Alterations of Hippocampal Acetylcholinesterase in Human Temporal Lobe Epilepsy

Robert C. Green, MD,* Howard W. Blume, MD, PhD,† Seth B. Kupferschmid, MS,* and M-Marsel Mesulam, MD*

Hippocampal sclerosis is the most common pathological finding associated with human temporal lobe epilepsy. Histochemical study with acetylcholinesterase (AChE) staining was used to investigate 7 surgically resected temporal lobes with hippocampal sclerosis from patients with temporal lobe epilepsy. In all 7 specimens, an abnormal but consistent pattern of staining was noted. In the hilum of the dentate gyrus, AChE-rich polymorphic cells were relatively preserved in comparison to the pyramidal neurons. In Ammon's horn, AChE fibers were lost in regions corresponding to the pyramidal cell dropout. AChE fibers were also lost along the inner portion of the molecular layer of the dentate gyrus, yet they were preserved within the outer portions of the molecular layer. These findings provide additional evidence for the relative selectivity of hippocampal pathology in human temporal lobe epilepsy.

Green RC, Blume HW, Kupferschmid SB, Mesulam M-M. Alterations of hippocampal acetylcholinesterase in human temporal lobe epilepsy. Ann Neurol 1989;26:347–351

A common form of human epilepsy is partial epilepsy of temporolimbic origin (TLE). Surgical resection of the anterior temporal lobe is commonly used to treat TLE patients whose seizures are intractable to anticonvulsant medication [1–3]. The resected tissue sometimes reveals a tumor or cortical malformation, but the most common pathologic finding is hippocampal (or Ammon's horn) sclerosis. Hippocampal sclerosis occurs in 58% to 75% of surgical cases and is characterized by a shrunken, gliotic hippocampus, with loss of granule cells in the dentate and of pyramidal cells in Ammon's horn [4]. In classic descriptions, this loss is heaviest in the CA1 and CA3–4 sectors and less extensive among the dentate granule cells and in the CA2 sector.

Animal models of generalized seizures can reproduce a pathological pattern similar to that found in hippocampal sclerosis [5, 6], but it is unclear whether hippocampal sclerosis in humans reflects the sequelae of long-term epileptic activity or represents the causative lesion [7–9]. In animal models of hippocampal sclerosis produced by kainic acid–induced seizures, axonal sprouting and physiological alteration of the granule cells have been demonstrated [10]. The neuronal cell death in human hippocampal sclerosis and the resulting reorganization of neural connectivity could contribute to the complex behavioral alterations observed in some patients with TLE [11].

The morphological alterations in hippocampal sclerosis have been described in great detail. However, the associated neurochemical and histochemical alterations are less well understood. This report presents evidence for altered acetylcholinesterase (AChE) fiber distribution in the hippocampi of 7 patients with TLE and hippocampal sclerosis.

Methods

The patients in this study had intractable TLE and were evaluated for possible temporal lobe resection by well-established neurological, neuropsychological, and neurophysiological criteria [2, 12]. After lateralization and localization of the epileptic focus, the anterior 5 to 8 cm of the temporal lobe were removed by subpial resection. Tissue from patients with suspected tumor was sent for routine pathological examination, and these specimens were excluded from this study. All other specimens (n = 10) in this sequential series of patients were immersed immediately after removal from the patient in 4% paraformaldehyde for 24 hours and sunk in an increasingly concentrated series of 0.1 M phosphate-buffered 10 to 40% (w/v) sucrose solutions (pH 7.4).

From the *Division of Neuroscience and Behavioral Neurology and the †Division of Neurosurgery, Charles A. Dana Research Institute, Beth Israel Hospital and Harvard Medical School, Boston, MA.

Received May 20, 1988, and in revised form Dec 8, 1988, and Feb 15, 1989. Accepted for publication Feb 23, 1989.

Address correspondence to Dr Green, Emory Neurobehavioral Program, Wesley Woods Hospital, 1821 Clifton Road, NE, Atlanta, GA 30329.

Serial 1-in-10 coronal sections were cut at 40 μm on a freezing microtome into a 0.1 M phosphate-buffered solution (pH 7.4) and mounted onto chrome-alum subbed slides. Serial sections were stained with cresyl violet and adjacent sections were stained for AChE at incubation times of 2, 3, and 4 hours by a modification of the copper thiocholine method that we recently used to describe the normal pattern of AChE fiberarchitecture in the human hippocampus and parahippocampal gyrus [13]. Control sections were processed with 4 mM butyrylthiocholine iodide as a substitute for acetylthiocholine iodide, and both substrates were used with and without ethopropazine, a relatively selective inhibitor of nonacetyl cholinesterase.

Ten lobectomy specimens were obtained for this study. The histochemical pattern of AChE staining in these specimens was compared to that obtained from 5 brains removed at autopsy from nonepileptic persons in the same age range as the TLE patients. The nonepileptic tissue had an autolysis time (death-to-fixation interval) of 2 to 18 hours, whereas this interval was routinely less than 15 minutes in the epileptic tissue. Therefore, the overall AChE enzyme preservation (and hence, the histochemical staining) was expected to be more intense in the material from the TLE patients. However, this difference in baseline staining should have had no effect on the patterns of relative intensity, which constituted the only focus of our study.

Results

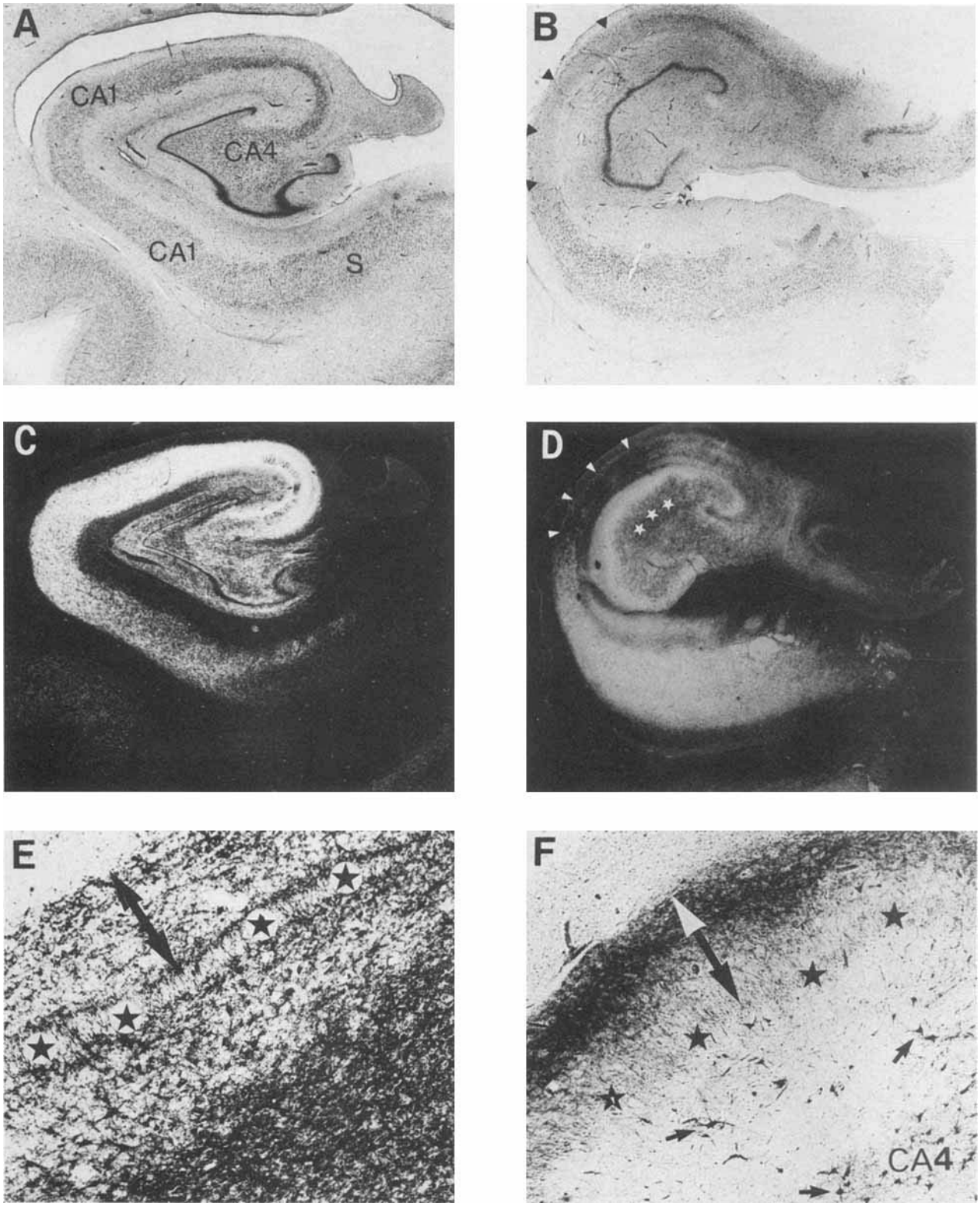
When serial sections were examined with Nissl stain, 7 of the 10 hippocampal specimens were observed to have hippocampal sclerosis. Only these 7 specimens are described in this report. In each of these specimens, pyramidal cell dropout followed the characteristic pattern of severe loss in CA4, CA3, and particularly portions of CA1, with relative sparing of CA2, the subiculum, and the portion of CA1 adjacent to the subiculum (see Figure, A and B). Evaluation of the sections from all 7 patients with hippocampal sclerosis revealed 3 consistent alterations in the distribution of hippocampal AChE staining. (1) In the normal brain, the hilum of the dentate gyrus contains AChE-rich polymorphic neurons and relatively AChE-poor CA4 pyramidal neurons. In the patients with hippocampal sclerosis, relative preservation of the AChE-rich neurons within the hilum of the dentate gyrus was seen in comparison to the widespread loss of CA4 pyramidal neurons (see Figure, B and F). (2) In the normal brain, AChE staining is present in all sectors of Ammon's horn but is most intense in CA2 and CA3 (see Figure, C). In the specimens with hippocampal sclerosis, AChE fibers were selectively depleted in those regions with the greatest loss of pyramidal cells, namely CA3 and parts of CA1 (see Figure). (3) The pattern of AChE fiber distribution within the molecular layer of the dentate gyrus was also altered. In the normal human hippocampus, a thin juxtagranular band in the molecular layer of the dentate gyrus stains more intensely than the rest of the molecular layer (see Figure, E). In each of the resected sclerotic hippocampi, this thin juxtagranular band of staining and some of the adjacent AChE staining within the molecular layer of the dentate disappeared, whereas staining within the outer half of the molecular layer of the dentate was preserved (see Figure, F).

In the material we examined, it often appeared as if the AChE staining in the outer portion of the molecular layer of the dentate gyrus was enhanced when compared to the nonepileptic control brains. Moreover, the nonsclerotic hippocampi demonstrated enhanced overall staining when compared to that obtained in the postmortem specimens. However, these differences in intensity are very difficult to interpret since the nonepileptic brains were unavoidably characterized by a longer autolysis time, as described in the Methods section. Staining was not obtained with butyrylthiocholine as the substrate, indicating the specificity of the reaction product for AChE.

Discussion

The AChE-positive neurons in the hilum of the dentate gyrus are probably not cholinergic since homologous AChE-rich cells in the monkey do not stain with choline acetyltransferase immunocytochemistry [14]. It is likely that these neurons are cholinoceptive and that the AChE is used to hydrolyze the acetylcholine released by the cholinergic afferents. The actual neurotransmitter identity of these cells is unknown, although there is evidence that some AChE-positive cortical cells produce gamma aminobutyric acid (GABA) or

Coronal sections through the human temporal lobe. (A) Nissl stain of the normal right hippocampus of a nonepileptic 30-year-old man who died of sepsis (death-to-fixation interval, 8 hours). (B) Ammon's horn sclerosis in the resected right hippocampus of a 33-year-old man with temporal lobe epilepsy (tissue fixed immediately after removal). C and D are adjacent sections to A and B, respectively, stained for acetylcholinesterase (AChE) and photographed with dark-field optics. AChE fibers are lost in regions corresponding to neuronal dropout, particularly in the proximal portion of CA1, as shown by the arrowheads *in B and D. The small white* stars *in D show the location of the granule cell layer. In bright-field AChE-stained sections E and F, the dentate granule cell layer* (stars) *and molecular layer of the dentate gyrus are shown in the nonepileptic specimen (E) and in the patient with temporal lobe epilepsy (F).* Small arrows *in F show preserved AChE-rich hilar neurons, despite prominent AChE fiber loss in the CA4 region. The* double-headed arrows *in E and F indicate the width of the molecular layer of the dentate gyrus. As demonstrated here, the distribution of AChE fibers in the molecular layer of the dentate gyrus is consistently altered in epileptic patients with Ammon's horn sclerosis. The apparent increase of AChE staining in the outer parts of the molecular layer in the epileptic patients cannot be interpreted because of the longer autolysis time in the nonepileptic tissue (see Methods section).*


A
CA1
CA4
CA1
S
B
C
D
E
F
CA4

somatostatin [15]. In all 7 specimens that we examined, these AChE-positive hilar neurons appeared to represent a population of cells that is relatively resistant to the pathological changes associated with hippocampal sclerosis.

AChE-rich fibers provide a reasonably good marker for incoming cholinergic (choline acetyltransferase-containing) pathways in the hippocampus [16–18]. The cell bodies for these afferents are located in the septal and diagonal band nuclei of the basal forebrain. In all 7 specimens, AChE staining was lost or diminished over the areas of pyramidal cell loss within Ammon's horn (see Figure, B, D, and E). This finding may reflect a general destruction of all neuronal elements in the regions of pyramidal cell dropout, including the afferent axons that normally synapse on the pyramidal cells. Alternatively, the primary lesion may be confined to pyramidal neurons and the loss of AChE fibers in these regions may represent retrograde transsynaptic degeneration of afferent axons, perhaps related to an interference in the delivery of essential trophic factors.

In rodents and monkeys, the majority of cholinergic afferents to the hippocampus originate in the septum and the nucleus of the diagonal band and synapse on the pyramidal cells of Ammon's horn and within the molecular layer of the dentate gyrus [14, 18–20]. When the fornix (containing septohippocampal axons) is lesioned, septal cholinergic neurons die because of their dependency on nerve growth factor (NGF) retrogradely transported from the target cell region in the hippocampus [21–24]. There are no data to suggest how extensive the depletion of target cells must be before degeneration of septal cholinergic neurons occurs. However, the death of pyramidal neurons in hippocampal sclerosis and the associated AChE fiber loss may interfere with the production and transport of NGF, causing retrograde cell degeneration within the septum and diagonal band nuclei. In turn, this may lead to further loss of efferent cholinergic projections, including those destined for the molecular layer in the dentate gyrus. This mechanism offers one explanation for the loss of AChE staining in the dentate gyrus despite the absence of marked cell loss among the granular neurons of the dentate in our specimens.

In the rat, repetitive electrical stimulation of limbic pathways has produced evidence of synaptic reorganization of mossy fibers in the molecular layer of the dentate gyrus [25]. Preliminary observations suggest that hippocampal sclerosis in TLE patients may also be associated with an enhancement of zinc-containing mossy fibers in the supragranular section of the dentate molecular layer [26, 27]. This reinnervation may be occurring in an attempt to repopulate the synaptic sites that would be denuded as a consequence of the loss of AChE fibers, as demonstrated by our observations.

It is tempting to interpret the apparent increase in staining of the outer portion of the molecular layer of the dentate gyrus (see Figure, F) as evidence for hyperinnervation, but this is difficult to substantiate using inherently nonquantitative histochemical techniques. Further efforts are under way to clarify this possibility. However, our data clearly suggest that in epileptic patients with hippocampal sclerosis, the distribution of AChE-rich (presumably cholinergic) afferents becomes markedly altered. It remains to be determined whether this pathological pattern is a result or a cause of the epileptic activity and whether it triggers a secondary reorganization of limbic circuitry, which may, in turn, contribute to the hormonal, cognitive, and behavioral changes that occur in some patients with temporal lobe epilepsy [28–32].

Dr Green was supported by the Lennox Fellowship of the American Epilepsy Society and the Penfield Fellowship of the Epilepsy Foundation of America. This project was also supported in part by a Javits Neuroscience Investigator Award (MMM). We thank Drs Anna Sotrel (neuropathologist at Beth Israel Hospital), William Q. Sturner, and Kristin Sweeney (of the Rhode Island Medical Examiner's Office), who provided autopsy tissue used in this study.

Presented in part at the 17th Annual Meeting of the Society for Neuroscience, New Orleans, LA, November 19, 1987.

References

1. Engel JE, ed. Surgical treatment of the epilepsies. New York: Raven Press, 1987
2. Blume HW, Schomer DL. Surgical approaches to epilepsy. Ann Rev Med 1988;39:301–313
3. Green RC, Adler JR, Erba G. Epilepsy surgery in children. J Child Neurol 1988;3:155–166
4. Babb TL, Brown WJ. Pathological findings in epilepsy. In: Engel JE, ed. Surgical treatment of the epilepsies. New York: Raven Press, 1987:511–540
5. Meldrum BS, Brierley JB. Neuronal loss and gliosis in the hippocampus following repetitive epileptic seizures induced in adolescent baboons by allylglycine. Br Res 1972;48:361–365
6. Nadler JV. Kainic acid as a tool for the study of temporal lobe epilepsy. Life Sci 1981;29:2031–2042
7. Cavanagh JB, Meyer A. Aetiological aspects of Ammon's horn sclerosis associated with temporal lobe epilepsy. Br Med J 1956;2:1403–1407
8. Mouritzen-Dam A. Hippocampal neuron loss in epilepsy and after experimental seizures. Acta Neurol Scand 1982;66:601–642
9. Babb TL, Brown WJ, Pretorius J, et al. Temporal lobe volumetric cell densities in temporal lobe epilepsy. Epilepsia 1984; 25:729–740
10. Tauck DL, Nadler JV. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid–treated rats. J Neurosci 1985;5:1016–1022
11. Spiers PA, Schomer DL, Blume HW, et al. Temporolimbic epilepsy and behavior. In: Mesulam M-M, ed. Principles of behavioral neurology. Philadelphia: Davis, 1985:289–326
12. Andermann F. Identification of candidates for surgical treatment of epilepsy. In: Engel JE, ed. Surgical treatment of the epilepsies. New York: Raven Press, 1987:51–70

13. Green RC, Mesulam M-M. Acetylcholinesterase fiber staining in the human hippocampus and parahippocampal gyrus. J Comp Neurol 1988;273:488–499
14. Mesulam M-M, Mufson EJ, Levey AI, et al. Atlas of cholinergic neurons in the forebrain and upper brainstem of the macaque based on monoclonal choline acetyltransferase immunohistochemistry and acetylcholinesterase histochemistry. Neuroscience 1984;12:669–686
15. Mesulam M-M, Dichter M. Concurrent acetylcholinesterase staining and g-aminobutyric acid uptake of cortical neurons in culture. J Histochem Cytochem 1981;29:306–308
16. Fonnum F. Topographic and subcellular localization of choline acetyltransferase in the rat hippocampal region. J Neurochem 1970;17:1029–1037
17. Matthews DA, Salvaterra PM, Crawford GD, et al. Distribution of choline acetyltransferase positive neurons and terminals in the hippocampus. Soc Neurosci Abstr 1983;9:79
18. Amaral DG, Eckenstein F. An immunohistochemical analysis of the cholinergic innervation of the monkey hippocampal formation using a polyclonal antiserum to choline acetyltransferase. Soc Neurosci Abstr 1986;12:442
19. Lewis PR, Shute CCD. The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. Brain 1967;90:521–542
20. Mellgren SI, Srebro B. Changes in acetylcholinesterase and distribution of degenerating fibers in the hippocampal region after septal lesions in the rat. Brain Res 1973;52:19–36
21. Hefti F. Nerve growth factor promotes survival of septal cholinergic neurons after fimbrial transections. J Neurosci 1986;6: 2155–2162
22. Crutcher KA, Collins F. Entorhinal lesions result in increased nerve growth factor–like growth-promoting activity in medium conditioned by hippocampal slices. Brain Res 1986;399:383–389
23. Kromer LF. Nerve growth factor treatment after brain injury prevents neuronal death. Science 1987;235:214–216
24. Hsiang J, Wainer BH, Shalaby IA, et al. Neurotrophic effects of hippocampal target cells on developing septal cholinergic neurons in culture. Neuroscience 1987;21:333–343
25. Sutula T, Xiao-Xian H, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. Science 1988;239:1147–1150
26. Sutula T, Cascino G, Cavazos J, et al. Hippocampal synaptic reorganization in partial complex epilepsy: evidence for mossy fiber sprouting in epileptic human temporal lobe. Ann Neurol 1988;24:134–135
27. Babb TL, Kupfer WR, Pretorius JK. Synaptic reorganization of mossy fibers into inner molecular layer in human epileptic fascia dentata. Soc Neurosci Abstr 1988;14:881
28. Bear D, Fedio P. Quantitative analysis of interictal behavior in temporal lobe epilepsy. Arch Neurol 1977;34:454–467
29. Weiser HG. Depth recorded limbic seizures and psychopathology. Neurosci Biobehav Rev 1983;7:427–440
30. Mendez MF, Cummings JL, Benson DF. Depression in epilepsy: significance and phenomenology. Arch Neurol 1986;43: 766–770
31. Engel J Jr, Caldecott-Hazard S, Bandler R. Neurobiology of behavior: anatomic and physiologic implications related to behavior. Epilepsia 1986; 27:S3–13
32. Schachter SC. Hormonal considerations in women with seizures. Arch Neurol 1988;45:1267–1270