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Experimental Status Epilepticus Alters γ -Aminobutyric Acid Type A Receptor Function in CA1 Pyramidal Neurons

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Abstract

There is a reduction of γ -aminobutyric acid (GABA)-mediated inhibition in the CA1 pyramidal region of the hippocampus during status epilepticus (SE). The cellular basis of this loss of GABA-mediated inhibition is not known. This study tested the possibility that GABA type A (GABA_A) receptor function in CA1 pyramidal neurons was reduced or blocked during SE, at least in part by postsynaptic cellular mechanisms. GABA_A receptor currents (I_{GABA}) were studied by whole-cell patch-clamp techniques in CA1 pyramidal neurons acutely dissociated from rats undergoing lithium/pilocarpine-induced limbic status epilepticus (SE neurons) and from naive rats (naive neurons). SE neurons had more depolarized resting membrane potential (−17.3 mV) compared with naive neurons (−56 mV). I_{GABA} was absent in 47% of SE neurons and reduced in 55% of the remainder, compared with naive neurons. The reduction in I_{GABA} in SE neurons resulted from a combination of factors, including reduced potency and reduced efficacy of GABA in activating chloride channels, and diminished driving force for the GABA-induced chloride currents once activated. These postsynaptic cellular mechanisms resulted in a net reduction or loss in GABA-mediated inhibition and may explain previous *in vivo* findings reporting a loss of inhibition in hippocampus during limbic SE.

Convulsive status epilepticus (SE) is a medical emergency that can result in brain damage or even death. While death following treated SE is usually a consequence of the underlying cause of SE, mortality is also related to seizure duration [1]. Neurologic morbidity including neuropsychological dysfunction [2], increased risk for subsequent seizures, and a low incidence of spontaneous remission of underlying epilepsy is associated with SE [2]. Improvement in prognosis of patients with SE will result from a better understanding of the pathophysiology of SE. A key to understanding the pathophysiology of SE was the development and validation of various animal models of this disorder. One such model initially described by Honchar and colleagues [3] was based on cholinergic stimulation of the brain by sequential administration of lithium and pilocarpine. Treiman and associates [4] found that a predictable sequence of electroencephalographic (EEG) changes that occurred during the course of human convulsive SE was replicated in lithium/pilocarpine-induced SE in rats [5]. In addition, the profile of efficacy of anticonvulsants in an animal model of SE was similar to that in human convulsive SE [6].

There is a critical role for loss of γ -aminobutyric acid type A (GABA_A)-mediated inhibition in the CA1 region of the hippocampus in partial onset SE in experimental animals [7]. SE induced by lithium and pilocarpine resulted in reduction of the number of GABA_A receptors (GABARs) in the rat forebrain; and injection of a combination of lithium and pilocarpine resulted in reduction of GABA_A-mediated inhibition in the CA1 region of the hippocampus

[8]. Loss of GABA_A-mediated inhibition in the CA1 region of the hippocampus was demonstrated in several other models of SE [9–11].

The cellular basis of loss of GABA-mediated inhibition in the CA1 region of the hippocampus during SE remains unknown. In this report, the possibility that the reduction of GABA-mediated inhibition in the CA1 region of the hippocampus observed during SE is at least partly due to a postsynaptic reduction of GABAR currents in CA1 pyramidal neurons is directly addressed. Several possible changes in CA1 pyramidal neurons could result in a net reduction of GABAR currents including a reduced number of GABARs available for binding, a reduced efficacy of GABA in opening the ion channel after binding, or a reduced driving force for GABAR current due to an altered Cl⁻ reversal potential.

Methods

SE was induced by the method of Honchar and colleagues [3]. Eighteen- to 28-day-old rats of both sexes weighing 50–100 gm were injected intraperitoneally with 3 mEq/kg LiCl followed 20 hours later with 25 mg/kg pilocarpine. Naive littermates were used as controls. Following pilocarpine injection, the rats were observed continuously for occurrence of behavioral seizures. Time to onset of behavioral seizures was recorded and seizures were allowed to continue for 45 minutes, thereby establishing SE in each animal. SE was terminated by ether anesthesia and rats killed.

Pyramidal neurons from the CA1 region of the hippocampus were isolated from brains of naive rats (naive neurons) and rats undergoing SE (SE neurons) according to a protocol modified from Kay and Wong [12,13]. The brain was dissected free, chilled to 4°C for 1 minute in a bicarbonate buffer (solution composed of the following [in mM]: NaCl 120, KCl 5, CaCl₂ 1.5, MgCl₂ 1, D-glucose 25, NaHCO₃ 25) and the region containing the hippocampus was blocked. After blot drying, the brain was mounted on a vibratome stage and 500-μm coronal sections containing hippocampus were cut. Hippocampal sections were incubated in Sigma type XXIII protease enzyme from *Aspergillus oryzae* in a piperazine-*N,N'*-bis(2-ethanesulphonic acid) (PIPES)-buffered incubation medium at 32°C for 30 minutes. The PIPES-buffered solution contained the following (in mM): NaCl 120, KCl 5, CaCl₂ 1.5, MgCl₂ 1, D-glucose 25, PIPES 20 (pH 7.0). After a minimum of 30 minutes of recovery, the CA1 region of the hippocampus was dissected from the rest of the section under a microscope and cut into 0.5-mm³ chunks. These chunks were triturated through fire-polished glass pipettes and the isolated neurons were plated on 35-mm sterile plastic petri dishes.

Whole-cell attached perforated patch recordings of GABA currents were made from SE neurons or from naive neurons. The acutely isolated neurons were studied on the stage of an inverted microscope equipped with Hoffman modulation contrast optics. Patch electrodes of 4 to 8 MΩ resistance were pulled and fire polished and filled with recording solution. The recording solution contained the following (in mM): Trizma phosphate (dibasic) 110, Trizma base 28, ethylene glycol bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid 11, MgCl₂ 2, CaCl₂ 0.5, pH 7.40. The “perforated patch” method of Horn and Marty [14] was employed. The recording solution contained amphotericin, a pore-forming antibiotic, in a concentration of 300 μg/ml. Amphotericin was excluded from the electrode tip since it interfered with formation of seals. The recording medium contained the following (in mM): NaCl 155, KCl 3, MgCl₂ 1, CaCl₂ 3, HEPES-Na⁺ 10, and tetrodotoxin 0.0005, pH 7.4. All recordings were made at room temperature. Patch electrodes were pulled on a Flaming Brown P-87 model puller and fire polished on Narishige forge.

Recording was initiated by lowering the electrode to the neuron and forming a high-resistance seal by application of gentle suction. Following seal formation, amphotericin diffused to the tip and inserted into the neuronal membrane, giving an access resistance of 5 to 15 M Ω . The GABA current recordings were conducted in whole-cell voltage-clamp mode using an Axopatch 1D amplifier and filtered at 3 kHz with an eight-pole Bessel filter before digitization. The data were displayed on-line on a Gould chart recorder.

In voltage-clamp technique, the voltage is held constant (i.e., “clamped”) while the current flowing through the membrane is measured. Once the membrane potential is clamped the membrane conductance change produced by GABA is linearly proportional to the measured current. Voltage-clamp techniques traditionally assigned the role of current measurement and voltage clamping to two pipettes. The “whole-cell patch” voltage clamp is a technique wherein only one micropipette is used full time for both voltage clamping and current recording. A method known as series-resistance compensation attempts to eliminate the aberrations arising when one pipette is made to perform the task of two. For series-resistance compensation to work, the micropipette resistance must be low compared with the impedance of the cell. To achieve low-access resistance a blunt pipette is tightly sealed on to a *patch* of membrane. The patch of membrane is subsequently ruptured so that the micropipette provides a low-resistance access to the *whole cell*, hence the term whole-cell patch clamp. A disadvantage of rupturing the cell membrane in the whole-cell patch-clamp technique is that cytoplasm is dialyzed out of the cell over time and second messenger cascades may be disturbed. In this study a perforated patch technique was used to study acutely isolated neurons. The pipette solution contained the antibiotic amphotericin B. Amphotericin forms small “perforations” (channels) on the cell membrane allowing low-resistance electrical access to the cell, without allowing the intracellular contents to be dialyzed out. Perforated patch technique offers the advantage over “ruptured patch” technique in that intracellular contents are minimally disturbed by the recording technique.

GABA-containing solutions were applied by a “sewer pipe” concentration clamp system consisting of Teflon tubes cemented together in a flat array [13]. This technique consisted of applying drugs to the cells via a 1-mm-diameter Teflon pipe. Since the pipe diameter was large compared with the cell diameter, the entire cell was bathed in a single fixed drug concentration achieving a “concentration clamp.” Solution changes were accomplished by moving the perfusing system laterally so that the barrel covering the recorded cell was changed from one to another, each barrel constantly flowing with one or another solution at a rate of 0.5 ml/hr. No cross contamination was evident between the tubes, since the response to GABA was completely and rapidly terminated by moving the assembly. After recording from each cell the medium in the entire plate was changed. A maximum of three neurons were recorded from each plate. GABA was applied for 2 seconds and there was an interval of 2 minutes between two applications of the drug. The concentration–response curve for GABA was obtained for GABA concentrations ranging from 1 to 300 μ M at 0.5 log₁₀ intervals. The reversal potential for GABAR currents was determined by applying 3 μ M GABA to neurons voltage clamped at various potentials between –60 mV and + 30 mV at 10-mV intervals.

Results

Behavioral Effects

The behavioral progression of seizure activity was similar to that described in mature adult rats. Staring and inactivity started 5 to 10 minutes after the injection of pilocarpine. This was followed by automatisms including wet dog shakes, grooming, and chewing. This activity progressed to forelimb myoclonus with rearing and falling followed by constant forelimb

clonus. There was a shorter latency (10–15 minutes) to the onset of convulsive seizures than that observed in mature adult animals.

Morphology and Resting Membrane Potential

The primary dendrites were preserved in both naive and SE neurons. Viable neurons were pyramidal shaped, agranular, and three dimensional (“shadowed”) under modulation contrast optics. SE neurons were morphologically indistinguishable from naive neurons (Fig 1). One to 3 minutes after formation of a gigohm seal, access to intracellular currents was obtained as indicated by a reduction in input resistance, an increase in capacitance, and the recording of a stable resting membrane potential. The resting membrane potential of the neurons was measured in current clamp mode. The membrane potential obtained 1 minute after establishing access was recorded. The resting membrane potential of 23 naive neurons from 8 animals ranged from -78 to -25 mV and had a mean value of -56 ± 5 mV. Resting membrane potential recorded in 32 SE neurons from 12 animals ranged from -62 mV to 0 mV with a mean value of -17.29 ± 2.9 mV. However, resting membrane potential of SE neurons was asymmetrically distributed about the mean (see frequency histogram of the distribution, Fig 2). The median resting potential for SE neurons was -10 mV in contrast to -55 mV for naive neurons.

GABA Concentration–Response Curve in SE Neurons

Since GABA activates both GABAR and GABA_B receptors, it was important to determine whether GABA_B receptors were activated in acutely isolated hippocampal neurons. In preliminary experiments, GABA currents in naive neurons were completely blocked by picrotoxin (not shown); GABA produced a monophasic response in all experiments (Fig 3). Both of these observations suggested that the current elicited by GABA application was due to activation of GABARs and not GABA_B receptors.

The potency and efficacy of GABA for activating GABAR was studied by generating a GABA concentration, GABAR current response curve by applying 1 to 100 μ M GABA to neurons (see Fig 3). Each neuron used for analysis was exposed to at least two concentrations of GABA, one of which was 100 μ M. GABAR currents elicited by various GABA concentrations were normalized to the current elicited by 100 μ M GABA. The concentration–response curve generated from 12 naive neurons is displayed in Figure 4A. The data were fitted to a sigmoidal dose–response (variable slope) equation by least-squares method, and the equation for this fit yielded an EC_{50} , for GABA of 4.01 ± 1.37 μ M.

Concentration–response data were obtained from 12 of the 19 SE neurons that showed a response to GABA. The GABAR current elicited by 3 μ M GABA in SE neurons was used to screen for changes in sensitivity of GABAR in these neurons to GABA. This concentration of GABA was close to EC_{50} in naive neurons and was above threshold for all naive cells. SE neurons showed a wide range of responses to 3 μ M GABA, ranging from being unresponsive to eliciting 90% of the evoked current at 100 μ M GABA (Fig 4B). The mean elicited current in response to 3 μ M GABA was 0.21 ± 0.11 of that evoked in response to 100 μ M GABA in SE neurons in contrast to 0.54 ± 0.09 in naive neurons. However, as displayed in Figure 4B, for greater than 50% of these SE neurons, 3 μ M GABA was subthreshold for eliciting any current. Thus, the median for response to 3 μ M GABA in GABA-responsive SE neurons was 0 , in contrast to 0.55 for the naive neurons. These findings suggested a shift in the GABA concentration–response relationship to the right in a majority of GABA-responsive SE cells. Consistent with this finding of a SE-induced rightward shift of the concentration–response relationship, maximal currents were obtained at 300 μ M GABA in three SE neurons in contrast to 10 μ M GABA in naive neurons.

GABAR Current Reversal Potential in SE Neurons

The driving force for GABAR currents is provided by the difference between the potential at which the cell is voltage clamped and the Cl^- -reversal potential. Since the GABA-induced currents in these cells were entirely GABAR mediated, the GABA reversal potential was the Cl^- -reversal potential. The GABA reversal potential was determined for both naive and SE neurons. The GABAR currents from seven naive neurons voltage clamped at various potentials were pooled and computer fitted to a straight line that crossed the abscissa at -35 mV (Fig 5A). Thus, the GABA reversal potential (hence Cl^- -reversal potential) in naive neurons was -35 mV. The protocol for measuring the GABAR current reversal potential in SE neurons was modified when SE cells had an elevated threshold for GABAR currents. In these neurons, GABA concentration sufficient to produce a one-half maximal response was used (Fig 5B). An additional measure of altered GABAR current driving force, the direction of current in response to GABA application with cell voltage clamped at -10 mV, was used to screen for changes in GABA reversal potential. At this potential all naive neurons responded to GABA by generating an outward current while nine of 19 SE neurons had inward currents.

The GABA reversal potential was formally determined in eight SE neurons. These SE neurons were divided into two subsets, neurons with a GABAR current reversal potential more depolarized than -10 mV and the remainder of SE neurons. Four of these eight SE neurons showed GABA reversal potential or more depolarized than -10 mV (see Fig 5B). The remaining four of the SE neurons showed GABAR current reversal potentials similar to that in naive neurons, ranging from -20 to -40 mV.

SE Neurons: GABA Unresponsive Cells

An attempt to measure GABAR current was made in 36 SE neurons from 12 animals. Several of the SE neurons showed no GABAR currents in response to GABA in the conditions used to record these currents in naive cells. These SE neurons were further tested by applying $300\text{ }\mu\text{M}$ GABA instead of $10\text{ }\mu\text{M}$ and remained unresponsive to this high concentration of GABA. Furthermore, when these neurons were voltage clamped at $+30$ mV, and exposed to $300\text{ }\mu\text{M}$ GABA, they remained unresponsive. These procedures again assessed the possibility that changes in the GABA reversal potential or the threshold for GABA response resulted in apparent unresponsiveness to GABA. Seventeen of a total of 36 SE neurons studied met all these criteria and were considered unresponsive to GABA. Morphologically, the GABA-unresponsive SE neurons were indistinguishable from the GABA-responsive SE neurons or the naive neurons. The mean resting membrane potential of the 17 GABA-unresponsive neurons was -16.6 mV. One explanation of the GABA unresponsiveness of these depolarized neurons was that they were insensitive to any neurotransmitter or even dead despite appearing viable under the microscope. To test this possibility, seven GABA-unresponsive SE neurons were tested for glutamate sensitivity. Six of these seven SE neurons showed an inward current in response to glutamate, the neuron voltage clamped at -25 mV (not shown).

Variability of SE Neurons

Eight SE neurons from a single animal were studied to distinguish between two possible explanations of the different results obtained, i.e., variability of electrophysiological characteristics in SE neurons was a consequence of either interanimal variability or intercellular variability within a single animal. Within SE neurons isolated from a single animal, the resting membrane potential varied between -3 mV and -56 mV, two neurons were not responsive to GABA, and of the responsive cells, three showed inward (depolarizing) GABA currents at -10 mV holding potential and two showed evidence of a rightward shift of the GABA concentration–GABAR current curve. These results

demonstrated that the variability of GABAR currents in SE neurons could be reproduced in neurons isolated from a single animal and, therefore, represented intercellular rather than interanimal variability.

Discussion

The primary conclusions of this study are (1) neurons can be acutely isolated preserving their morphology, and studied electrophysiologically after an acute neurologic insult, and (2) a wide range of changes in GABAR currents occurs in SE neurons. The most dramatic of these changes are (1) 47% of SE neurons become unresponsive to GABA, (2) 55% of SE neurons that retain GABA sensitivity have diminished sensitivity to it, and (3) Cl^- reversal potential is shifted close to 0 mV in 46% of GABA-responsive SE cells. In some SE neurons, however, the GABAR currents are similar to those in naive neurons. In addition, the resting membrane potential is depolarized in a majority of SE neurons when compared with naive cells.

In this study, a perforated patch technique was used to study acutely isolated neurons. Perforated patch technique offered the advantage over ruptured patch technique in that intracellular contents were minimally disturbed by the recording technique. Using the perforated-patch technique, the naive CA1 neurons had electrophysiological properties comparable with those recorded with the conventional whole-cell patch and sharp intracellular electrode techniques [13,15,16]. The resting membrane potential in these neurons was similar to that reported in other studies. The threshold concentration of GABA for eliciting a Cl^- current, EC_{50} , and the concentration required to elicit the maximal response was also similar to that previously reported [17]. Finally, GABA currents were purely GABAR currents with no GABA_B receptor current contribution, as has been reported by others [18]. The enzymatic isolation of CA1 pyramidal hippocampal neurons from animals undergoing SE offered several advantages over whole-animal and slice preparations. Postsynaptic neurons were studied directly by whole-cell patch-clamp recording techniques. The SE neurons could be adequately space clamped following truncation of the dendritic processes in the isolation procedure. Finally, GABA and other drugs were applied directly to the neurons in known, rigidly controlled concentrations to the cells.

There was large variability of electrophysiological effects of SE within the population of SE neurons. This variability parallels the variability of morphological changes in the CA1 region following SE. In vivo, some of CA1 pyramidal neurons die immediately after SE, other neurons show variable changes ranging from delayed death to morphological features of neuronal injury, to no change in morphology [19].

Eighteen- to 28-day-old rats were used in this study to facilitate acute isolation of CA1 pyramidal neurons. Because cell survival worsens with increasing age of the animals, and injury to neurons during isolation procedure could potentially confound the results of the study, animals were studied early in adulthood. Since the study focused on the GABARs, 18- to 28-day-old rats were chosen because it was the age by which the GABAR in the rat hippocampus becomes adult type. Laurie and colleagues [20] reported that GABAR subunit mRNA expression pattern in the rat hippocampus was identical to that of adults by postnatal day 18. The findings in this study were consistent with earlier studies performed on mature adult rats [7,8], suggesting that these results may apply to mature adults.

Reduction in GABAR Sensitivity for GABA

The complete loss of responsiveness to GABA in 47% of SE neurons was the most dramatic consequence of SE noted. This loss of responsiveness was not due to changes in the GABA

reversal potential or a diminished affinity of the receptor, since GABAR currents could not be recorded even following application of 300 μ M GABA at a holding potential of +30 mV. These neurons were determined to be viable because of preserved morphological integrity, membrane potential, and responsivity to glutamate. There was also evidence of a rightward shift of the GABA concentration–response curve in GABA-responsive SE neurons; the threshold for GABAR currents was raised and peak currents required higher concentration of GABA in more than 50% of the neurons. The concentration–response curve for individual SE neurons could not be determined, since our drug application system allowed only application of a maximum of three different concentrations of GABA to any neuron. Since the responsivity of SE neurons showed a wide range and a one-tailed distribution from the median (see Fig 3), pooling of data from different neurons was not possible.

This reduced sensitivity of GABAR to GABA could represent diminished affinity of the receptor for GABA and a diminished efficacy of GABA in opening the Cl^- ionophore after binding to the receptor. We speculate that GABAR protein undergoes posttranslational modification during SE, producing the observed loss of affinity and activity of the receptor. Stimulation of second messenger systems may have resulted in a reduction of GABAR currents probably by phosphorylation of GABAR protein. Cyclic AMP and the adenylate cyclase activator forskolin decreased GABA-gated Cl^- flux in rat cortical synaptoneurosome [21]. The catalytic subunit cyclic AMP–dependent protein kinase (cPKA) when injected into cultured mouse spinal cord neurons reduced GABAR currents [22]. Activation of Ca^{2+} -phospholipid–dependent protein kinase (protein kinase C) reduced GABA-induced whole-cell currents in recombinant receptors expressed in *Xenopus* oocytes and kidney cells, and cultured sympathetic neurons [23].

Chloride Loading in SE Neurons

Since the entire GABA current was GABAR mediated, it was concluded that the GABA reversal potential was the Cl^- reversal potential. The GABA reversal potential in naive neurons was –35 mV, more depolarized than that reported using high-resistance sharp electrodes to record from CA1 pyramidal neurons in hippocampal slice preparation. This probably was a consequence of voltage clamping the neuron at –10 mV for the recording of GABA currents. Since the holding potential can alter the intracellular Cl^- concentration and the neurons were commonly held at –10 mV for recording GABA currents, only those SE neurons that had a GABA reversal potential more depolarized than –10 mV were considered Cl^- loaded.

The GABA reversal potential, and hence the Cl^- reversal potential, was shifted close to 0 mV in 47% of GABA-responsive SE neurons. This suggested that the intracellular Cl^- concentration in many SE neurons was markedly elevated, approaching the extracellular Cl^- concentration. Cl^- flux into neurons was determined by the driving force (the difference between the membrane potential and Cl^- equilibrium potential) and the resting Cl^- permeability of the neuron. In hippocampal pyramidal neurons, the resting Cl^- permeability was low [24]; however, small quantities of GABA sufficiently increased Cl^- permeability to allow intracellular Cl^- accumulation [18]. Several studies have demonstrated that the GABA reversal potential was very labile, being influenced by the membrane potential and application of GABA [18,24]. It was possible that CA1 pyramidal neurons were exposed to a significant amount of GABA during prolonged SE, which provided permeability for entry of Cl^- ions. Since the membrane potential of CA1 neurons was depolarized during SE, there was an adequate driving force for Cl^- entry.

The SE neurons show depolarization of the membrane potential for extended periods of time after the episode of SE. A similar phenomenon occurs in cultured hippocampal pyramidal neurons exposed to excitotoxic concentrations of glutamate [25,26]. This phenomenon was

dependent upon activation of NMDA receptors on the neurons. Activation of NMDA receptors on the hippocampal CA1 pyramidal neurons was believed to be responsible for the neuropathological changes in the brain during SE [19]. It was likely that SE caused protracted activation of NMDA receptors, resulting in the extended neuronal depolarization described in cultured hippocampal neurons.

Clinical Significance

Alteration of GABAergic inhibition has long been known to play a role in the pathogenesis of SE. The convulsant drugs penicillin, picrotoxin, and bicuculline cause SE by antagonizing GABAR-mediated inhibition. Furthermore, benzodiazepines enhance GABA-mediated inhibition and are the most potent and rapidly acting agents used for the treatment of SE. When the benzodiazepines fail, the barbiturates phenobarbital and pentobarbital, which also enhance GABA-mediated inhibition, are recommended for treatment of SE. Loss of GABAergic inhibition in the CA1 region of the hippocampus during SE has been repeatedly demonstrated [9,11,27]. The loss of inhibition in the hippocampus could be caused by diminished release of GABA or diminished postsynaptic GABAR responsiveness. While the release of GABA from neurons during SE has not been studied, amino acid neurotransmitter levels during SE have been measured. Walton and co-workers [28] have studied amino acid neurotransmitter levels in SE induced by lithium and pilocarpine during various stages of SE as determined by the EEG. GABA levels rise in early SE and continue to do so in the late stages. It is difficult to interpret these observations, since increased GABA levels could represent increased synthesis, diminished release, or diminished breakdown of this neurotransmitter.

This study demonstrated diminished postsynaptic GABAR responsiveness during SE. A reduction of GABA currents due to a loss of GABA responsivity, a rightward shift of the concentration–response curve, or a reduced or even reversed driving force could severely impair GABAR-mediated inhibition in CA1 region of hippocampus during SE. This could at least partly explain the observed loss of GABA-mediated inhibition during partial SE described previously [7]. Studies in the past have suggested that changes in the postsynaptic neuron and altered GABAR function occur during SE. There was reduction of ³H muscimol binding to synaptic plasma membranes of animals undergoing lithium/pilocarpine-induced SE [8], and benzodiazepines lost efficacy after 35 minutes of seizures in experimental SE [5]. In human partial SE, it is a common clinical observation that the longer the period of SE the less likely it will be controlled by benzodiazepines [6]. The current study suggests that the loss of GABA-mediated inhibition observed during SE is at least partially due to changes in the postsynaptic neuron.

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References

1. Towne AR, Pellock JM, KOD, DeLorenzo RJ. Determinants of mortality in status epilepticus. *Epilepsia* 1994;35:27–34. [PubMed: 8112254]
2. Lothman EW, Bertram EH. Epileptogenic effects of status epilepticus. *Epilepsia* 1993;34(suppl 1):S59–S70. [PubMed: 8462492]

3. Honchar MP, Olney JW, Sherman WR. Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. *Science* 1983;220:323–325. [PubMed: 6301005]
4. Treiman DM, Walton NY, Kendrick C. A progressive sequence of electroencephalographic changes during generalized convulsive status epilepticus. *Epilepsy Res* 1990;5:49–60. [PubMed: 2303022]
5. Walton NY, Treiman DM. Response of status epilepticus induced by lithium and pilocarpine to treatment with diazepam. *Exp Neurol* 1988;101:267–275. [PubMed: 3396644]
6. Treiman DM. The role of benzodiazepines in the management of status epilepticus. *Neurology* 1990;40:32–42. [PubMed: 2159132]
7. Lothman E. The biochemical basis and pathophysiology of status epilepticus. *Neurology* 1990;40:13–23. [PubMed: 2185436]
8. Kapur J, Lothman EW, DeLorenzo RJ. Loss of GABA_A receptors during partial status epilepticus. *Neurology* 1994;44:2407–2408. [PubMed: 7991140]
9. Kapur J, Lothman EW. Loss of inhibition precedes delayed spontaneous seizures in the hippocampus after tetanic electrical stimulation. *J Neurophysiol* 1989;61:427–434. [PubMed: 2918364]
10. Lothman EW, Bertram EH, Kapur J, Stringer JL. Recurrent spontaneous hippocampal seizures in the rat as a chronic sequela to limbic status epilepticus. *Epilepsy Res* 1990;6:110–118. [PubMed: 2387285]
11. Michelson HB, Kapur J, Lothman EW. Reduction of paired pulse inhibition in the CA1 region of the hippocampus by pilocarpine in naive and in amygdala-kindled rats. *Exp Neurol* 1989;104:264–271. [PubMed: 2721629]
12. Kay AR, Wong RK. Isolation of neurons suitable for patch-clamping from adult mammalian central nervous systems. *J Neurosci Methods* 1986;16:227–238. [PubMed: 3523050]
13. Oh K-S, Lee C-J, Gibbs JW, Coulter DA. Postnatal development of GABA_A receptor function in somatosensory thalamus and cortex: whole-cell voltage clamp recordings in acutely isolated rat neurons. *J Neurosci* 1995;15:1341–1351. [PubMed: 7869102]
14. Horn R, Marty A. Muscarinic activation of ionic currents measured by a new whole-cell recording method. *J Gen Physiol* 1995;92:145–159. [PubMed: 2459299]
15. Coulter DA, Huguenard JR, Prince DA. Differential effects of petit mal anticonvulsants and convulsants on thalamic neurones: GABA current blockade. *Br J Pharmacol* 1990;100:807–813. [PubMed: 2119843]
16. Stelzer A, Kay AR, Wong RK. GABA_A-receptor function in hippocampal cells is maintained by phosphorylation factors. *Science* 1988;241:339–341. [PubMed: 2455347]
17. Stelzer A, Wong RK. GABA_A responses in hippocampal neurons are potentiated by glutamate. *Nature* 1989;337:170–173. [PubMed: 2563149]
18. Huguenard JR, Alger BE. Whole-cell voltage-clamp study of the fading of GABA-activated currents in acutely dissociated hippocampal neurons. *J Neurophysiol* 1986;56:1–18. [PubMed: 3746390]
19. Fujikawa DG, Daniels AH, Kim JS. The competitive NMDA receptor antagonist CGP 40116 protects against status epilepticus-induced neuronal damage. *Epilepsy Res* 1994;17:207–219. [PubMed: 7912191]
20. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen GABA_A receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci* 1992;12:4151–4172. [PubMed: 1331359]
21. Heuschneider A, Schwartz RD. CAMP and forskolin decrease g-amino butyric acid-gated chloride flux in rat brain synaptoneurosome. *Proc Natl Acad Sci USA* 1989;86:2938–2942. [PubMed: 2468163]
22. Porter NM, Twyman RE, Uhler MD, Macdonald RL. Cyclic AMP-dependent protein kinase decreases GABA_A receptor current in mouse spinal neurons. *Neuron* 1990;5:789–796. [PubMed: 2176510]
23. Krishek BJ, Xie X, Blackstone C, et al. Regulation of GABA_A receptor function by protein kinase C phosphorylation. *Neuron* 1994;12:1081–1095. [PubMed: 8185945]

24. Thompson SM, Gahwiler BH. Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in hippocampus in vitro. *J Neurophysiol* 1989;61:501–511. [PubMed: 2709096]
25. Coulter DA, Sombati S, DeLorenzo RJ. Electrophysiology of glutamate neurotoxicity in vitro: induction of a calcium-dependent extended neuronal depolarization. *J Neurophysiol* 1992;68:362–373. [PubMed: 1388200]
26. Sombati S, Coulter DA, DeLorenzo RJ. Neurotoxic activation of glutamate receptors induces an extended neuronal depolarization in cultured hippocampal neurons. *Brain Res* 1991;566:316–319. [PubMed: 1687663]
27. Kapur J, Stringer JL, Lothman EW. Evidence that repetitive seizures in the hippocampus cause a lasting reduction of GABAergic inhibition. *J Neurophysiol* 1989;61:417–426. [PubMed: 2918363]
28. Walton NY, Gunawan S, Treiman DM. Brain amino acid concentration changes during status epilepticus induced by lithium and pilocarpine. *Exp Neurol* 1990;108:61–70. [PubMed: 1969357]

Fig 1. Photographs of: (A) CA1 pyramidal neurons acutely isolated from 18- to 28-day-old rats undergoing 45 minutes of status epilepticus (SE) induced by lithium and pilocarpine injections (SE neurons). (B) CA1 Pyramidal neurons acutely isolated from naive rats (naive neurons). The calibration bar is 50 µm.

Fig 2. Relative frequency distribution histograms of resting membrane potentials of 17 naive neurons and 31 status epilepticus (SE) neurons. The resting membrane potentials were binned into 7.5-mV intervals; median resting membrane potential of SE neurons was -10 mV and that of naive neurons was -55 mV.

Fig 3.
 γ -Aminobutyric acid (GABA) concentration–response relationship for a CA1 pyramidal neuron isolated from a 30-day-old rat. Traces were from a single neuron and show responses to three concentrations of GABA. The concentration of GABA eliciting the current appears below the trace, and the bar indicates the duration of GABA application. The neuron was voltage clamped at -10 mV.

Fig 4.

(A) Pooled data from seven naive neurons; γ -aminobutyric acid (GABA) concentration–response relationship for naive neurons. Each point represents the mean of five observations and error bars show SEMs. The line was the best fit (least-squares method) of the data to a sigmoidal curve. The EC_{50} was derived from the equation for the sigmoid curve that best fit the data. (B) Relative frequency distribution histogram of normalized GABA receptor (GABAR) current to 3 μ M GABA in SE neurons and naive neurons binned by 0.1 interval.

Fig 5.

(A) I-V curve (I = GABA-evoked current; V = potential to which neuron was voltage clamped) generated from data (mean $\pm$ SEM) pooled from eight naive neurons. The line was fit by linear regression and crosses the abscissa at -35 mV. (B) Individual I-V curves for eight S E neurons with the lines fitted by linear regression.