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Acute Cellular Alterations in the Hippocampus After Status Epilepticus

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Summary: The critical, fundamental mechanisms that determine the emergence of status epilepticus from a single seizure and the prolonged duration of status epilepticus are uncertain. However, several general concepts of the pathophysiology of status epilepticus have emerged: (a) the hippocampus is consistently activated during status epilepticus; (b) loss of GABA-mediated inhibitory synaptic transmission in the hippocampus is critical for emergence of status epilepticus; and, finally (c) glutamatergic excitatory synaptic transmission is important in sustaining status epilepticus. This review focuses on the alteration of GABAergic inhibition in the hippocampus that occurs during the prolonged seizures of status epilepticus. If reduction in GABAergic inhibition leads to development of status epilepticus, enhancement of GABAergic inhibition would be expected to interrupt status epilepticus. Benzodiazepines and barbiturates are both used in the treatment of status epilepticus and both drugs enhance $GABA_A$ receptor-mediated inhibition. However, patients often become refractory to benzodiazepines when seizures are prolonged, and barbiturates are often then used for these refractory cases of status epilepticus. Recent evidence suggests the presence of multiple $GABA_A$ receptor isoforms in the hippocampus with different sensitivity to benzodiazepines but similar sensitivity to barbiturates, thus explaining why the two drug classes might have different clinical effects. In addition, rapid functional plasticity of $GABA_A$ receptors has been demonstrated to occur during status epilepticus in rats. During status epilepticus, there was a substantial reduction of diazepam potency for termination of the seizures. The loss of sensitivity of the animals to diazepam during status epilepticus was accompanied by an alteration in the functional properties of hippocampal dentate granule cell $GABA_A$ receptors. Dentate granule cell $GABA_A$ receptor currents from rats undergoing status epilepticus had reduced sensitivity to diazepam and zinc but normal sensitivity to GABA and pentobarbital. Therefore, the prolonged seizures of status epilepticus rapidly altered the functional properties of hippocampal dentate granule cell $GABA_A$ receptors, possibly explaining why benzodiazepines and barbiturates may not be equally effective during treatment of the prolonged seizures of status epilepticus. A comprehensive understanding of the cellular and molecular events leading to the development, maintenance, and cytotoxicity of status epilepticus should permit development of more effective treatment strategies and reduction in the mortality and morbidity of status epilepticus. **Key Words:** GABA—Hippocampus—Seizures—Status epilepticus.

Most seizures are relatively brief and self-terminating. During some seizures, however, early termination fails and status epilepticus ensues. One hypothesis for development of status epilepticus is that activation of γ-aminobutyric acid (GABA) receptor-mediated inhibition is responsible for normal termination of a seizure. If the GABAergic inhibition fails to terminate the seizure, a progressive reduction in $GABA_A$ receptor-mediated inhibition develops which, when severe enough, results in a prolonged self-sustained seizure (1,2). There is experimental support for this hypothesis. Prolonged hippocampal seizures reduce $GABA_A$ receptor-mediated inhibition, and this reduction in inhibition can reliably predict occurrence of status epilepticus (3). Status epilepticus in humans also suggests a role for $GABA_A$ receptors because it is treated with the benzodiazepines diazepam, lorazepam, and midazolam and the barbiturates phenobarbital and pentobarbital, all of which exert their anticonvulsant effect by enhancing $GABA_A$ receptor-mediated inhibition (4). The $GABA_A$ receptor is the site of action of many of the antiepileptic drugs (AEDs) used to treat status epilepticus in humans, and there is direct evidence for altered properties of hippocampal $GABA_A$ receptors during status epilepticus. Therefore, it is important to characterize the functional properties of $GABA_A$ receptors and the effect of status epilepticus on these properties to understand the pathogenesis and treatment of status epilepticus.

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RECOMBINANT AND NATIVE $GABA_A$ RECEPTORS

Properties and structure of recombinant GABA receptors

GABA is the major inhibitory neurotransmitter in the CNS. It is released from GABAergic neurons and binds to several types of GABA receptors: $GABA_A$, $GABA_B$, and $GABA_C$ receptors. $GABA_A$ receptors mediate the majority of fast inhibition in the CNS. They are macromolecular proteins that contain specific binding sites for GABA and for a number of allosteric regulators, including picrotoxin, barbiturates, benzodiazepines, and the anesthetic steroids, and they form a chloride ion channel.

On the basis of sequence similarity, six different $GABA_A$ receptor-subunit families have been identified and named: α-, β-, γ-, δ-, ϵ-, and π-subunits. There is 30–40% sequence identity among the subunit families. About 20–30% sequence homology exists among all $GABA_A$ receptor-subunit candidates and other gene products of the superfamily (5–7). Most of the subunit families have multiple members (α1–6; β1–4; γ1–4; and ρ1–2), and all of the sequences within each subunit family are homologous, with about 70–80% amino acid sequence identity (Table 1). Additional diversity arises from RNA splice variants, described thus far for γ2 (8) and β4 (9,10). Each $GABA_A$ receptor-subunit cDNA encodes for a polypeptide of about 50 kDa, with putative *N*-glycosylation sites and four α-helical, hydrophobic membrane-spanning regions (5,6). Between the third and fourth membrane-spanning regions is a putative hydrophilic cytoplasmic region of highly variable sequence involved in intracellular regulatory mechanisms such as phosphorylation.

The current understanding of the molecular structure of the $GABA_A$-receptor–ion channel complex is that it is a heteropentameric glycoprotein of about 275 kDa, composed of combinations of multiple polypeptide subunits. The subunits form a quasi-symmetric structure around the ion channel, with each subunit contributing to the wall of the channel (6). The model is based on the nicotinic acetylcholine receptor, another member of the ligand-gated ion channel gene superfamily, and electron microscopic image analysis of the native $GABA_A$ receptors (11). However, the number of each subtype and their stoichiometry remain uncertain. $GABA_A$ receptors are generally believed to be composed of combinations of α-, β-, γ-, δ- and possibly ϵ-subunits. The ρ-subunit forms homopentameric $GABA_C$ receptors in the retina; the role of the π-subunit remains uncertain.

TABLE 1. *$GABA_A$ receptor subunit subtypes*

Subunits	α	β	γ	δ	ϵ	π
No. of subtypes	6	4	4	1	1	1
No. of splice variants	0	4	1	0	Multiple	0

Pharmacology of recombinant $GABA_A$ receptors

The pharmacologic properties of $GABA_A$ receptors are dependent on subunit subtype composition. The benzodiazepine sensitivity of $GABA_A$ receptors differs when different α subtypes are combined with β- and γ-subunits (Table 2) (12–16). The α1βγ-subunits produce diazepam- and zolpidem-sensitive BZ1 $GABA_A$ receptors, α2βγ- and α3βγ-subunits produce BZ2a,b $GABA_A$ receptors with high diazepam and low zolpidem sensitivity, α5βγ-subunits produce BZ2c $GABA_A$ receptors with high diazepam sensitivity but zolpidem insensitivity, and α4- or α6βγ-subunits produce BZ3 $GABA_A$ receptors that are benzodiazepine insensitive. Barbiturates bind to an allosteric regulatory site on the $GABA_A$ receptor, but the subunit location of the barbiturate binding site is unknown.

There is growing evidence for the structural diversity of native $GABA_A$ receptors derived from whole brain. The distribution of mRNAs in the CNS determined by in situ hybridization is very different for each subunit subtype. For example, the ρ-subunit is expressed primarily in the retina (17), whereas various α, β, and γ subtypes and the single δ subtype show very different regional as well as developmental distributions (18–20). Immunoprecipitation studies have shown that different specific combinations of $GABA_A$ receptor-subtypes occur in the different regions of the brain. McKernan et al. (21) found that a majority of native $GABA_A$ receptors contain only a single α-subtype and that α1-, α2-, α3-, or α5-subtypes exist in combination with a β- and a γ2-subtype. Serum specific for the δ subunit was found to precipitate a specific set of $GABA_A$ receptors that contained α1, α3 β2/3γ2 subtypes but not the α5 subtype (22). Partial colocalization suggests some tentative major oligomeric assemblies, e.g., Wisden et al. (19) propose the likely existence of at least five combinations: (a) α1β2γ2; (b) α2β3γx; (c) α5β1γx; (d) α1α4β2δ; and (e) α1α6β2δ. They also note that the γ1-subunit is limited to the limbic regions of amygdala, hypothalamus, and septum.

Hippocampal $GABA_A$ receptors

It is likely that multiple specific $GABA_A$ receptor isoforms are expressed in the hippocampus. The existence of $GABA_A$ receptor isoforms has been demonstrated by in vivo and in vitro pharmacologic studies. Immunopurification and in situ hybridization studies have shown that the subtypes display a heterogeneous distribution in the CNS. Experiments using subunit-specific antibodies for quantitative immunopurification of $GABA_A$ receptors from specific regions of the CNS and comparison of the identified subunit combinations with the localization of the corresponding mRNA have allowed initial identification of the subunit composition of some $GABA_A$ receptor isoforms. Hippocampal dentate granule cells can potentially express $GABA_A$ receptors with distinct

TABLE 2. *Subunit-specific pharmacologic properties of $GABA_A$ receptors*

Pharmacologic property	Subunit subtypes
Benzodiazepine sensitivity	α and β with γ2
High benzodiazepine affinity (nM BZ affinity)	α1, α2, α3, or α5 with γ2, β
BZ1 pharmacology (high zolpidem affinity)	α1 with γ2, β
BZ2a,b pharmacology (low zolpidem affinity)	α2, α3 with γ2, β
BZ2c pharmacology (zolpidem insensitive)	α5 with γ2, β
BZ3 pharmacology (BZR agonist insensitive)	α4 or α6 with γ2, β
High zinc sensitivity (IC_{50} < 10 μM)	α and β without γ2
Low zinc sensitivity (IC_{50} > 100 μM)	α1 and β with γ2
Moderate zinc sensitivity (IC_{50} 10–100 μM)	α4 or α6, β, γ2, δ or ε and any α, β
Enhancement by loreclezole	β2 or β3 present
High furosemide sensitivity (IC_{50} < 10 μM)	α6 and β2 or β3 present
Moderate furosemide sensitivity (IC_{50} 100 μM–1 mM)	α4 and β2 or β3 present
Low furosemide sensitivity (IC_{50} > 1 mM)	α1, α2, α3, or α5 or β1 present

properties because they express specific $GABA_A$ receptor-subtype mRNAs at high levels. In situ hybridization studies have demonstrated that dentate granule cells primarily express mRNAs for α1,2,4, β1,3, γ1,2, and δ1 $GABA_A$ receptor-subunit subtypes. Which subtypes assemble to form native granule cell $GABA_A$ receptors is unknown. The α4-, β1-, γ1-, and δ1-subtype mRNAs have a restricted distribution in the brain, and each subtype confers distinct pharmacologic properties. Although immunoprecipitation studies have not been performed on isolated preparations of dentate gyrus, a whole-brain immunopurification study using a δ-specific antibody has been reported, demonstrating that the δ-subunit is associated with the α1-, α3-, β2/3-, and γ2 subtypes. This study provided no information regarding expression of α3-, α4-, β1-, or γ1-subtypes by granule cells. Immunoprecipitation studies did not reveal if subtype combinations precipitated from brains were functionally expressed by granule cells. Finally, these studies did not determine if more than one $GABA_A$ receptor isoform was expressed by single granule cell (22).

Pharmacology of dentate granule cell $GABA_A$ receptors

The pharmacologic properties of $GABA_A$ receptor currents recorded from acutely dissociated hippocampal dentate granule cells from 28- to 35-day-old rats were characterized using the whole-cell patch-clamp technique (23). Granule cells were voltage-clamped to 0 mV and GABA was applied using a modified U-tube rapid application technique.

Concentration-dependent $GABA_A$ receptor currents were obtained from individual granule cells (Fig. 1A). Mean GABA EC_{50} was 46 ± 10 μM, maximal current was 842 ± 54 CpA, and the Hill slope was 1.2 (Fig. 1C). Diazepam-enhanced granule cell $GABA_A$ receptor currents were evoked by 10 μM GABA in a concentration-dependent fashion (Fig. 2A,B). Mean EC_{50} was 158 ± 13 nM, maximal enhancement was 210 ± 10%, and the Hill slope was 1.2 ± 0.3 (Fig. 2C). Zn^{++} reduced granule cell $GABA_A$ receptor currents evoked by 30 μM GABA in a concentration-dependent fashion (Fig. 3A,B). Mean IC_{50} was 28.5 ± 11 μM, maximal inhibition was 77 ± 3%, and the Hill slope was 2.0 ± 0.4 (Fig. 3C).

INVOLVEMENT OF HIPPOCAMPAL $GABA_A$ RECEPTORS IN DEVELOPMENT OF STATUS EPILEPTICUS

Hippocampal involvement in status epilepticus

Several lines of evidence suggest that the hippocampal–parahippocampal loop can sustain seizures during status epilepticus. In functional mapping studies combining EEG and 2-deoxyglucose mapping of metabolic activity during status epilepticus, increased glucose utilization occurred in hippocampal–parahippocampal structures (subiculum, parasubiculum, and entorhinal cortex), limbic structures, including the amygdala, and extralimbic structures (24). Similarly, $GABA_A$ receptor-combined hippocampal–parahippocampal slices sustain status epilepticus. Therefore, it is important to understand the functional properties of hippocampal $GABA_A$ receptors and how they are modified by status epilepticus.

Whole-animal studies of acute changes in the pharmacology of status epilepticus

Status epilepticus was induced in Sprague–Dawley rats by intraperitoneal injection of 3 mEq/kg LiCl, followed 20 h later by 50 mg/kg pilocarpine (25). After pilocarpine injection, the rats were observed continuously for occurrence of behavioral seizures. The time to onset of behavioral seizures was recorded and the seizures were observed. Behavioral seizures evoked by lithium/pilocarpine were characterized by immobility, repetitive chewing, head nodding, vibrissial twitching, forelimb clonus with or without rearing, and falling (26,27). Seizure termination was defined as the absence of forelimb clonus or falling, facial twitching, and stop and stare activity. In addition, resumption of normal behavior within 30 min of drug injection was assessed. Diazepam was administered 10 or 45 min after pilocarpine injection. The proportion of rats that ceased to have

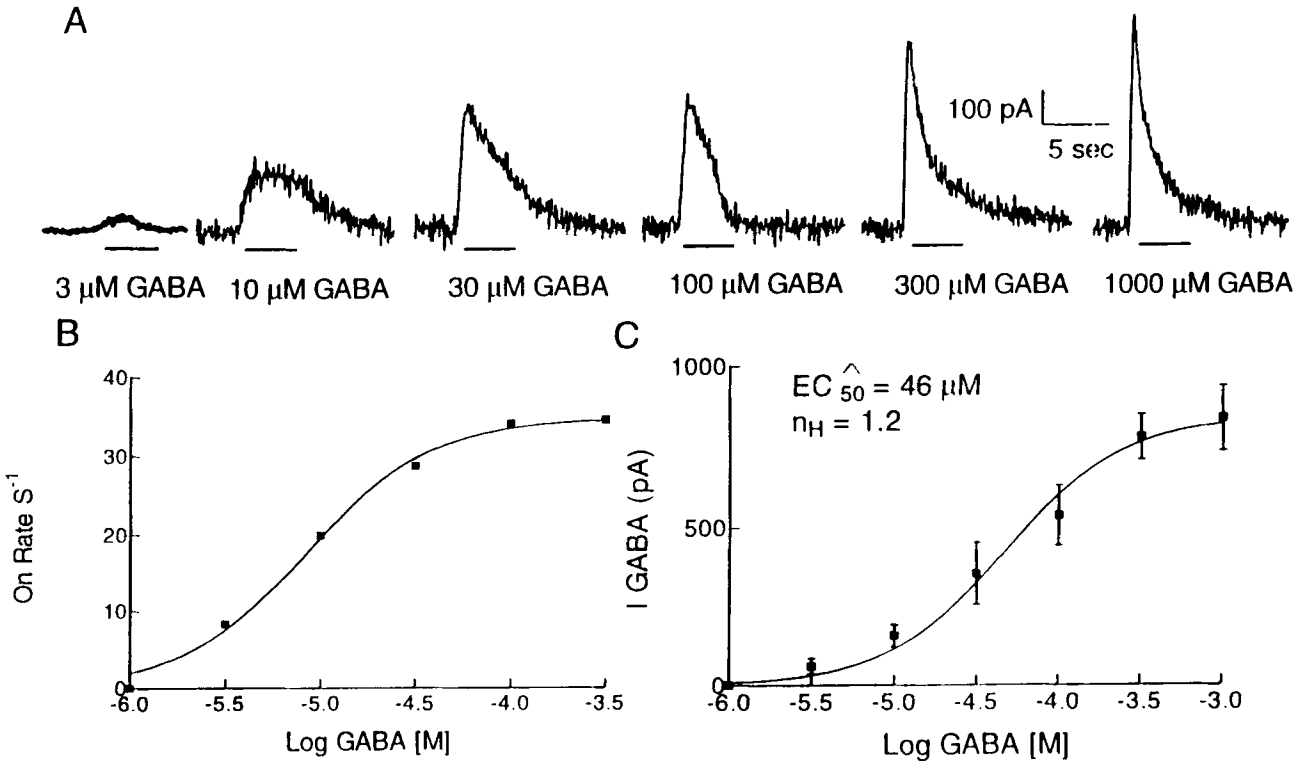

FIG. 1. GABA concentration–response curve for a dentate granule cell isolated from a 30-day-old rat. **A:** Traces were from a single neuron and show responses to six concentrations of GABA. The concentration of GABA eliciting the current appears below the trace, and the bar indicates the duration of GABA application. **B:** The on rate of GABAR current is plotted as a function of GABA concentration. **C:** Pooled data from six neurons: each point represents the mean of five observations, and error bars show SEMs. The line was the best fit of the data to a sigmoidal function. The EC_{50} was derived from the equation for the sigmoid function that best fit the data. From Kapur et al. (23).

seizures within 5 min of diazepam injection was plotted against log diazepam dose. The data were fitted to a sigmoidal dose–response curve, with the maximum fixed to 100% and minimum to 0%. The ED_{50}s were derived from the equation that best fitted the data.

Behavioral seizures began 3–5 min after injection with pilocarpine. A detailed diazepam dose–response analysis was performed using a total of 30 rats. After 10 min of seizures, increasing doses of diazepam from 2 mg/kg to 20 mg/kg were administered to four groups of rats: 2 mg/kg, $n = 5$; 7.5 mg/kg, $n = 3$; 10 mg/kg, $n = 3$; and 20 mg/kg, $n = 3$. After 45 min of seizures, three rats were treated with 20 mg/kg, 30 mg/kg, 50 mg/kg, and 100 mg/kg of diazepam. Four rats were not treated with an AED and these rats continued to have seizures for 2 h. After 10 min of seizures, diazepam (20 mg/kg) terminated seizures in all treated animals ($n = 3$). However, after 45 min of seizures (status epilepticus), diazepam (20 mg/kg) terminated the seizures in none of the animals ($n = 3$). At high doses of diazepam (50 mg/kg and 100 mg/kg), behavioral seizures appeared terminated, but rats were extremely sedated and resumption of normal activity did not occur. The dose–response data were fitted to a sigmoidal dose–response curve and the ED_{50}s for diazepam control of behavioral seizures after 10 min and 45 min of seizures were derived (Fig. 4). The dose–response curve showed that the ED_{50} for diazepam-induced termination of seizures shifted from 4.2 mg/kg when administered after 10 min of continuous seizures to 40 mg/kg when administered after 45 min of continuous seizures.

Because diazepam exerts its anticonvulsant effect primarily by enhancing GABAergic inhibition by acting on $GABA_A$ receptors (28), we hypothesized that seizures altered the functional properties of $GABA_A$ receptors. The seizures could potentially alter the modulation of $GABA_A$ receptor by various drugs, such as enhancement by benzodiazepines, barbiturates, and neurosteroids and antagonism by penicillin, picrotoxin, bicuculline, and Zn^{++}. We characterized $GABA_A$ receptor currents recorded from acutely isolated hippocampal dentate granule cells, their potentiation by benzodiazepines and barbiturates, and their inhibition by Zn^{++}.

Regulation of dentate granule cell $GABA_A$ receptor currents from rats undergoing status epilepticus

$GABA_A$ receptor currents were reduced in granule cells from rats undergoing status epilepticus

Whole-cell voltage-clamp recordings were made from dentate granule cells (29,30) acutely isolated from con-

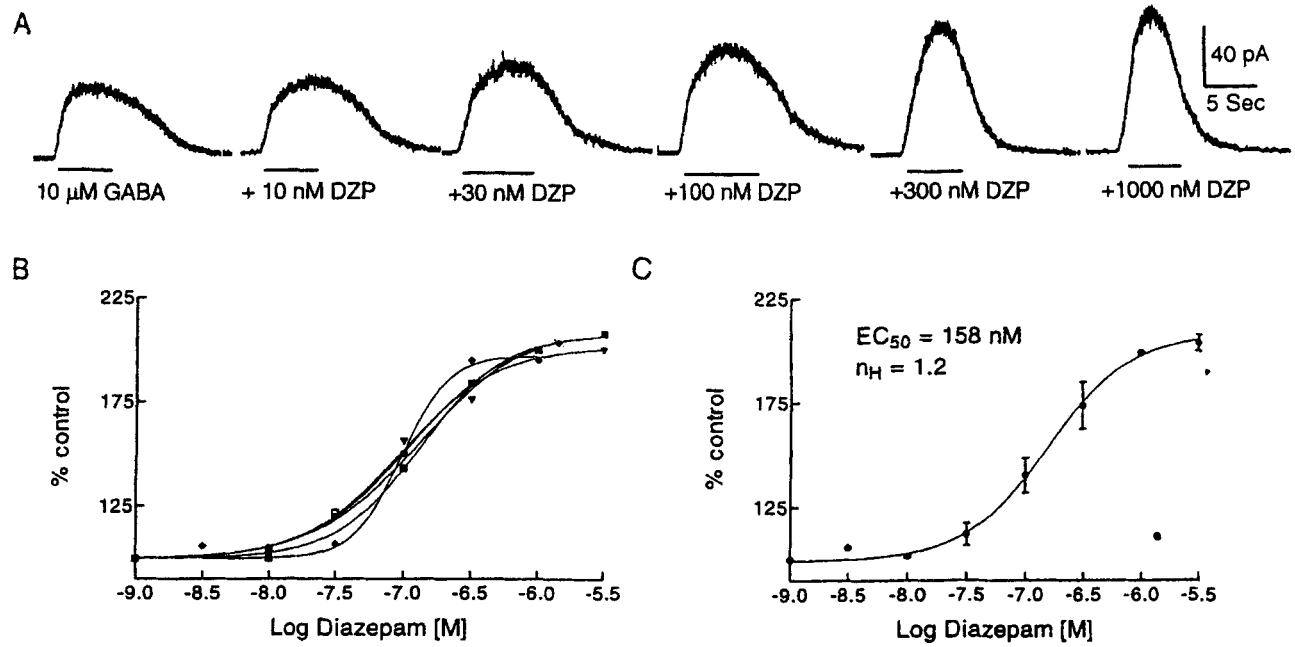

FIG. 2. *Diazepam-enhanced dentate granule cell $GABA_A$ receptor currents.* **A:** *The traces were from a single neuron. Concentrations of* diazepam (DZP) applied with 10 μM GABA are shown below the traces. Horizontal bars show the duration of application of the drug. Recovery between drug application is not shown. **B:** Diazepam concentration—response curves for enhancement of GABA currents are plotted for seven cells. **C:** Each point represents the mean of seven observations, and the error bars show SEMs. The lines are the best fit of the data to a sigmoidal function. The EC_{50} was derived from the equation for the sigmoid function that best fit the data. From Kapur et al. (23).

trol rats or from same-age rats that experienced 45 min of continuous seizures (status epilepticus) (1). When access was initially established in granule cells from control rats, 10 μM $GABA_A$ receptor currents increased slightly and became stable in 2–4 min (run-up) (Fig. 5A). The stable response compared with the first response increased by 174 ± 47% (n = 4). In contrast, $GABA_A$ receptor currents evoked from granule cells from animals

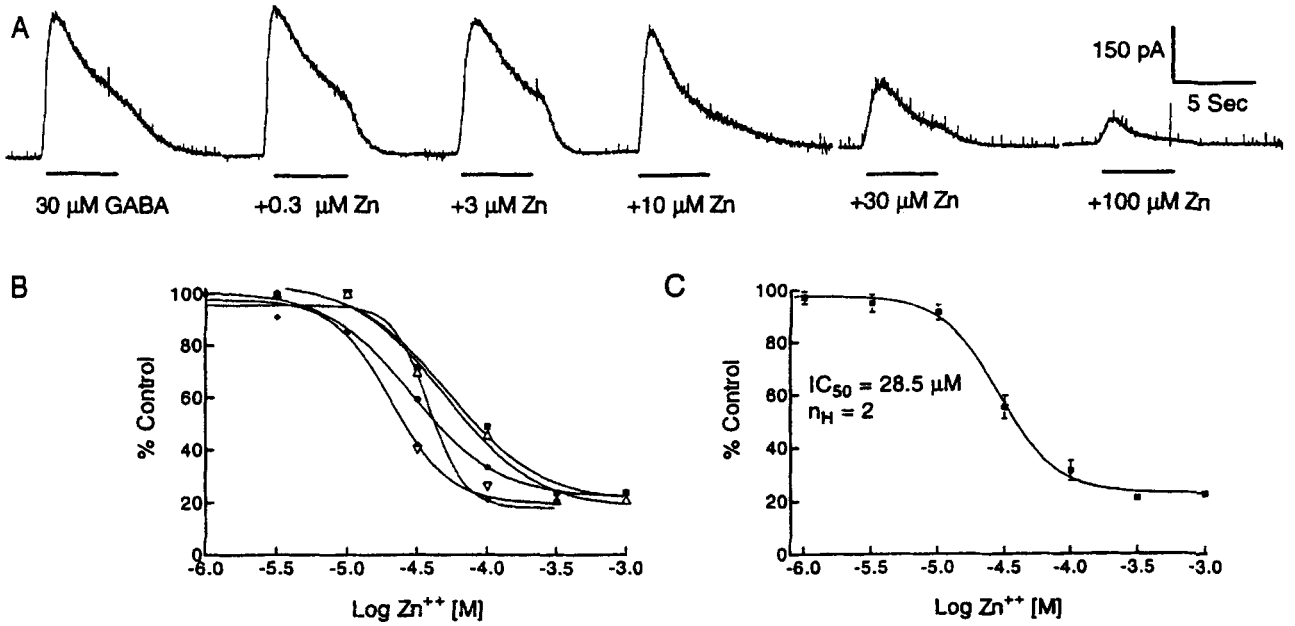

FIG. 3. Zn^{++} inhibited dentate granule cell $GABA_A$ receptor currents. **A:** The traces were from a single neuron. Drug concentrations applied with 30 μM GABA are shown below the trace. Horizontal bars show the duration of drug application. Recovery between drug applications is not shown. Note the Zn^{++} inhibition of $GABA_A$ receptor currents was incomplete. **B:** Zn^{++} concentration–response curves for inhibition of GABAR currents are plotted for eight cells. **C:** Each point represents the mean of the data from eight neurons, and the error bars show SEMs. The lines are the best fit of the data to a sigmoidal function. The IC_{50} was derived from the equation for the sigmoid function that fit the data. From Kapur et al. (23).

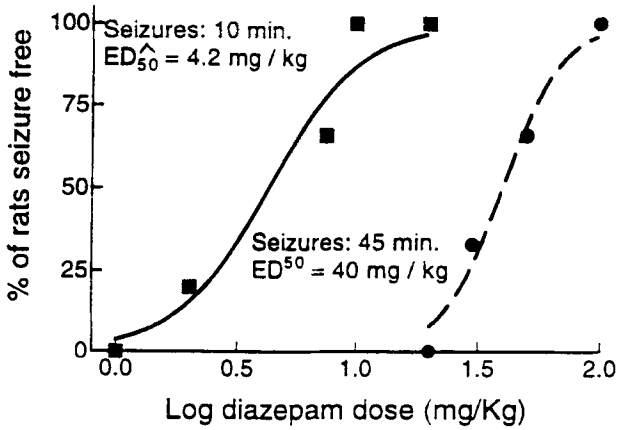

FIG. 4. Diazepam was effective in controlling brief (10-min) seizures but lost efficacy after prolonged (45 min) seizures. Seizures were induced in 70–150-g rats by i.p. injection of 3 mEq/kg LiCl, followed 16–24 h later by 50 mg/kg i.p. injection of pilocarpine. Behavioral seizures started within 1–5 min in all rats. Diazepam was administered 10 min (*filled boxes, solid line; n* = 14) or 45 min (*filled circles, dashed line; n* = 12) after pilocarpine injection. The proportion of rats that stopped having seizures within 5 min of diazepam injection was plotted against log diazepam dose. The data were fitted to a sigmoidal dose–response curve with the maximum fixed to 100% and minimum to 0%. The ED_{50}s were derived from the equation that best fit the data. From Kapur et al. (1).

undergoing status epilepticus required 10 min to stabilize, and the run-up was substantially larger (374 ± 66%; $n = 5$, $p < 0.05$; Fig. 5B). Once stable responses to 10 μM GABA were obtained, GABA was applied to granule cells at concentrations ranging from 1 to 1,000 μM (Fig. 6). For each of the groups, data from individual cells were pooled and fitted to a sigmoidal logistic equation. In neurons from control animals, the mean GABA EC_{50} for $GABA_A$ receptors was 42 ± 19 μM ($n = 17$), similar to that of neurons from animals undergoing status epilepticus: 33 ± 14 μM; $n = 9$; ($p > 0.05$). The maximal $GABA_A$ receptor current in cells from control animals was 962 ± 109 pA ($n = 19$), similar to that of cells from animals undergoing status epilepticus: 820 ± 188 pA ($n = 9$). Thus, after status epilepticus there was increased run-up of $GABA_A$ receptor currents after initial access, but once stable currents had been obtained, the potency and efficacy of GABA on dentate granule cell $GABA_A$ receptors were similar to those in neurons from control animals. Modulation of $GABA_A$ receptor currents was studied in dentate granule cells isolated from control rats or from those undergoing status epilepticus after stabilization of currents.

Diazepam enhancement of $GABA_A$ receptor currents was diminished in granule cells from rats undergoing status epilepticus

In hippocampal dentate granule cells from control animals, 10 μM GABA administered with 300 nM diazepam enhanced $GABA_A$ receptor currents in all neurons by 68 ± 10% ($n = 6$) (Fig. 7A). In contrast, in dentate granule cells from animals undergoing status epilepticus, 300 nM diazepam inconsistently enhanced 6 or 10 μM GABA-evoked $GABA_A$ receptor currents by 10 ± 6%; ($n = 5$; $p < 0.001$, grouped t test) (Fig. 7B). In neurons from control animals, 1 μM diazepam enhanced $GABA_A$ receptor currents by 92 ± 6% ($n = 6$), but in neurons from animals undergoing status epilepticus, 3 μM diazepam enhanced $GABA_A$ receptor currents by 51 ± 8% ($n = 5$; $p < 0.05$, grouped t test) (Fig. 7). The EC_{50} for diazepam enhancement of $GABA_A$ receptor currents in neurons from control animals was 195 ± 12 nM, and the EC_{50} in neurons from animals undergoing status epilepticus was 4.4 ± 0.25 μM (Fig. 8). Therefore, the prolonged seizures of status epilepticus reduced the potency and efficacy of diazepam for enhancement of granule cell $GABA_A$ receptor currents.

Zinc inhibition of $GABA_A$ receptor currents was diminished in granule cells from rats undergoing status epilepticus

Because Zn^{++} modulation of recombinant $GABA_A$ receptor currents varies inversely with benzodiazepine sensitivity (32,33), Zn^{++} inhibition of granule cell $GABA_A$ receptor currents was studied. Zn^{++} was less potent in inhibiting $GABA_A$ receptor currents recorded from granule cells isolated from animals undergoing status epilepticus than from control granule cells. In neurons from control animals, $GABA_A$ receptor currents were inhibited 59 ± 4% ($n = 8$) by 100 μM Zn^{++} (Fig. 9A), but in neurons isolated from animals undergoing status epilepticus the inhibition was reduced to 39 ± 6% ($n = 6$; $p < 0.05$, grouped t test) (Fig. 9B). In dentate granule cells from control rats, $GABA_A$ receptor currents were reduced by Zn^{++} in a concentration-dependent fashion with an IC_{50} of 30 ± 3.6 μM ($n = 12$) (Fig. 10). In dentate granule cells isolated from animals undergoing status epilepticus, the IC_{50} of Zn^{++} inhibition of $GABA_A$ receptor currents was 123 ± 15 μM ($n = 10$, $p < 0.01$, grouped t test) (Fig. 10). The maximal inhibition of $GABA_A$ receptor currents by Zn^{++} was unchanged (78 ± 3% in neurons from control animals and 90 ± 16% in neurons from animals undergoing status epilepticus). Therefore, the prolonged seizures of status epilepticus reduced the potency of Zn^{++} without altering the efficacy of inhibition of granule cell $GABA_A$ receptor currents.

Pentobarbital enhancement of $GABA_A$ receptor currents was unchanged in cells from rats undergoing status epilepticus

In neurons from control animals ($n = 6$), $GABA_A$ receptor currents elicited by 10 μM GABA were enhanced 77 ± 7% by 30 μM pentobarbital (Fig. 11A), whereas in neurons from animals undergoing status epilepticus ($n = 3$), $GABA_A$ receptor currents elicited by 10 μM GABA were enhanced 62 ± 11% by 30 μM pentobarbital ($P > 0.05$, grouped t test) (Fig. 11B). In

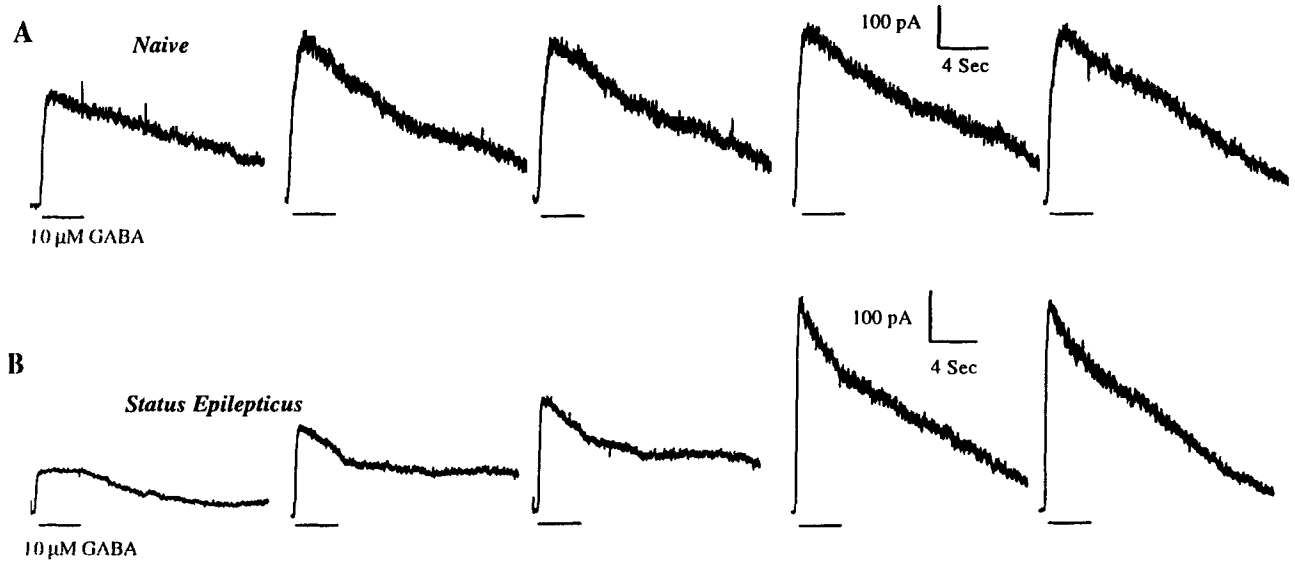

FIG. 5. Stabilization of $GABA_A$ receptor currents from dentate granule cells elicited immediately on access. Traces were from two neurons: **(top)** from a cell isolated from a control animal, and **(bottom)** from an animal undergoing status epilepticus. The durations of GABA application are indicated by bars. Two min elapsed between each GABA application. **A:** $GABA_A$ receptor currents elicited from hippocampal dentate granule cells isolated from control animals rapidly increased to a relatively stable amplitude. **B:** $GABA_A$ receptor currents elicited from hippocampal dentate granule cells isolated from animals undergoing status epilepticus took longer to stabilize and showed a greater increase in amplitude. From Kapur et al. (1).

dentate granule cells from control animals (n = 6), the pentobarbital EC_{50} was 42 ± 15 μM, and in neurons from animals undergoing status epilepticus (n = 6) the pentobarbital EC_{50} was not significantly different: 36 ± 8 μM (Fig. 12). Maximal enhancement of $GABA_A$ receptor currents by pentobarbital in neurons from control rats (190 ± 55%) and in neurons from animals undergoing status epilepticus (158 ± 20%) were not significantly different ($p > 0.05$, grouped t test). Therefore, the prolonged seizures of status epilepticus did not alter the efficacy or potency of pentobarbital enhancement of $GABA_A$ receptor currents in dentate granule cells.

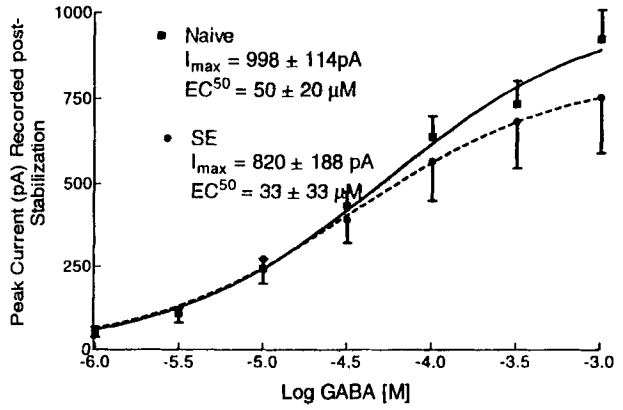

FIG. 6. GABA concentration dependency. GABA concentration-normalized $GABA_A$ receptor peak current relationships were plotted for 17 neurons isolated from control animals and nine neurons isolated from animals undergoing status epilepticus. Concentration–response data were obtained after stabilization of currents. Each point represents the mean of normalized peak currents; error bars show SEMs. The line is the best fit of data to a sigmoidal function. The EC_{50} and I_{max} were derived from the equation for the sigmoidal function that best fit the data. From Kapur et al. (1).

DISCUSSION

Diazepam loses effectiveness in the treatment of status epilepticus

This study demonstrates the prolonged seizures of status epilepticus reduce the ability of diazepam to terminate status epilepticus. The refractoriness to diazepam results from loss of the potency but not the efficacy of diazepam. Refractoriness to diazepam has been previously reported in both humans (34) and rats (27). Several possible mechanisms can be hypothesized to explain the loss of diazepam effectiveness in the treatment of the prolonged seizures of status epilepticus: seizures may become more intense; there may be enhanced excitatory transmission; or there may be altered inhibition. Past studies indicate the hippocampus is involved in the generation of status epilepticus (2,35,36) and that hippocampal GABAergic inhibition is altered during status epilepticus (3,37,38). These studies suggest that refractoriness of seizures to diazepam might result from altered $GABA_A$ receptor function in the hippocampus. The experiments reported here support this hypothesis.

Plasticity of $GABA_A$ receptor function during status epilepticus

During status epilepticus, $GABA_A$ receptor-mediated inhibition in the hippocampus is reduced in both the CA1

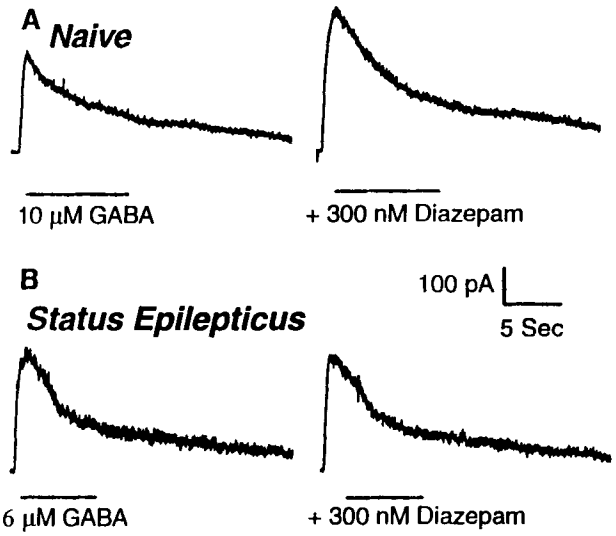

FIG.7. Diazepam enhancement of $GABA_A$ receptor currents from dentate granule cells of control animals and from cells isolated from rats after 45 min of seizures. Three hundred nanomolar diazepam-enhanced $GABA_A$ receptor currents in dentate granule cells from control animals but not those from cells isolated from rats after 45 min of seizures. The traces are from two different neurons. Horizontal bars show the duration of application of the drug. **A:** Three hundred nanomolar diazepam was applied with 10 μM GABA to a dentate granule cell from a control animal. **B:** 300 nM diazepam was applied with 6 μM GABA to a granule cell isolated from a rat after status epilepticus. A lower concentration of GABA was used to compensate for a small left shift of GABA concentration–response curve in cells from animals undergoing status epilepticus (equipotent GABA concentration). From Kapur et al. (1).

region and the dentate gyrus (3,38,39). One proposed mechanism for the reduction in inhibition is a specific alteration in the functional properties of $GABA_A$ receptors (38). We have demonstrated that two functional properties of $GABA_A$ receptors, diazepam enhancement and Zn^{++} inhibition of $GABA_A$ receptor currents, were altered by the prolonged seizures (1). This plasticity of $GABA_A$ receptors in the hippocampus may play a role in the pathogenesis and treatment of status epilepticus. Seizures in the hippocampus reduce GABAergic inhibition, and the findings presented here demonstrate that this is due in part to changes in $GABA_A$ receptor function. The reduction in diazepam sensitivity of dentate granule cell $GABA_A$ receptors parallels the loss of effectiveness of diazepam in the treatment of experimental status epilepticus. It is possible that changes in the diazepam sensitivity of dentate granule cell $GABA_A$ receptors reflect reduction of diazepam sensitivity in the treatment of status epilepticus. In addition, pentobarbital sensitivity of $GABA_A$ receptors on dentate granule cells isolated from animals undergoing status epilepticus was preserved. This suggested that status epilepticus alters specific properties of $GABA_A$ receptors rather than causing a generalized dysfunction of the receptor.

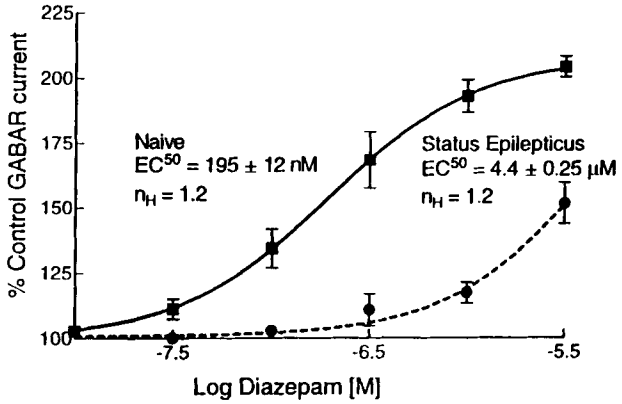

FIG. 8. Diazepam concentration–dentate granule cell $GABA_A$ receptor current enhancement relationships. Diazepam concentration–response curves were obtained for neurons isolated from control animals (*filled boxes, solid line; n* = 9) and for neurons isolated from animals undergoing status epilepticus (*filled circles, dashed line; n* = 12). Higher concentrations of diazepam inhibited $GABA_A$ receptor current as previously reported (31) n_H, Hill slope. From Kapur et al. (1).

Status epilepticus and temporal lobe epilepsy origin have distinct effects on hippocampal $GABA_A$ receptors

Studies investigating the role of $GABA_A$ receptor-mediated inhibition in the hippocampus in kindling and other models of temporal lobe epilepsy are the most

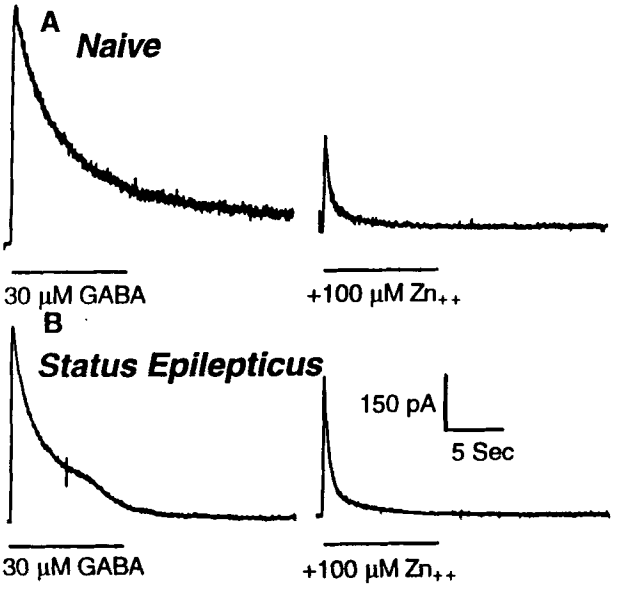

FIG. 9. Zn^{++} inhibition of $GABA_A$ receptor currents in dentate granule cells from control animals and from animals undergoing status epilepticus. One hundred micromolar Zn^{++} inhibited $GABA_A$ receptor currents in dentate granule cells from control animals more than in granule cells from animals undergoing status epilepticus. The traces are from two different neurons. One hundred μM Zn^{++} was co-applied with 30 μM GABA. Horizontal bars show the duration of application of the drug. **A:** Traces from a dentate granule cell isolated from a control animal. **B:** Traces from a granule cell isolated from an animal undergoing status epilepticus. From Kapur et al. (1).

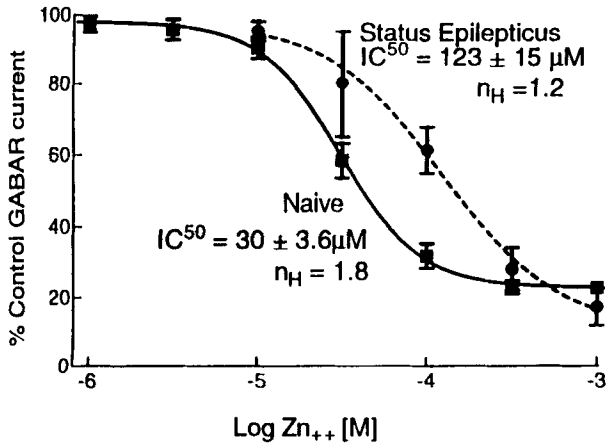

FIG. 10. Zn^{++} concentration–dentate granule cell $GABA_A$ receptor current reduction relationships. Zn^{++} concentration–dentate granule cell $GABA_A$ receptor current inhibition relationships were obtained from neurons isolated from control animals (*filled boxes, solid line; n* = 12) and from neurons isolated from animals undergoing status epilepticus (*filled circles, dashed line; n* = 12). The lines are the best fit of the data to a sigmoidal function. The IC_{50} and Hill slope (n_H) were derived from the equation for the sigmoidal function that best fit the data. From Kapur et al. (1).

comparable to the current study. However, brief seizures of temporal lobe epilepsy and prolonged seizures of status epilepticus are distinct phenomena. Almost 50% of patients who experience an episode of status epilepticus have not previously had a seizure (40). Epileptic seizures are brief, and data from epilepsy monitoring units indicate that the majority of seizures spontaneously terminate within 10 min (41). In contrast, status epilepticus is a syndrome consisting of a very prolonged seizure with continuous evolution of neurologic state, worsening cerebral metabolism, a steady rise in core temperature, a rise in blood pressure, lactic acidosis, hyperglycemia (42), and increased catecholamine levels (43). Hippocampal injury and neuronal loss occur as a result of status epilepticus in humans (44,45) and in most animal models of status epilepticus (46–50). However, whether individual brief seizures cause neuronal loss remains controversial (48,51,52). It is therefore expected status epilepticus and chronic temporal lobe epilepsy have different effects on hippocampal dentate granule cell $GABA_A$ receptors.

In kindling, subconvulsive electrical stimulation applied repeatedly to various regions of the brain evokes progressively prolonged behavioral and EEG seizures that terminate in generalized tonic–clonic seizures. However, there are important differences between the gradual plasticity occurring during the kindling process and the rapidly evolving changes of status epilepticus reported here. Several studies have reported enhanced [^{3}H]-muscimol and [^{3}H]-benzodiazepine binding in hippocampal membranes (53) and specifically in the hippocampal dentate gyrus (54,55). This increase in the hippocampal dentate granule cell $GABA_A$ receptors after kindling was associated with an increase in the amplitude of miniature inhibitory postsynaptic currents and enhancement of paired pulse depression of the kindled dentate gyrus (56). These long-term changes in $GABA_A$ receptor-mediated inhibition in the dentate gyrus were likely to be antiepileptic in nature. The findings of this study, however, do not contradict studies on the kindling model. Although inhibitory neurotransmission in the dentate gyrus was enhanced during kindling and diminished during status epilepticus, the changes in kindling were slower to develop compared with the rapid changes that occur during status epilepticus.

In electrical stimulation models of epilepsy, $GABA_A$ receptor-mediated inhibition in the dentate gyrus was chronically reduced, but this reduction was hypothesized to be due to circuit rearrangement and dormancy of basket cells (39). Recently, Buhl et al. (57) demonstrated enhanced Zn^{++} sensitivity of hippocampal dentate granule cell $GABA_A$ receptors after kindling and suggested that this increased sensitivity resulted in a collapse of the augmented inhibition during seizures. Gibbs et al. (58) found increased $GABA_A$ receptor density and enhanced $GABA_A$ receptor Zn^{++} sensitivity in another model of chronic temporal lobe epilepsy. Several important distinctions between these studies and this report pertain. First, the reduced diazepam sensitivity demonstrated here has not been reported in the past. Second, the

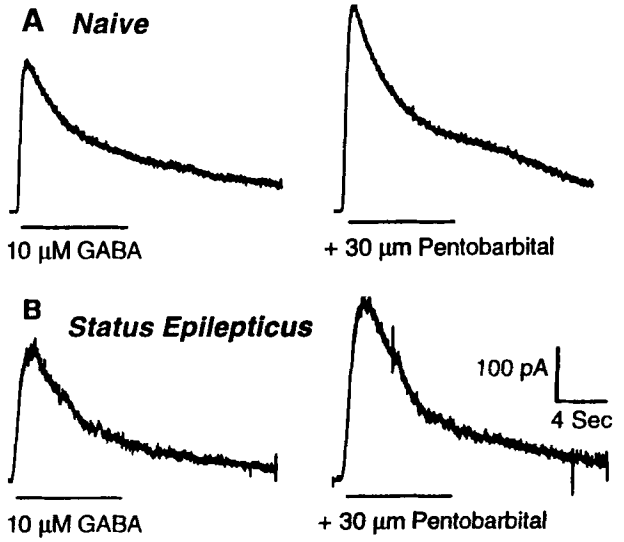

FIG. 11. Pentobarbital enhancement of $GABA_A$ receptor currents from dentate granule cells from control animals and from cells isolated from animals undergoing status epilepticus. Thirty micromolar pentobarbital equally enhanced $GABA_A$ receptor currents in dentate granule cells from control animals and granule cells from animals undergoing status epilepticus. The traces are from two different neurons. Thirty µM pentobarbital was co-applied with 10 µM GABA. Horizontal bars show the duration of application of the drug. **A:** Traces from a dentate granule cell isolated from a control animal. **B:** Traces from a granule cell isolated from an animal undergoing status epilepticus. From Kapur et al. (1).

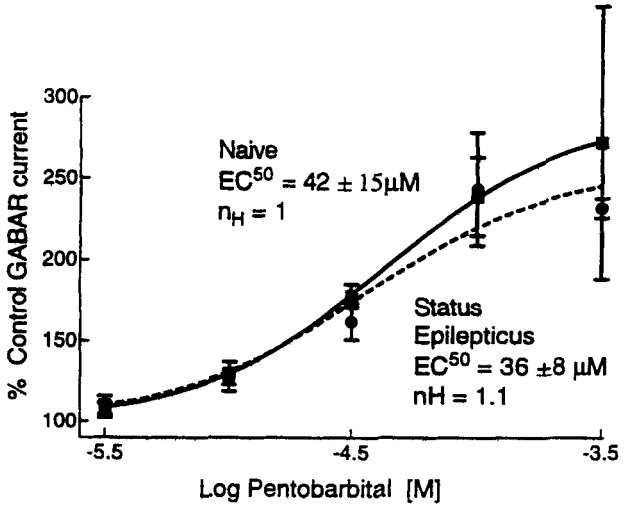

FIG. 12. Pentobarbital concentration–dentate granule cell $GABA_A$ receptor current enhancement relationships. Pentobarbital concentration–dentate granule cell $GABA_A$ receptor current enhancement relationships were obtained for neurons isolated from control animals (*filled boxes, solid line; n* = 7) and from neurons isolated from animals undergoing status epilepticus (*filled circles, dashed line; n* = 6). The lines were the best fit of the data to a sigmoidal function. The EC_{50} and Hill slope (n_H) were derived from the equation for the sigmoidal function that best fit the data. From Kapur et al. (1).

changes that were observed in these studies were acute, occurring over minutes, whereas previous reports documented changes that were chronic, occurring over several weeks. Finally, previous studies reported increased Zn^{++} sensitivity of hippocampal dentate granule cell $GABA_A$ receptors, whereas the current study reports diminished Zn^{++} sensitivity of granule cell $GABA_A$ receptors.

Possible molecular mechanisms for altered $GABA_A$ receptor function

This rapid, selective loss of benzodiazepine and Zn^{++} sensitivity is a novel form of $GABA_A$ receptor plasticity, and the underlying molecular basis is unclear. Diminished benzodiazepine sensitivity with development of benzodiazepine tolerance occurred over a prolonged period of time (59). During development of cerebellar granule cells, benzodiazepine sensitivity of $GABA_A$ receptors is lost during maturation, in parallel with increasing expression of the α6-subtype in the $GABA_A$ receptor. Similarly, development of tolerance to benzodiazepines requires chronic benzodiazepine administration.

This selective loss of benzodiazepine and Zn^{++} sensitivity may result from altered structural composition or altered state of phosphorylation of $GABA_A$ receptors. Diazepam sensitivity of $GABA_A$ receptors requires the presence of the γ2-subtype with a β-subtype and either α1-, α2-, α3-, or α5-subtype (13,60). Recombinant $GABA_A$ receptors expressed without the γ2-subtype are highly sensitive to Zn^{++} (IC_{50} < 10 μM, whereas $GABA_A$ receptors expressed with the γ2-subtype were relatively insensitive to Zn^{++} (32,33). Therefore, one explanation for acute reduction of diazepam sensitivity of hippocampal dentate granule cell $GABA_A$ receptors after seizures would be a loss of the γ2-subtype from the receptor. However, this would not explain the diminished Zn^{++} sensitivity of these receptors. Another potential explanation for diminished diazepam and Zn^{++} sensitivity would be altered α-subtype expression, because α-subtypes are known to alter both Zn^{++} and diazepam sensitivity of the $GABA_A$ receptors. For example, recombinant $GABA_A$ receptors with α4- or α6-subtype with a β-subtype and a γ2-subtype have low diazepam and Zn^{++} sensitivities (61).

Seizures may alter $GABA_A$ receptor function by other mechanisms, such as posttranslational modification of $GABA_A$ receptors or release of endogenous benzodiazepine-like substances. Modification of $GABA_A$ receptors by phosphorylation is well demonstrated (60,62,63), and seizures are known to modulate activities of cAMP-dependent protein kinase, calcium–calmodulin-dependent protein kinase, and calcium–phospholipid-dependent protein kinase (64,65). However, it remains to be shown that posttranslational modification can alter benzodiazepine and Zn^{++} sensitivity of $GABA_A$ receptors.

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