Zinc Inhibition of \(\gamma\)-Aminobutyric Acid\(\text{A}\) Receptor Function Is Decreased in the Cerebral Cortex during Pilocarpine-Induced Status Epilepticus\(^1\)

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ABSTRACT

Functional modulation of \(\gamma\)-aminobutyric acid\(\text{A}\) (GABA\(\text{A}\)) receptors by Zn\(^{2+}\), pentobarbital, neuroactive steroid alphaxalone, and flunitrazepam was studied in the cerebral cortex and cerebellum of rats undergoing status epilepticus induced by pilocarpine. Under control conditions, Zn\(^{2+}\) dose-dependently inhibited muscimol-stimulated uptake of \(^{36}\text{Cl}\) in cortical and cerebellar membranes. However, Zn\(^{2+}\) inhibition of stimulated \(^{36}\text{Cl}\) uptake was selectively decreased in the cortex (but not in the cerebellum) 1 to 2 h after the onset of status epilepticus. This loss of Zn\(^{2+}\) response in the cortex appeared to be selective to Zn\(^{2+}\) only, because pentobarbital-, alphaxalone-, or flunitrazepam enhancement of muscimol-stimulated \(^{36}\text{Cl}\) uptake did not change in this brain region either at 1 or 2 h after seizures. Because this loss of Zn\(^{2+}\) response in the cortex was apparent only about 1 h after the onset of status epilepticus but not earlier, we tested whether status epilepticus was critical for the development of the loss of Zn\(^{2+}\) response. We found that, in rats where status epilepticus was terminated by diazepam within 30 min after seizure onset, Zn\(^{2+}\) response was preserved in the cortex. These findings suggest that continuous seizures of pilocarpine-induced status epilepticus caused a rapid and selective decrease in Zn\(^{2+}\) inhibition of GABA\(\text{A}\) receptor function in the cortex. The possible relevance of such rapid seizure-induced GABA\(\text{A}\) receptor plasticity in the cerebral cortex is discussed.

Fast inhibitory neurotransmission in the mammalian central nervous system is mainly mediated by \(\gamma\)-aminobutyric acid\(\text{A}\) (GABA\(\text{A}\)) receptors. The GABA\(\text{A}\) receptor is a heteropentameric protein that contains specific binding sites for various positive and negative allosteric modulators (e.g., GABA, benzodiazepines, steroids, barbiturates, picrotoxin, and Zn\(^{2+}\)), and regulates chloride channel conductance (Macdonald and Olsen, 1994). Zn\(^{2+}\), a naturally occurring trace element in the central nervous system, inhibits GABA\(\text{A}\) receptor-operated chloride conductance in a noncompetitive fashion (Legendre and Westbrook, 1990; Smart et al., 1994), and is suggested to participate in synaptic signaling process (Choi and Koh, 1998). There is evidence that Zn\(^{2+}\) is accumulated in degenerating neurons after transient ischemia and seizure activity (Fredrickson et al., 1989; Koh et al., 1996). Zn\(^{2+}\) is released in a Ca\(^{2+}\)-dependent manner from hippocampal mossy fiber terminals during spontaneous activity, K\(^+\) stimulation, and epileptic seizures (Assaf and Chung, 1984; Howell et al., 1984; Mody and Miller, 1985).

The synaptic levels of Zn\(^{2+}\) may reach as high as 200 to 300 \(\mu\text{M}\) in the dentate gyrus during chronic seizures (Cavazos et al., 1991; Buhl et al., 1996). Also, Zn\(^{2+}\) has been reported to induce convulsions and exacerbate kainate-induced neurotoxicity (Pei and Koyama, 1983; Nave and Connor, 1993). These findings suggest that increased brain levels of Zn\(^{2+}\) are likely associated with brain hyperexcitability.

Epileptic seizures alter GABA\(\text{A}\) receptor sensitivity to allosteric modulators. For example, in kindling and other chronic seizure models of temporal lobe epilepsy, Zn\(^{2+}\)-inhibition and benzodiazepine-enhancement of GABA\(\text{A}\) receptor function have been shown to increase in the dentate gyrus (Buhl et al., 1996; Gibbs et al., 1997). Also in acute seizure models of other generalized epilepsy, seizures cause more rapid changes in GABA\(\text{A}\) receptor properties. For example, generalized absence seizures selectively decrease steroid modulation of \([35\text{S}]\text{t-butylbicycrophosphorothionate}\) binding to GABA\(\text{A}\) receptors in thalamus (Banerjee et al., 1998a). During status epilepticus, Zn\(^{2+}\) inhibition and benzodiazepine enhancement of GABA\(\text{A}\) receptor function decrease very rapidly in the dentate gyrus. The development of such rapid seizure-induced loss of functional modulation of

ABBREVIATIONS: GABA\(\text{A}\), \(\gamma\)-aminobutyric acid\(\text{A}\); EEG, electroencephalogram.
GABA<sub>A</sub> receptor function by steroids or benzodiazepines has been suspected to play a role in epileptogenesis or maintenance of seizure activity (Kapur and Macdonald, 1997; Banerjee et al., 1998a). However, it remains unclear whether Zn<sup>2+</sup> inhibition of GABA<sub>A</sub> receptor function has any role in the pathophysiology of status epilepticus or temporal lobe epilepsy.

Most of the previous observations of seizure-induced GABA<sub>A</sub> receptor plasticity have been made in the dentate gyrus because of the presence of high Zn<sup>2+</sup>-containing synaptic terminals in this brain region that are known to undergo aberrant sprouting during chronic epilepsy (Cavazos et al., 1991). Axonal sprouting or aberrant reorganization of neurons is not a phenomenon typical to hippocampus only, but it may occur in other brain regions. For example, increased synaptogenesis or aberrant reorganization of cortical neurons has been observed after focal cortical seizures induced by intracortical kainic acid (Chen et al., 1996). This increase in cortical synaptogenesis occurs in a manner similar to that observed in the dentate gyrus a few weeks after systemic administration of kainic acid (Chen et al., 1996).

Cerebral cortex is rich in intravascular Zn<sup>2+</sup> (Choi and Koh, 1998), and Zn<sup>2+</sup> (100 μM) has been shown to cause cell death in cortical neurons in culture (Choi et al., 1988). Although seizure-induced changes in GABAergic inhibition in hippocampus have been studied extensively, there is no data available whether GABA<sub>A</sub> receptors are altered in the cerebral cortex during status epilepticus. In the present study, we determined whether the modulation of GABA<sub>A</sub> receptor function by Zn<sup>2+</sup> and other allosteric modulators was altered in the cerebral cortex during pilocarpine-induced status epilepticus. For this, we measured the effect of Zn<sup>2+</sup>, pentobarbital, alphaxalone (a neuroactive steroid), and flunitrazepam (a benzodiazepine) on muscimol-stimulated uptake of <sup>36</sup>Cl<sup>-</sup> in cortical and cerebellar synaptoneurosomes prepared from rats undergoing status epilepticus induced by pilocarpine.

The assay of <sup>36</sup>Cl<sup>-</sup> uptake provides a functional measurement of actions of GABA<sub>A</sub> receptor agonists and antagonists, and this assay system responds in a pharmacologically appropriate manner for a chloride channel coupled with a GABA<sub>A</sub> receptor (Harris and Allan, 1985). Moreover, the technique of <sup>36</sup>Cl<sup>-</sup> influx allowed us to examine the majority of the GABA<sub>A</sub> receptor-operated Cl<sup>-</sup> channels, even those on interneurons or on distal dendrites of larger neurons that are difficult to record from using electrophysiologic techniques. The cerebellum was chosen as an internal control because cerebellum stains positively for Zn<sup>2+</sup>, but pilocarpine-induced seizures do not evolve from this brain region.

**Experimental Procedures**

**Materials.** <sup>36</sup>Cl<sup>-</sup> was purchased from New England Nuclear (Boston, MA). Diazepam, zinc chloride, pentobarbital, pilocarpine, scopolamine, and the neuroactive steroid alphaxalone were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were obtained from commercial sources and were of highest available purity.

**Surgery and Electroencephalogram (EEG) Recording of Pilocarpine-Induced Status Epilepticus.** Adult male Sprague-Dawley rats were used in all experiments. Animals were maintained on a 12-h light/dark cycle and given free access to food and water. Four monopolar EEG recording electrodes were surgically implanted bilaterally on the surface of frontal/parietal cortex under halothane anesthesia. Seven days after surgery, EEG recordings were made continuously for 30 to 40 min before inducing seizures, with the animals freely moving in a heated shielded Plexiglas container.

Rats were injected first with scopolamine (1 mg/kg i.p.) to minimize the peripheral effects of pilocarpine. Thirty minutes after scopolamine treatment, pilocarpine seizures were induced by i.p. administration of pilocarpine (320–340 mg/kg). Control rats were also implanted with recording electrodes and had EEG recorded after scopolamine + saline injections. In our study, we defined status epilepticus as occurrence of continuous ictal discharge in the EEG for at least 40 min. Rats exhibiting continuous seizures for 1 and 2 h were sacrificed and their cortices and cerebellas dissected out and processed for <sup>36</sup>Cl<sup>-</sup> uptake assay (see below).

**Diazepam Treatment.** To determine whether the observed change in <sup>36</sup>Cl<sup>-</sup> uptake by Zn<sup>2+</sup> during status epilepticus (see Results below) was mediated by seizures themselves, pilocarpine seizures in a separate group of animals were terminated within 15 to 20 min after the seizure onset by diazepam. Diazepam (10 mg/kg) was administered i.p. 15 min after the onset of pilocarpine seizures. The control group of rats received saline (instead of diazepam) 15 min after the onset of pilocarpine seizures. Both pilocarpine + saline- and pilocarpine + diazepam-treated rats were sacrificed 45 or 105 min after saline or diazepam treatment (for 1- and 2-h time points, respectively), and their cortices were assayed for <sup>36</sup>Cl<sup>-</sup> uptake.

**Preparation of Synaptoneurosomes.** Cortical and cerebellar synaptoneurosomes were prepared by the method of Buck and Harris (1990). Briefly, the dissected tissue was homogenized by hand (10–12 strokes) in ice-cold assay buffer containing 145 mM NaCl, 5 mM KCl, 1 mM MgCl<sub>2</sub>, 10 mM d-glucose, 1 mM CaCl<sub>2</sub>, and 10 mM HEPEs (pH 7.5). The homogenate was filtered through three layers of nylon mesh (160 μm) and the filtrate was centrifuged at 1000 g for 15 min. The pellet was washed twice with the assay buffer (1000 g for 15 min each) and the final pellet was suspended in assay buffer. Protein content was determined using a modified method of the Lowry assay (Peterson, 1977).

**<sup>36</sup>Cl<sup>-</sup> Uptake Assay.** The uptake of <sup>36</sup>Cl<sup>-</sup> was measured as reported by Buck and Harris (1990). Aliquots (200 μl) of cortical or cerebellar tissue were incubated in the shaking water bath at 34°C for 10 min. Tissue uptake of <sup>36</sup>Cl<sup>-</sup> was then initiated by adding 200 μl of assay buffer containing 0.2 μlCU/ml of <sup>36</sup>Cl<sup>-</sup>. All drugs, including muscimol, were added to the tissue along with <sup>36</sup>Cl<sup>-</sup>. Five seconds later, <sup>36</sup>Cl<sup>-</sup> influx was terminated by adding 4 ml of ice-cold assay buffer containing 100 μM picrotoxin and rapid filtration under vacuum onto premoistened 2.4 cm Whatman GF/C glass microfilter filters. The filters were then washed twice with assay buffer and radioactivity counted. The amount of <sup>36</sup>Cl<sup>-</sup> bound to the filters in the absence of tissue was used as “no-tissue blank” and was subtracted from all values.

**Data Analysis.** <sup>36</sup>Cl<sup>-</sup> uptake was expressed as cpm retained/mg of protein/5 s. The effect of Zn<sup>2+</sup> and other modulators on <sup>36</sup>Cl<sup>-</sup> uptake was determined on muscimol-stimulated <sup>36</sup>Cl<sup>-</sup> uptake. Each membrane uptake assay was performed in triplicate and repeated in four to five different rats. The nonlinear curve fitting of the concentration-effect curves was determined using Frizm (GraphPad, San Diego, CA). In case of Zn<sup>2+</sup> inhibition assays, the data were fit to a partial (bottom plateau > 0) instead of a full (bottom plateau = 0) inhibition model. The maximal extent of inhibition (I<sub>max</sub>) or enhancement (E<sub>max</sub>) was determined as the difference between the top and bottom plateaus of the concentration-effect curves.

**Statistics.** All data are expressed as mean ± S.E. Data comparing the effects of allosteric modulators on <sup>36</sup>Cl<sup>-</sup> uptake in epileptic and control animals were analyzed by one-way ANOVA followed by Bonferroni’s test for post hoc comparisons between multiple group means. Two individual group means were compared using a two-tailed, independent Student’s t test.
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Results

Zn<sup>2+</sup> Inhibition of Muscimol-Stimulated <sup>36</sup>Cl<sup>-</sup> Uptake Was Selectively Decreased in the Cortex but Not in Cerebellum during Status Epilepticus. In control cortical and cerebellar synaptoneurosomes, muscimol (1–100 μM) dose-dependently increased the uptake of <sup>36</sup>Cl<sup>-</sup> \( [EC_{50} \sim 5 \mu M] \); maximal extent of enhancement \( (E_{max}) \) was about 170% in cortex and 120% in cerebellum; see Fig. 1. A concentration of muscimol (10 μM) that produced ~120% increase (in cortex) and ~90% increase (in cerebellum) over basal uptake was chosen for subsequent experiments.

Neither basal- nor muscimol (10 μM)-stimulated <sup>36</sup>Cl<sup>-</sup> uptake in the cortex or cerebellum was altered during status epilepticus (at 1- or 2-h time points) (Table 1). In control cortex and cerebellum, Zn<sup>2+</sup> dose-dependently inhibited muscimol-stimulated uptake of <sup>36</sup>Cl<sup>-</sup> \( [I_{max} \sim 26 \pm 6\% \text{ (in cortex)} \) and 23 ± 4% (in cerebellum); Fig. 2]. However, during status epilepticus (both at 1- and 2-h time points), Zn<sup>2+</sup> inhibition of stimulated <sup>36</sup>Cl<sup>-</sup> influx was decreased in the cortex but not in cerebellum \( [I_{max} \sim 9 \pm 3\% \text{ (epileptic cortex)} \) versus 26 ± 6% (control cortex); see Table 2]. This loss of Zn<sup>2+</sup> effect in the cortex was significant at both 10 and 100 μM concentrations \( (P < .01; n = 5 \text{ for each concentration; see Fig. 2]). \) Cortices from pilocarpine-treated rats sacrificed earlier than 1 h (i.e., 20–30 min after the onset of seizures) did not show any significant loss of Zn<sup>2+</sup> response (Table 2).

To determine whether the observed loss of Zn<sup>2+</sup> response in the cortex during status epilepticus was selective to Zn<sup>2+</sup> only, we tested whether the modulation of <sup>36</sup>Cl<sup>-</sup> uptake by pentobarbital, flunitrazepam, or the neuroactive steroid alphaxalone was also altered in the cortex during status epilepticus. In control cortical preparations, both pentobarbital (1–100 μM) and alphaxalone (0.1–10 μM) dose-dependently increased the influx of stimulated <sup>36</sup>Cl<sup>-</sup> \( (E_{max} \sim 30\% \text{ for pentobarbital and } \sim 17\% \text{ for alphaxalone, } n = 4 \text{ in each experiment; see Table 3}). \) Flunitrazepam (1–10 μM) only moderately increased the stimulated uptake of <sup>36</sup>Cl<sup>-</sup> \( (E_{max} \sim 10\%, \ n = 4). \) During status epilepticus (both at 1- and 2-h time points), we did not observe any significant change in the dose response of either pentobarbital or alphaxalone or flunitrazepam (Table 3).

The above results suggested that the decrease in Zn<sup>2+</sup> inhibition of <sup>36</sup>Cl<sup>-</sup> uptake in the cortex (which occurred 1–2 h after the onset of status epilepticus) might have resulted from status epilepticus-induced alterations in the functional properties of GABA<sub>A</sub> receptors. Therefore, we tested whether status epilepticus was critical for the loss of Zn<sup>2+</sup> response in the cerebral cortex. For this, we determined Zn<sup>2+</sup> modulation of muscimol-stimulated uptake of <sup>36</sup>Cl<sup>-</sup> in the cortex of rats where status epilepticus was terminated within 15 min after its onset.

Zn<sup>2+</sup> Inhibition of Muscimol-Stimulated <sup>36</sup>Cl<sup>-</sup> Influx in the Cortex Was Not Altered when Pilocarpine-Induced Status Epilepticus Was Blocked. Both clinical and experimental status epilepticus are blocked by benzodiazepines if administered in the early stages of seizures (Ramsey, 1993). In our study, we attempted to terminate the development of status epilepticus by administering diazepam (10 mg/kg i.p.) within 15 to 20 min after the onset of continuous seizures in the EEG. Within 5 to 10 min after its administration, diazepam completely terminated pilocarpine seizures. Continuous spikes in the EEG were replaced with low-voltage occasional spikes. We sacrificed diazepam-treated rats at 45 and 105 min after diazepam administration (for 1- and 2-h time points, respectively), and found that Zn<sup>2+</sup> continued to inhibit muscimol-stimulated <sup>36</sup>Cl<sup>-</sup> uptake both at 1- or 2-h time-points (Fig. 3). It may be noted that under control conditions, diazepam (up to a concentration of 100 μM) did not significantly enhance stimulated uptake of <sup>36</sup>Cl<sup>-</sup> in either cortical or cerebellar membrane preparations (data not shown). Therefore, we did not run a parallel control group of saline + diazepam to determine whether diazepam alone had any effect on Zn<sup>2+</sup> inhibition of <sup>36</sup>Cl<sup>-</sup> uptake.

Discussion

Our data indicate that Zn<sup>2+</sup> inhibition of GABA<sub>A</sub> receptor function was decreased in the cerebral cortex during pilocarpine-induced status epilepticus. Other modulators of GABA<sub>A</sub> receptors (e.g., barbiturates, steroids, or benzodiazepines), however, continued to increase receptor function in this brain region during status epilepticus. This suggested that the ability of Zn<sup>2+</sup> to inhibit GABA<sub>A</sub> receptor function was selectively decreased in the cortex during pilocarpine seizures. Also, the observed loss of Zn<sup>2+</sup> inhibition of <sup>36</sup>Cl<sup>-</sup> uptake (during seizures) appeared to be mediated by seizures themselves because: 1) when seizures were stopped at 15 to 20 min, Zn<sup>2+</sup> response did not decrease; and 2) the loss of Zn<sup>2+</sup> response was observed only in the cerebral cortex but...
not in the cerebellum, an area from which pilocarpine seizures do not evolve.

Both clinical and experimental status epilepticus are known to cause extensive brain damage (Lemos and Cavalheiro, 1995). Status epilepticus induced by systemic or intra-hippocampal kainic acid produces severe neuropathological changes in the hippocampus and other limbic areas. However, in the kainic acid model, status epilepticus-induced neuronal damage is relatively contained within the limbic structures. In contrast, pilocarpine-induced status epilepticus is a more generalized phenomenon where seizure-induced neuronal damage is more extensive and involves the neocortex and thalamus, as well as the limbic structures (Lemos and Cavalheiro, 1995).

Several studies in the past have shown that GABA-mediated inhibition decreases very rapidly in the CA1 pyramidal neurons during status epilepticus (Kapur et al., 1994; Kapur and Coulter, 1995). Status epilepticus also alters the sensitivity of GABA_A receptors to allosteric modulators, and such seizure-induced changes in GABA_A receptor sensitivity are thought to have important functional consequence. For example, benzodiazepines (which increase GABA_A receptor function in the brain) effectively terminate status epilepticus if given during the early stages of seizures. However, as the seizures progress, benzodiazepines lose their antiepileptic potential (Ramsey, 1993). It seems that the development of such tolerance to benzodiazepine response during status epilepticus may arise from seizure-induced decrease in GABA_A receptor sensitivity to benzodiazepines in the hippocampus (Kapur and Macdonald, 1997). In the present study, we did not observe any significant decrease in either GABA responses or in flunitrazepam (a benzodiazepine) enhancement of 36Cl^- influx in the cortex during status epilepticus. However, hippocampal synaptoneurosomes do show a loss of benzodiazepine enhancement of 36Cl^- influx in rats undergoing pilocarpine-induced status epilepticus, where flunitrazepam fails to enhance stimulated 36Cl^- influx 1 or 2 h after pilocarpine-induced status epilepticus (Banerjee et al., 1998b). This suggests that benzodiazepine enhancement of GABA_A receptor function is selectively altered in the hippocampus, but not in the cerebral cortex during status epilepticus. Although the mechanism for such selective resistance to loss of benzodiazepine effect in the cerebral cortex during status epilepticus is not clear, seizure-induced down-regulation of GABA_A receptor z subunits (which confer benzodiazepine sensitivity to the receptor; Pritchett et al., 1989a) or a subunit isoforms (which determine benzodiazepine pharmacology; Pritchett et al., 1989b) in the hippocampus but not in the cortex is a possibility.

Unlike the benzodiazepine response, we found that Zn^{2+} (which inhibits GABA_A receptor function in the brain) lost its ability to inhibit muscimol-stimulated uptake of 36Cl^- in the cerebral cortex during status epilepticus. No such loss of Zn^{2+} inhibition was observed in cerebellum. A similar loss of Zn^{2+} inhibition of GABA-activated chloride current was found to occur in the dentate gyrus of rats undergoing status epilepticus (Kapur and Macdonald, 1997). Although seizure-induced selective loss of benzodiazepine response in the dentate gyrus may play a role in the development of prolonged and continuous seizures of status epilepticus, it is not clear whether seizure-induced loss of Zn^{2+} inhibition of receptor function in the cortex and hippocampus facilitates seizures or provides an anticonvulsant effect. The findings from chronic epilepsy models suggest that increased Zn^{2+} inhibition of
GABA<sub>A</sub> receptor function is likely associated with brain hyperexcitability. For example, in chronic seizure models of temporal lobe epilepsy, GABA<sub>A</sub> receptor binding, Zn<sup>2+</sup> inhibition, and benzodiazepine enhancement of GABA<sub>A</sub> receptor function all increase in the dentate gyrus (Shin et al., 1985; Buhl et al., 1996; Gibbs et al., 1997). Both increased GABA<sub>A</sub> receptor binding and enhanced functional response to benzodiazepine are indicators of increased inhibition or hypoxecitability. Recent studies suggest that chronic seizure-induced increase in Zn<sup>2+</sup> inhibition of GABA<sub>A</sub> receptor function in the dentate gyrus may, in fact, promote hyperexcitability in the dentate gyrus (and in other related brain regions) by offsetting seizure-induced augmented inhibition (Buhl et al., 1996; Gibbs et al., 1997). By this analogy, it would appear that hippocampal and the cortical GABA<sub>A</sub> receptors that become less sensitive to functional modulation by Zn<sup>2+</sup> during status epilepticus may make the brain less susceptible to seizures.

The possibility that status epilepticus-induced decrease in Zn<sup>2+</sup> inhibition of GABA<sub>A</sub> receptor function in cortex and/or dentate gyrus is somehow acting to terminate pilocarpine seizures is interesting. In rats, a single systemic injection of pilocarpine induces status epilepticus, which typically lasts for about 5 to 6 h. About 2 to 3 weeks after status epilepticus, rats exhibit spontaneous seizures that are believed to model temporal lobe epilepsy (Cavaleiro et al., 1991). In our study, the loss of Zn<sup>2+</sup> inhibition was apparent only about 1 h after the onset of status epilepticus (but not earlier). Therefore, it appeared unlikely that the loss of Zn<sup>2+</sup> response in cortex was causally related to pilocarpine-induced status epilepticus. On the contrary, we suspected that pilocarpine seizures themselves might have caused the loss of Zn<sup>2+</sup> effect. We tested this possibility and determined whether status epilepticus was critical for the development of the loss of Zn<sup>2+</sup> response. We found that in rats where status epilepticus was not allowed to continue for more than 20 to 30 min (by administering diazepam 15 min after the seizures become continuous), the Zn<sup>2+</sup> response in the cortex was completely preserved. This suggested that the observed loss of Zn<sup>2+</sup> response during pilocarpine-induced status epilepticus was indeed caused by seizures. It may also be noted that in our study, status epilepticus did not dissipate as GABA<sub>A</sub> receptors become less sensitive to Zn<sup>2+</sup>. The loss of Zn<sup>2+</sup> inhibition of GABA<sub>A</sub> receptor function was apparent within the first hour of seizures, although seizures typically continued for more than 5 h. Therefore, based on our data, it is unlikely that the loss of Zn<sup>2+</sup> response was responsible for seizure termination in the pilocarpine model of status epilepticus. Although this might be the purpose of change, it is not sufficient to terminate seizures.

The time course for the development of the observed loss of Zn<sup>2+</sup> and benzodiazepine response in the hippocampus and cerebral cortex (during status epilepticus) suggests that the initial 40 to 60 min of seizures of status epilepticus are critical for the development of such loss of drug effects. Interestingly, there is evidence that continuous limbic seizures of status epilepticus (for ~1 h) produce moderate neuronal damage in the hippocampus and cerebral cortex. When status epilepticus is allowed to continue for more than 3 to 4 h, seizure-induced cell damage increases (Lemos and Cavaleiro, 1995; Fujikawa, 1996). Therefore, in the present study the possibility that continuous pilocarpine seizures (for 1 h or more) caused cell damage in the cortex which, in turn, led to the loss of Zn<sup>2+</sup> effect, may be considered. However, this does not explain a selective loss of Zn<sup>2+</sup> (but not of benzodiazepine or steroid) effect. Also, the dentate granule cells (where both benzodiazepine and Zn<sup>2+</sup> effects are reported to be lost during the first hour of status epilepticus; Kapur and Macdonald, 1997) have been found resistant to seizure-induced cell damage during the first few hours of status epilepticus (Lemos and Cavaleiro, 1995; Dube et al., 1998; Motte et al., 1998). This suggests that the selective loss of drug effect on GABA<sub>A</sub> receptor function during the first few hours of status epilepticus may not be related to seizure-induced cell or neuronal damage. It is also interesting to ask how such a relatively rapid change in pharmacology of GABA<sub>A</sub> receptors could occur, although gene expression changes can be triggered rapidly by seizures (e.g., Banerjee et al., 1998c), including GABA<sub>A</sub> receptor subunits. Large changes as observed in the present study are more likely caused by modifications, e.g., phosphorylation of proteins affecting receptor channel.

### Table 3

<table>
<thead>
<tr>
<th>Region/Drugs</th>
<th>36Cl&lt;sup&gt;-&lt;/sup&gt; Uptake (cpm)</th>
<th>Control</th>
<th>Status Epilepticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscimol (10 µM)</td>
<td>960 ± 61</td>
<td>17 ± 6</td>
<td>943 ± 40</td>
</tr>
<tr>
<td>+ Alphaxalone (1 µM)</td>
<td>1114 ± 66</td>
<td>30 ± 5</td>
<td>1142 ± 53</td>
</tr>
<tr>
<td>+ Pentobarbital (100 µM)</td>
<td>1248 ± 53</td>
<td>11 ± 6</td>
<td>1265 ± 41</td>
</tr>
<tr>
<td>+ Flunitrazepam (10 µM)</td>
<td>1065 ± 67</td>
<td>20 ± 5</td>
<td>1027 ± 60</td>
</tr>
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**Fig. 3.** Concentration-dependent inhibition of 10 µM muscimol-stimulated uptake of 36Cl<sup>-</sup> by Zn<sup>2+</sup> in the cortex of rats where status epilepticus was terminated by diazepam within 30 min after its onset. Values are mean ± S.E. from five rats. Termination of status epilepticus restored the "lost" Zn<sup>2+</sup> response in the cortex. *P < .01 when compared with respective control values.

**TABLE 3**

Maximal extent of enhancement (E<sub>max</sub> [%]) of 10 µM muscimol-stimulated uptake of 36Cl<sup>-</sup> by pentobarbital, alphaxalone, and flunitrazepam in nonepileptic (control) and epileptic (2 h after the onset of status epilepticus) cortex.

36Cl<sup>-</sup> uptake is expressed as cpm retained/mg protein/5 s. Values are mean ± S.E. of four independent experiments, each performed in triplicate. The effect of the above drugs on 36Cl<sup>-</sup> uptake did not change significantly 2 h after the onset of status epilepticus.

These data are consistent with the idea that status epilepticus-induced decrease in Zn<sup>2+</sup> inhibition of GABA<sub>A</sub> receptor function in cortex and/or dentate gyrus is somehow acting to terminate pilocarpine seizures.
function directly, or the synaptic localization, clustering, assembly, or turnover of the protein.

References

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