell cycle disturbances associated with an apoptotic mechanism of hippocampal neuronal cell death in TLE (49). The analysis of other components of the apoptotic cascade in the hippocampus of patients with medically intractable TLE reveals an enhanced expression and phosphorylation of the death-associated protein kinase (DAPK), a calcium/calmodulin-activated kinase, which functions in apoptosis mediated by death receptors. DAPK and DAPK-interacting protein 1 (DIP-1) are localized in mitochondria in control tissue, whereas in epileptic hippocampus they are found in cytoplasm and endoplasmic reticulum, a translocation usually associated with apoptotic death (50). In addition, epilepsy samples display increased DAPK binding to calmodulin, DIP-1, and the Fas-associated death domain protein (35).

Conclusions

There is an increasing amount of evidence that associates markers of neuronal death in blood and brain with a history of repeated single seizures; however, the evidence is still indirect and retrospective. Detailed studies that reported seizure type and seizure count in patients undergoing temporal lobectomy for intractable epilepsy would permit precise correlations between clinical seizure types and neuronal death mechanisms in specific brain regions. These investigations also may offer novel targets for anticonvulsant therapy aimed at reducing seizure-induced damage and epileptogenesis.

Acknowledgments

References

1. Meldrum BS. Concept of activity-induced cell death in epilepsy: historical and contemporary perspectives. *Prog Brain Res* 2002;135:3–11.

2. Cavazos JE, Das I, Sutula TP. Neuronal loss induced in limbic pathways by kindling: evidence for induction of hippocampal sclerosis by repeated brief seizures. *J Neurosci* 1994;14:3106–3121.

3. Tuunanen J, Pitkanen A. Do seizures cause neuronal damage in rat amygdala kindling? *Epilepsy Res* 2000;39:171–176.

4. Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M, Lindvall O. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc Natl Acad Sci USA* 1997;94:10432–10437.

5. Pitkanen A, Nissinen J, Nairismagi J, Lukasiuk K, Grohn OH, Miettinen R, Kauppinen R. Progression of neuronal damage after status epilepticus and during spontaneous seizures in a rat model of temporal lobe epilepsy. *Prog Brain Res* 2002;135:67–83.

6. Buttner T, Lack B, Jager M, Wunsche W, Kuhn W, Muller T, Przuntek H, Postert T. Serum levels of neuron-specific enolase and s-100 protein after single tonic-clonic seizures. *J Neurol* 1999;246:459–461.

7. Rabinowicz AL, Correale J, Boutros RB, Couldwell WT, Henderson CW, DeGiorgio CM. Neuron-specific enolase is increased after single seizures during inpatient video/EEG monitoring. *Epilepsia* 1996;37:122–125.

8. Tumani H, Otto M, Gefeller O, Wiltfang J, Herrendorf G, Mogge S, Steinhoff BJ. Kinetics of serum neuron-specific enolase and prolactin in patients after single epileptic seizures. *Epilepsia* 1999;40:713–718.

9. DeGiorgio CM, Correale JD, Gott PS, Ginsburg DL, Bracht KA, Smith T, Boutros R, Loskota WJ, Rabinowicz AL. Serum neuron-specific enolase in human status epilepticus. *Neurology* 1995;45:1134–1137.

10. Sankar R, Shin DH, Wasterlain CG. Serum neuron-specific enolase is a marker for neuronal damage following status epilepticus in the rat. *Epilepsy Res* 1997;28:129–136.

11. Reisinger J, Hollinger K, Lang W, Steiner C, Winter T, Zeindlhofer E, Mori M, Schiller A, Lindorfer A, Wiesinger K, Siostrzonek P. Prediction of neurological outcome after cardiopulmonary resuscitation by serial determination of serum neuron-specific enolase. *Eur Heart J* 2007;28:52–58.

12. Briellmann RS, Newton MR, Wellard M, Jackson GD. Hippocampal sclerosis following brief generalized seizures in adulthood. *Neurology* 2001;57:315–317.

13. Briellmann R, Berkovic S, Syngeniotis, King M, Jackson GD. Seizure associated hippocampal volume loss: a longitudinal magnetic resonance study of temporal lobe epilepsy. *Ann Neurol* 2002;51:641–644.

14. Mathern GW, Pretorius JK, Babb TL. Influence of the type of initial precipitating injury and at what age it occurs on course and outcome in patients with temporal lobe seizures. *J Neurosurg* 1995;82:220–227.

15. Theodore WH, Bhatia S, Hatta J, Fazilat S, DeCarli C, Bookheimer SY, Gaillard WD. Hippocampal atrophy, epilepsy duration, and febrile seizures in patients with partial seizures. *Neurology* 1999;52:132–136.

16. Fuerst D, Shah J, Shah A, Watson C. Hippocampal sclerosis is a progressive disorder: a longitudinal volumetric MRI study. *Ann Neurol* 2003;53:413–416.

17. Tasch E, Cendes F, Li LM, Dubeau F, Andermann F, Arnold DL. Neuroimaging evidence of progressive neuronal loss and dysfunction in temporal lobe epilepsy. *Ann Neurol* 1999;45:568–576.

18. Kalviainen R, Salmenpera T, Partanen K, Vainio P, Riekkinen P, Pitkanen A. Recurrent seizures may cause hippocampal damage in temporal lobe epilepsy. *Neurology* 1998;50:1377–1382.

19. Liu RS, Lemieux L, Bell GS, Sisodiya SM, Bartlett PA, Shorvon SD, Sander JW, Duncan JS. The structural consequences of newly diagnosed seizures. *Ann Neurol* 2002;52:573–580.

20. Holtkamp M, Schuchmann S, Gottschalk S, Meierkord M. Recurrent seizures do not cause hippocampal damage. *J Neurol* 2004;251:458–463.

21. Thom M, Zhou J, Martinian L, Sisodiya S. Quantitative postmortem study of the hippocampus in chronic epilepsy: seizures do not inevitably cause neuronal loss. *Brain* 2005;128:1344–1357.

22. Lu ZH, Chakraborty G, Ledeen RW, Yahya D, Wu G. N-Acetylaspartate synthase is bimodally expressed in microsomes and mitochondria of brain. *Brain Res Mol Brain Res* 2004;122:71–78.

23. Demougeot C, Garnier P, Mossiat C, Bertrand N, Giroud M, Beley A, Marie C. N-Acetylaspartate, a marker of both cellular dysfunction and neuronal loss: its relevance to studies of acute brain injury. *J Neurochem* 2001;77:408–415.

24. Lentz MR, Kim JP, Westmoreland SV, Greco JB, Fuller RA, Ratai EM, He J, Sehgal PK, Halpern EF, Lackner AA, Masliah E, Gonzalez RG. Quantitative neuropathologic correlates of changes in ratio of N-acetylaspartate to creatine in macaque brain. *Radiology* 2005;235:461–468.

25. Mathern GW, Adelson PD, Cahan LD, Leite JP. Hippocampal neuron damage in human epilepsy: Meyer's hypothesis revisited. *Prog Brain Res* 2002;135:237–251.

26. Srinivasula SM, Ahmad M, Fernandez-Alnemri T, Alnemri ES. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol Cell* 1998;1:949–957.

27. Martin DA, Siegel RM, Zheng L, Lenardo MJ. Membrane oligomerization and cleavage activates the caspase-8 (FLICE/MACHalpha1) death signal. *J Biol Chem* 1998;273:4345–4349.

28. Lamkanfi M, Festjens N, Declercq W, Vanden Berghe T, Vandenabeele P. Caspases in cell survival, proliferation and differentiation. *Cell Death Differ* 2007;14:44–55.

29. Chen J, Nagayama T, Jin K, Stetler RA, Zhu RL, Graham SH, Simon RP. Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia. *J Neurosci* 1998;18:4914–4928.

30. Niquet J, Baldwin RA, Allen SG, Fujikawa DG, Wasterlain CG. Hypoxic neuronal necrosis: protein synthesis-independent activation of a cell death program. *Proc Natl Acad Sci USA* 2003;100:2825–2830.

31. Niquet J, Liu H, Wasterlain CG. Programmed neuronal necrosis and status epilepticus. *Epilepsia* 2005;46(suppl 5):43–48.

32. Niquet J, Seo DW, Allen SG, Wasterlain CG. Hypoxia in presence of blockers of excitotoxicity induces a caspase-dependent neuronal necrosis. *Neuroscience* 2006a;141:77–86.

33. Eguchi K. Apoptosis in autoimmune diseases. *Intern Med* 2001;40:275–284.

34. Henshall DC, Clark RS, Adelson PD, Chen M, Watkins SC, Simon RP. Alterations in bcl-2 and caspase gene family protein expression in human temporal lobe epilepsy. *Neurology* 2000;55:250–257.

35. Henshall DC, Schindler CK, So NK, Lan JQ, Meller R, Simon RP. Death-associated protein kinase expression in human temporal lobe epilepsy. *Ann Neurol* 2004;55:485–494.

36. Shinoda S, Schindler CK, Meller R, So NK, Araki T, Yamamoto A, Lan JQ, Taki W, Simon RP, Henshall DC. Bim regulation may determine hippocampal vulnerability after injurious seizures and in temporal lobe epilepsy. *J Clin Invest* 2004;113:1059–1068.

37. Yamamoto A, Murphy N, Schindler CK, So NK, Stohr S, Taki W, Prehn JH, Henshall DC. Endoplasmic reticulum stress and apoptosis signaling in human temporal lobe epilepsy. *J Neuropathol Exp Neurol* 2006a;65:217–225.

38. Uysal H, Cevik IU, Soylemezoglu F, Elibol B, Ozdemir YG, Evrenkaya T, Saygi S, Dalkara T. Is the cell death in mesial temporal sclerosis apoptotic? *Epilepsia* 2003;44:778–784.

39. Yamamoto A, Schindler CK, Murphy BM, Bellver-Estelles C, So NK, Taki W, Meller R, Simon RP, Henshall DC. Evidence of tumor necrosis factor receptor 1 signaling in human temporal lobe epilepsy. *Exp Neurol* 2006b;202:410–420.

40. Schindler CK, Pearson EG, Bonner HP, So NK, Simon RP, Prehn JH, Henshall DC. Caspase-3 cleavage and nuclear localization of caspase-activated DNase in human temporal lobe epilepsy. *J Cereb Blood Flow Metab* 2006;26:583–589.

41. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 1999;397:441–446.

42. Daugas E, Susin SA, Zamzami N, Ferri KF, Irinopoulou T, Larochette N, Prevost MC, Leber B, Andrews D, Penninger J, Kroemer G. Mitochondrio-nuclear translocation of AIF in apoptosis and necrosis. *FASEB J* 2000;14:729–739.

43. Zhang X, Chen J, Graham SH, Du L, Kochanek PM, Draviam R, Guo F, Nathaniel PD, Szabo C, Watkins SC, Clark RS. Intranuclear localization of apoptosis-inducing factor (AIF) and large scale DNA fragmentation after traumatic brain injury in rats and in neuronal cultures exposed to peroxynitrite. *J Neurochem* 2002;82:181–191.

44. Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, Poirier GG, Dawson TM, Dawson VL. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. *Science* 2002;297:259–263.

45. Fonfria E, Dare E, Benelli M, Sunol C, Ceccatelli S. Translocation of apoptosis-inducing factor in cerebellar granule cells exposed to neurotoxic agents inducing oxidative stress. *Eur J Neurosci* 2002;16:2013–2016.

46. Plesnila N, Zhu C, Culmsee C, Groger M, Moskowitz MA, Blomgren K. Nuclear translocation of apoptosis-inducing factor after focal cerebral ischemia. *J Cereb Blood Flow Metab* 2004;24:458–466.

47. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. *Science* 1998;281:1322–1326.

48. Niquet J, Wasterlain CG. Bim, Bad and Bax: a deadly combination in epileptic seizures. *J Clin Invest* 2004;113:960–962.

49. Nagy Z, Esiri MM. Neuronal cyclin expression in the hippocampus in temporal lobe epilepsy. *Exp Neurol* 1998;150:240–247.

50. Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katayama T, Tohyama M. Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J Biol Chem* 2001;276:13935–13940.