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Enhanced GABAergic inhibition preserves hippocampal structure and function in a model of epilepsy

(perforant pathway stimulation/excitotoxicity/somatostatin/vigabatrin)

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ABSTRACT **Extensive electrical stimulation of the perforant pathway input to the hippocampus results in a characteristic pattern of neuronal death, which is accompanied by an impairment of cognitive functions similar to that seen in human temporal lobe epilepsy. The excitotoxic hypothesis of epileptic cell death [Olney, J. W. (1978) in *Kainic Acid as a Tool in Neurobiology*, eds. McGeer, E., Olney, J. W. & McGeer, P. (Raven, New York), pp. 95–121; Olney, J. W. (1983) in *Excitotoxins*, eds. Fuxe, K., Roberts, P. J. & Schwartch, R. (Wenner-Gren International Symposium Series, Macmillan, London), Vol. 39, pp. 82–96; and Rothman, S. M. & Olney, J. W. (1986) *Ann. Neurol.* 19, 105–111] predicts an imbalance between excitation and inhibition, which occurs probably as a result of hyperactivity in afferent pathways or impaired inhibition. In the present study, we investigated whether the enhancement of γ-aminobutyric acid (GABA)-mediated (GABAergic) inhibition of neurotransmission by blocking the GABA-metabolizing enzyme, GABA transaminase, could influence the histopathological and/or the behavioral outcome in this epilepsy model. We demonstrate that the loss of pyramidal cells and hilar somatostatin-containing neurons can be abolished by enhancing the level of synaptically released GABA, and that the preservation of hippocampal structure is accompanied by a significant sparing of spatial memory as compared with placebo-treated controls. These results suggest that enhanced GABAergic inhibition can effectively block the pathophysiological processes that lead to excitotoxic cell death and, as a result, protect the brain from seizure-induced cognitive impairment.**

Human limbic epilepsy is associated with learning and memory impairment (1–3) and sclerotic changes in the hippocampal formation (4, 5). Hippocampal cell loss is most prominent in the pyramidal cell layers of the CA1 and CA3 areas but also has been found in other subfields. In recent years, it has been shown that the local interneurons, especially those in the hilus of the dentate gyrus, are also affected in patients with focal limbic seizures. These cells include somatostatin- and neuropeptide Y-containing cells and mossy cells (6–8), whereas a population of nonpyramidal cells visualized by immunostaining for glutamic acid decarboxylase seems to have remained unaffected in the human epileptic hippocampus (9).

During epileptic seizures of limbic or cortical origin, epileptic activity spreads to the hippocampus mainly through the perforant pathway (PP), which represents the major route for excitatory input from the entorhinal cortex into the hippocampus. In an animal model of epilepsy, stimulation of the PP has been used to mimic the hippocampal effects of limbic seizures or status epilepticus (10–12). After sustained PP stimulation, the hippocampal cellular damage resembles that associated with human temporal lobe epilepsy (11–15) and is also accompanied by cognitive impairment as measured by the Morris water maze test (14, 15). The pyramidal cells in the CA1 and in the CA3c areas degenerate, whereas CA3a, CA3b, and CA2 pyramidal cells and dentate granule cells remain relatively well preserved (4, 5, 13). Hilar interneurons containing somatostatin (SS) have been shown to be exceptionally vulnerable, while other populations of nonpyramidal cells are quite resistant in this model of epilepsy (12). Pretreatment with glutamate receptor antagonists has been shown to alleviate the stimulation-induced pyramidal cell damage and interictal spiking activity (14, 15).

The aim of the present experiments was to demonstrate whether an increase in γ-aminobutyric acid (GABA)-mediated (GABAergic) inhibition of neurotransmission can prevent seizure-induced neuronal damage in the hippocampus and the associated spatial memory impairment. GABAergic inhibition was enhanced by γ-vinyl-GABA (vigabatrin, VGB), which elevates the GABA levels in the brain by blocking the GABA-metabolizing enzyme, GABA transaminase (16–18).

METHODS

The PP stimulation lasting 60 min (with 20 Hz, 0.1-ms duration, and 2-mA current of each stimuli) was applied to freely moving adult rats by using a modification of the method of Sloviter (10–12). One group of rats received 500 mg of VGB per kg of body weight intraperitoneally as a pretreatment 24 hr before the stimulation, and the other group received the same amount of saline as placebo treatment. Electroencephalograms (EEG) were recorded before the injections (baseline recordings) and again 6–9 days after the stimulation (follow-up recordings). For the study of spatial memory, the rats were tested in the Morris water maze 7–10 days after the stimulation and then were perfused intracardially for histology 14–18 days after the stimulation.

Male Wistar rats (n = 31) were used in the study. The animals were anesthetized with intraperitoneal sodium pentobarbital and inserted into a stereotaxic frame (lambda and bregma in the same horizontal level). For EEG recordings, pairs of insulated stainless steel electrodes (1.0-mm tip separation) were implanted bilaterally into the hippocampus (4.1 mm posterior, 2.6 mm lateral, and 3.6 mm ventral to the bregma) with the lower tip at the upper granular cell layer of

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Abbreviations: GABA, γ-aminobutyric acid; SS, somatostatin; VGB, vigabatrin; PP, perforant pathway; EEG, electroencephalogram; SS-I, SS immunoreactive.

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the dentate gyrus and the upper tip at the pyramidal cell layer of the CA1 area. A similar pair of electrodes (but with 0.5-mm tip separation) was implanted in the angular bundle (7.0 mm posterior, 4.5 mm lateral, and 4.1 mm ventral to the bregma) for stimulating the PP. Two stainless steel watch screws were inserted bilaterally as indifferent and ground electrodes in the parietal skull above the cerebellum. The electrodes were fixed with dental acrylate.

After a recovery period of 2 weeks, the hippocampal EEG and evoked potentials were recorded. Only the rats showing evoked population spikes in the dentate gyrus were chosen for PP stimulation; the others were then used as controls with electrodes in the Morris water maze test and in the histological evaluation. Rats with acceptable population spikes were divided into two groups: one group received vigabatrin, and the other received physiological saline intraperitoneally 24 hr before stimulation. The EEGs were recorded by a polygraph (Grass 78, 7P511 amplifiers). Quantification of the epileptiform activity was made manually from the charts, in which the behavioral correlates of the rats were also simultaneously recorded.

After the recording of the control EEGs, the rats were tested in the Morris water maze for 5 days (for the detailed description of the method used, see ref. 19). The daily test consisted of 10 trials (60 sec each). The location of the platform was changed daily so that on days 1, 3, and 5 it was in quadrant 1, and on days 2 and 4 it was in quadrant 3 (on the opposite side of the maze pool).

For the intracardial perfusion, the rats were deeply anesthetized 14–18 days after the stimulation. They were perfused first with saline for 2 min followed by a 30-min perfusion with a fixative containing 0.05% glutaraldehyde, 4% paraformaldehyde, and 0.2% picric acid in 0.1 M phosphate buffer. Then the brains were removed from the skull and the blocks of the dorsal hippocampus and overlying neocortex were dissected. After that, the blocks were immersed in 10% and 20% sucrose in 0.1 M phosphate buffer until they sank and were frozen in liquid nitrogen and thawed before being sectioned on a Vibratome at 60 μm. Alternate sections were processed for silver impregnation and immunocytochemistry.

The silver impregnation and immunocytochemical procedures have been described (20). For the detection of SS, a monoclonal mouse antibody (Soma 8, ref. 21) raised to cyclic SS was used. The antibody recognizes the C-terminal portion of the peptide in either cyclic or linear form and was used at 1 μg/ml of TBS (0.05 M Tris in buffered saline, pH 7.4) for 24–48 hr at 4°C (for a more detailed description, see ref. 20).

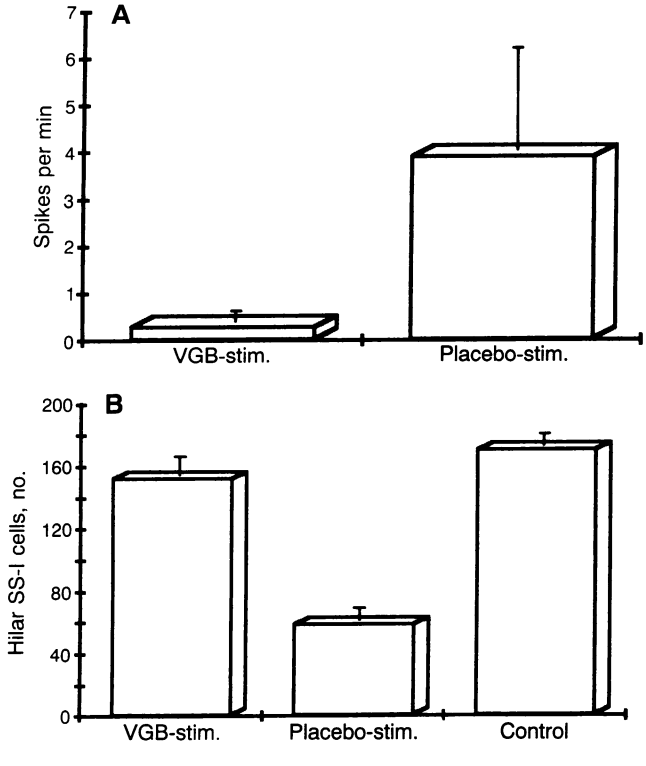

FIG. 1. (*A*) Number (group mean ± SEM) of interictal spikes 1 week after PP stimulation ($P < 0.05$ between groups by the Mann–Whitney *U* test). (*B*) Number (group mean ± SEM) of SS-I cells per mm^2 in the hilus of the dentate gyrus 2 weeks after PP stimulation ($P < 0.01$ between placebo- and VGB-treated group by the Mann–Whitney *U* test). VGB-stim, VGB-treated stimulated animals; Placebo-stim, placebo-treated stimulated animals; Control, nonstimulated controls.

RESULTS

At the beginning of stimulation, all rats exhibited evoked population spikes immediately and had wet-dog-shakes (WDS) within 2 min. The evoked responses of the hippocampus were monitored during the whole stimulation session by an oscilloscope. Most of the rats showed spiking for more than 50% of the duration of the stimulation session (six of nine rats in the VGB-pretreated group and seven of eight in the placebo-treated group). All rats in both of these groups had partial seizures, and most of them also had generalized seizures (five rats in both groups) during the stimulation period, and five placebo-treated rats continued to exhibit seizures 10–20 min afterwards. The seizure types observed in the VGB-treated rats were similar and as strong as those of

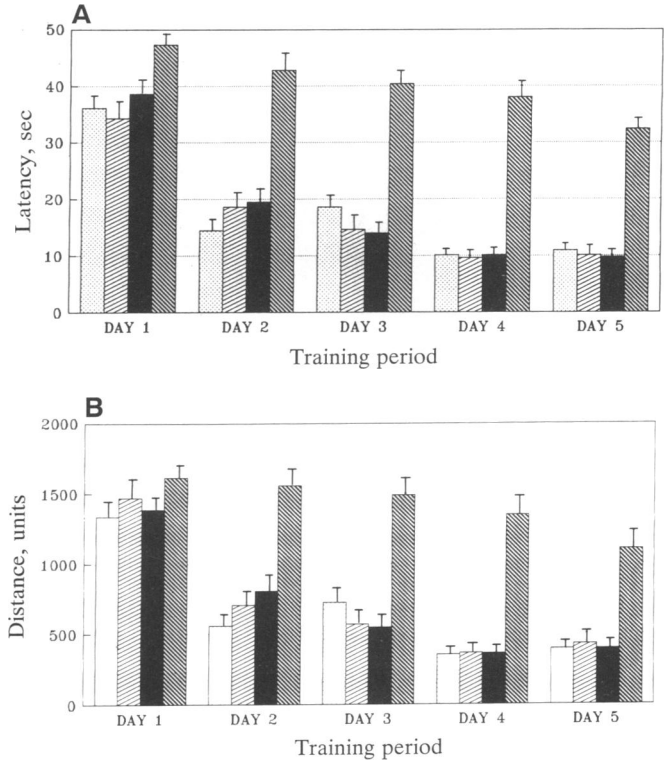

FIG. 2. Acquisition of the water maze task expressed as escape latency (sec) (*A*) and distance (units) (*B*) to a submerged platform. The groups of rats consisted of unoperated rats (▭), operated nonstimulated rats (■), PP-stimulated rats, which received either 500 mg of VGB per kg of body weight (■) or saline (placebo) (▨) before stimulation. The results are expressed as group means of the daily trials ± SEM. The analysis of variance revealed a group difference in both latency and distance on every training day [$F(3,256) = 17.54 - 39.72$, $P < 0.001$] except in the first training day [latency: $F(3,256) = 4.05$, $P = 0.019$; distance: $F(3,256) = 221$, $P = 0.112$].

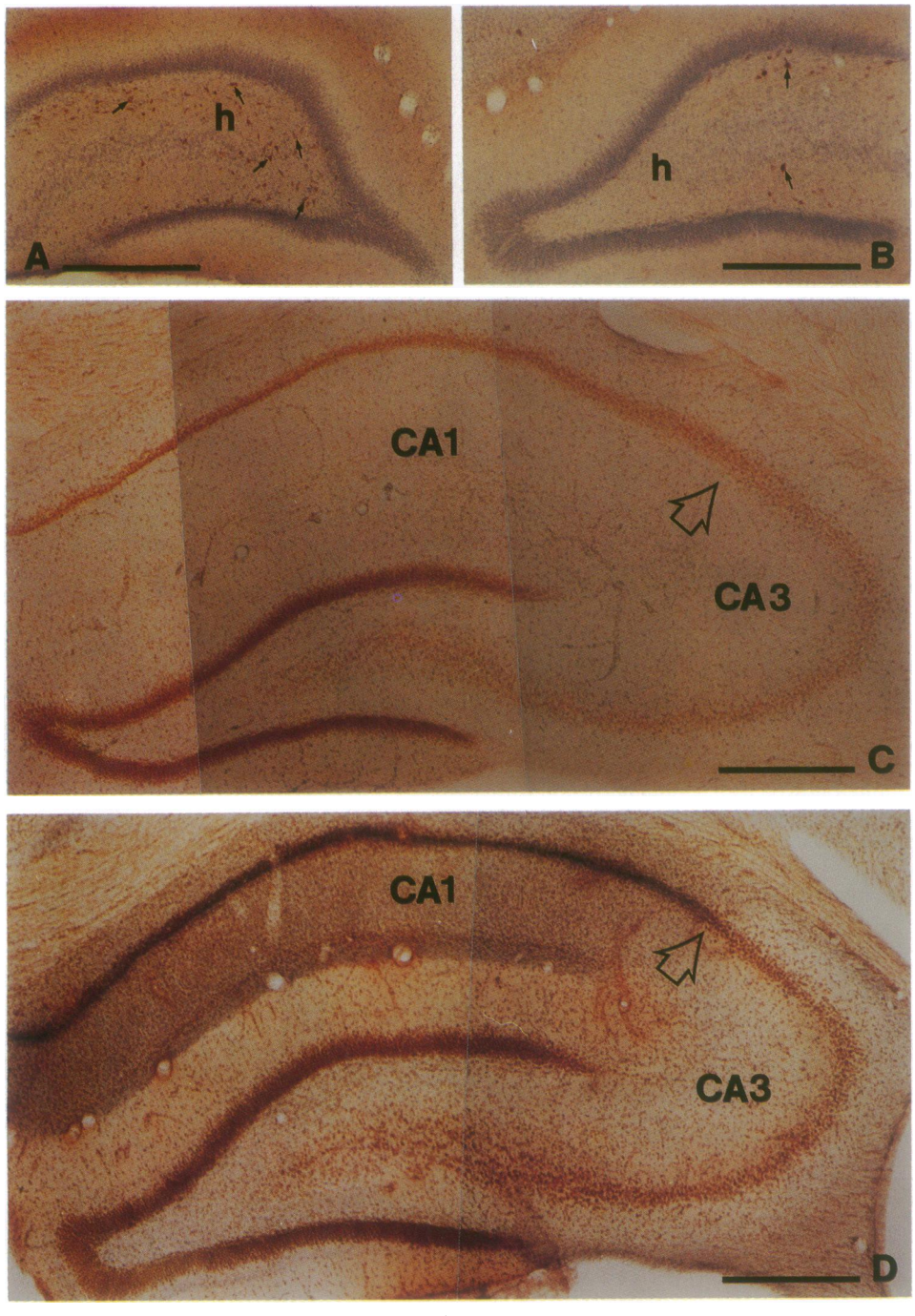

FIG. 3. (*A* and *B*) SS-immunostained sections of the dentate gyrus counterstained with cresyl violet from a PP-stimulated rat treated with VGB (*A*) or placebo (*B*). The frequency of SS-I neurons (brown; arrows) in the hilus of the VGB-treated animal appears normal, whereas in the placebo-treated animal, it is remarkably reduced. (*C* and *D*) Silver-impregnated sections. (*D*) Degenerated neurons (in black) are seen in the CA1 region of a PP-stimulated rat treated with placebo. (*C*) Degenerating neurons are rarely visible in VGB-treated animals. The open arrow marks the border region between CA1 and CA3. (Bars = 500 μm.)

the placebo-treated rats. However, the number and duration of seizures were higher in the placebo-treated group. Five of eight rats in this group and none in the VGB-treated group had a seizure duration that lasted more than 50% of the stimulation period.

In the follow-up EEGs, all rats in the placebo-treated group (n = 8) showed interictal spiking [maximum 17.6, minimum 0.13, mean 3.97, and median 1.39 spike(s) per min], but only four of nine rats in the VGB-treated group showed any spiking [maximum 1.21, minimum 0.0, mean 0.34, and median 0.09 spike(s) per min]. The difference was significant between the groups (Fig. 1*A*; for the criteria of spikes, see ref. 22).

During the 10 trials on day 1 of the Morris water maze test, no differences were found in learning ability between the two groups of controls or the PP-stimulated rats in the placebo- or VGB-treated groups. However, during the second day and each testing day after that (when the location of the platform was changed daily), the placebo-treated animals found the platform significantly slower ($P < 0.001$) than VGB-treated rats or normal controls, whereas the latter two groups did not differ from each other on any testing day (Fig. 2).

Three of five placebo-treated rats processed for histological analysis showed degeneration in the CA1 area and occasionally in the CA3c area [one grade 1, one grade 2, and one grade 3; the hemispheres were scored on the basis of CA1

neurons irreversibly damaged (see ref. 20): 0, normal; 1, up to 10%; 2, 10–50%; 3, >50%. Two placebo-treated stimulated rats did not show any degeneration in silver-impregnated sections. None of the rats in the VGB-treated group ($n = 7$) or in the normal control group had any degeneration either in the CA3 or the CA1 areas of the hippocampus (Fig. 3; $P < 0.05$ between the VGB- and placebo-treated groups by Fischer's exact test). The cell loss, when present, was always bilateral, although its grade varied between the two hemispheres.

There was also a marked reduction in the number of SS-immunoreactive (SS-I) neurons in the dentate gyrus of the PP-stimulated rats in the placebo-treated group. In the stimulated rats of the VGB-treated group, the number of SS-I neurons did not differ from that of the normal controls (Fig. 1*B* and Fig. 3). In the placebo-treated group, the reduction was more marked on the stimulated side.

DISCUSSION

The pattern of pyramidal cell death in the CA1 and CA3c areas agrees with our previous study (13). The differences in the stimulation protocol and the fact that our animals were awake during stimulation may be responsible for the difference between our results and those of Sloviter, who found more damage in the CA3 area after sustained PP stimulation of rats anesthetized with urethane (10–12). The reduced number of SS-I cells after PP-stimulation also agrees with our previous study and is in accordance with earlier results (12).

The hilar SS cells occupy a key position in the circuitry of the dentate gyrus. They have been shown to receive a convergent excitatory drive from mossy fiber collaterals and the perforant pathway (23), and they in turn terminate along the dendrites of the granule cells in close association with the entorhinal terminals (24, 25). This circuit may play a role in gating entorhinal excitation of the hippocampus as a function of granule cell discharge in the dentate gyrus (26). Malfunctioning of this gating mechanism may lead to the apparent decrease in inhibition (10–12) and to the interictal spiking noted in the present study.

However, by enhancing GABAergic inhibition through the systemic administration of VGB, we could prevent both pyramidal cell damage in the CA1 and CA3c areas and the disappearance of SS-I cells from the dentate gyrus. The finding that enhanced GABAergic inhibition will improve the neurological and histopathological outcome after seizure activity suggests that GABAergic inhibition may normally be responsible for limiting seizure progression and/or for protecting neurons from excitotoxic cell death. However, GABAergic inhibition may be unable to cope with the degree of hyperactivity reaching the hippocampus during limbic seizures in humans and in the present animal model of epilepsy.

We have speculated earlier in the PP-stimulation model that cell death is likely to be induced by an excitotoxic mechanism, which can be acute or delayed (13). The acute edema and ion influx may occur as a result of excessive stimulation of the excitatory amino acid receptors, causing depolarization and opening voltage-dependent ion channels, most importantly those permeable to calcium. Alternatively, hyperactivity may result in consumptive hypoxia and ischemic depolarization, which also would lead to an influx of ions and water. The transiently increased intracellular concentration of free Ca^{2+} during the nonselective acute phase is likely to be responsible for the activation of the delayed mechanism, but only in the selectively vulnerable neurons. Once the delayed mechanism has been activated, increased inhibition is unlikely to be effective. Thus, the protective effect of the increased GABAergic inhibition must take place during the acute phase, probably by reducing afferent depolarization and the resulting hyperactivity and presumed consumptive hypoxia. The stimulation-induced spiking in the dentate gyrus is not reduced by VGB during stimulation; thus, the enhanced inhibition is likely to stop the spread of hyperactivity along a later stage in the intrahippocampal trisynaptic pathway.

The present results open up new perspectives in the treatment of human limbic seizures by demonstrating that an enhancement of the level of synaptically released GABA can protect hippocampal structure and function in a model of epilepsy. Blocking seizure progression in this manner is most likely to improve the neurological outcome in patients with recurrent complex partial seizures.

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