Kainic Acid-Induced Seizures Enhance Dentate Gyrus Inhibition by Downregulation of GABA<sub>B</sub> Receptors

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Seizures cause a persistent enhancement in dentate synaptic inhibition concurrent with, and possibly compensatory for, seizure-induced hippocampal hyperexcitability. To study this phenomenon, we evoked status epilepticus in rats with systemic kainic acid (KA), and 2 weeks later assessed granule cell inhibition with paired-pulse stimulation of the perforant path (PP) \textit{in vitro}. Controls demonstrated three components of paired-pulse inhibition: early inhibition (10–30 msec), intermediate facilitation (30–120 msec), and late inhibition (120 msec to 120 sec). After seizures, inhibition in all components was enhanced significantly. The GABA<sub>A</sub> antagonist bicuculline blocked only early enhanced inhibition, demonstrating that both GABA<sub>A</sub> and GABA<sub>B</sub> postsynaptic receptors contribute to seizure-induced enhanced inhibition. In controls, the GABA<sub>B</sub> antagonist CGP 35348 blocked both GABA<sub>A</sub> and GABA<sub>B</sub> responses in granule cells, suggesting that CGP 35348 acts presynaptically, blocking receptors that suppress GABA release. In contrast, slices from KA-treated rats were markedly less sensitive to CGP 35348. To test the hypothesis that GABA<sub>B</sub> receptors regulating GABA release are downregulated after seizures, we measured paired-pulse suppression of recurrent IPSPs, or disinhibition, using mossy fiber stimuli. Early disinhibition (<200 msec) was reduced after seizures, whereas late disinhibition remained intact. CGP 35348 blocked the early component of disinhibition in controls and, to a lesser extent, reduced disinhibition in KA slices. However, paired monosynaptic IPSPs recorded intracellularly showed no difference in disinhibition between groups. Our findings indicate that seizure-induced enhancement in dentate inhibition is caused, at least in part, by reduced GABA<sub>B</sub> function in the polysynaptic recurrent inhibitory circuit, resulting in reduced disinhibition and heightened GABA release.

**Key words:** hippocampus; dentate gyrus; paired-pulse inhibition; GABA<sub>B</sub> disinhibition; presynaptic; autoreceptors; kainic acid; bicuculline; CGP 35348; epilepsy; seizures

Neuronal alterations produced by seizures have been implicated in promoting cellular hyperexcitability and seizure propagation. In animal models of epilepsy, including kainic acid (KA)-induced status epilepticus (Holmes and Thompson, 1988; Stafstrom et al., 1992; Lothman and Bertram, 1993) and kindling (Goddard et al., 1969; Wong and Moshe, 1987; Holmes et al., 1993), seizures alter the brain, increasing susceptibility to subsequent epileptogenic stimuli. Seizure-induced changes that contribute to this heightened excitability include enhanced NMDA receptor function (Mody et al., 1988; Martin et al., 1992; Kohr et al., 1993; Kohr and Mody, 1994), reduced inhibition in CA1 (King et al., 1985; Kapur et al., 1989) and CA3 (Zhao and Leung, 1992), death of hilar cells that normally excite recurrent inhibitory interneurons (Sloviter, 1987; Cavazos and Sutula, 1990), axonal sprouting, and formation of aberrant excitatory synapses (Sutula, 1988).

In contrast to these persistent changes that promote hyperexcitability, inhibition of dentate gyrus granule cells is lost only transiently during and immediately after seizures (Maru and Goddard, 1987; Milgram et al., 1991; Spiller and Racine, 1994). Within 24 hr, PP-evoked inhibition of granule neurons recovers to levels higher than those preceding KA administration (Milgram et al., 1991) or kindled stimulation (Tuff et al., 1983; King et al., 1985; Oliver and Miller, 1985; de Jonge and Racine, 1987; Maru and Goddard, 1987; Gilbert, 1991; Milgram et al., 1995), although this inhibition may be subject to more rapid fatigue (Sloviter, 1992; Buhl et al., 1996). Little is known about the underlying mechanisms or the functional role of this enhanced inhibition. The concurrent expression of increased dentate inhibition and hyperexcitability throughout the hippocampus (King et al., 1985; Cronin et al., 1992; Zhao and Leung, 1992; Bekenstein et al., 1993) suggests that enhanced inhibition may be a compensatory mechanism to suppress the spread of seizure activity.

Although studies have indicated an increase of late Cl\textsuperscript{−}-independent synaptic potentials (Oliver and Miller, 1985), GABA<sub>A</sub> receptor number- (Otis et al., 1994) or both postsynaptic GABA<sub>A</sub> and GABA<sub>B</sub> receptor response- (Sperber, 1991; de Jonge and Racine, 1987; Gilbert, 1991) enhanced paired-pulse inhibition might not be mediated solely by postsynaptic mechanisms, because there is a complex presynaptic regulation of inhibitory transmission. Repeated stimuli produce a decrease in inhibitory postsynaptic potential (IPSP) amplitude, a phenomenon called disinhibition, produced in large part by activation of presynaptic GABA<sub>B</sub> receptors (Thompson, 1992; Lambert and Wilson, 1993; Pitter and Alger, 1994; Pearce, 1995). GABA<sub>B</sub> receptors that suppress neurotransmitter release have been found on presynaptic terminals of both GABAergic (Davies and Col-
lingridge, 1993; Mott et al., 1993) and glutamateergic (Harrison et al., 1990; Scanziani et al., 1992; Davies et al., 1993) neurons in the hippocampus. Therefore, inhibition of granule neurons also could be enhanced either by raising the excitatory drive on inhibitory interneurons (Collins et al., 1982) or by reducing GABA<sub>B</sub> receptor-mediated suppression of GABA release from inhibitory terminals (Davies et al., 1990; Mott et al., 1993).

In the dentate gyrus of in vitro hippocampal slices, we have used selective antagonists to remove GABA<sub>A</sub> or GABA<sub>B</sub> inhibition to determine which receptor populations show seizure-induced alterations. In addition, extracellular and intracellular recordings from dentate granule neurons were used to examine frequency-dependent disinhibition of IPSPs in the recurrent inhibitory pathway. Our results demonstrate that although both postsynaptic GABA<sub>A</sub> and GABA<sub>B</sub> responses are enhanced by seizures, the underlying mechanism is a decreased GABA<sub>B</sub> receptor-mediated suppression of presynaptic GABA release. Portions of these results have been presented elsewhere (Sperber et al., 1991; Haas et al., 1994).

**MATERIALS AND METHODS**

**KA status epilepticus.** Seizures were induced in 60-d-old, male Sprague-Dawley rats (Taconic Farms, NY) by systemic KA injection (15 mg/kg, i.p.) (K 0250, Sigma, St. Louis, MO). Rats were monitored for seizure behavior for 24 hr after injection. Only rats displaying severe status epilepticus, defined as continuous tonic–clonic seizure behavior for at least 30 min, were used in this study.

**Electrophysiology.** Two weeks after exposure to KA status epilepticus, KA-treated rats (n = 28) and age-matched controls (n = 26) were decapitated under deep ether anesthesia and the brains rapidly removed. The hippocampus plus attached entorhinal cortex were dissected out, submerged in a reservoir containing chilled artificial CSF (ACSF), and decapitated under deep ether anesthesia and the brains rapidly removed.

**Recruitment.** The hippocampus plus attached entorhinal cortex were dissected out, submerged in a reservoir containing chilled artificial CSF (ACSF), and decapitated under deep ether anesthesia and the brains rapidly removed. The hippocampal slices were transferred to an interface perfusion chamber (Haas et al., 1979) and bathed continuously with ACSF containing (in mM): NaCl 126, KCl 5, MgCl<sub>2</sub> 2, Na<sub>H</sub>PO<sub>4</sub> 1.25, NaHCO<sub>3</sub> 26, d-glucose 10, pH 7.2, 32–34°C. Slices were incubated for at least 30 min before recording. For extracellular recording of field potentials, glass microelectrodes (microfilament capillary 1.2 mm outer diameter; ±10 MΩ) (A-M Systems) filled with NaCl (2 M) were placed in the granule cell body layer (stratum granulosum) of the suprapyramidal blade of the dentate gyrus. Bipolar, twisted tungsten stimulating electrodes (tip distance 50 μm) (Frederick Haer) were placed in the perforant path (PP) for orthodromic activation of granule cells and at the CA3–hilar border to stimulate mossy fibers antidromically, and stimuli were applied as DC square pulses (20–400 μA, 100 μsec duration). Extracellular population spikes were recorded with an Axoclamp-2A amplifier, and data were digitized and analyzed on an IBM PC-AT. To block GABA receptors, either the GABA<sub>A</sub> antagonist bicuculline (10 μM) (Tocris Cookson, St. Louis) or the GABA<sub>B</sub> antagonist 3-aminopropyl-diethoxymethyl-phosphonic acid, CGP 53348 (Olpe et al., 1990), 400 μM (a generous gift from Ciba-Geigy, Basel, Switzerland) was bathed applied for 1 hr before electrophysiological recording.

**Intracellular recordings from granule cells were made with glass electrodes filled with 3 m K<sup>+</sup> acetate (120–170 MΩ). Stimulating electrodes were placed in the stratum granulosum proximal to the recording electrode to activate interneurons directly and evoke monosynaptic IPSPs. Excitatory amino acid neurotransmission was blocked with the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX (10 μM)) plus the NMDA receptor antagonist d-APV (40 μM), bath applied for 30 min before recording. Intracellular data were filtered at 3 kHz and sampled at 20 kHz. CNQX and d-APV were purchased from Tocris Neuramin (Bristol, England).

**Stimulation paradigms.** The amplitude and time course of feedforward and feedback inhibition of dentate granule cells were assessed with paired-pulse stimulation of the PP. PP stimuli evoked EPSPs, and action potentials (population spikes) were recorded extracellularly in the stratum granulosum (see Fig. 2A, Control PSI, 10 msec). Two identical stimuli were delivered with interstimulus intervals (ISIs) ranging from 10 msec to 9 sec. Stimulus intensities were chosen such that the first evoked population spike was ~75% of maximum. Paired-pulse inhibition was measured as the ratio of the second, test, population spike (PS2) amplitude to the amplitude of the first, conditioning, population spike (PS1). A decrease in the test spike amplitude compared with the conditioning spike indicated paired-pulse inhibition (Fig. 2A, Control, 10 msec), whereas a relative increase represented facilitation (Fig. 2A, Control, 50 msec). No paired-pulse effects were observed with intervals longer than 10 sec, so the time between pairs of stimuli was maintained at 20 sec to avoid interference between trials.

Disinhibition of recurrent inhibition, the decrease in IPSP amplitude with repeated stimuli, was measured using paired-pulse stimulation of mossy fibers. Stimulation of mossy fibers at the hilar–CA3 border evoked a negative-going antidromic population action potential without an EPSP (see Fig. 5A) and activated feedback inhibition. Because pure IPSPs cannot be directly recorded extracellularly (Bruner and Misgeld, 1993), IPSPs which was inferred from the decrease in amplitude that an antidromic mossy fiber stimulus produced on a subsequent orthodromic PP-evoked population spike (see Fig. 5A, compare amplitude of the PP response alone, left, to the PP response in the MF–PP pair, right). Mossy fiber and PP stimulus intensities were chosen such that the mossy fiber-evoked recurrent IPSP inhibited the orthodromic population spike by 50% at an interpulse interval of 5 msec. To produce disinhibition of recurrent inhibition, paired mossy fiber stimuli were delivered with ISIs ranging from 10 msec to 9 sec, with the second mossy fiber stimulus followed 5 msec later by a test orthodromic stimulus to the PP (see Fig. 5B). The decrease in the second mossy fiber-evoked IPSP was apparent as an increase in amplitude of the test PP-evoked population spike (Fig. 5B) compared with inhibition produced by a single MF stimulation (Fig. 5A, MF–PP).

In intracellular recordings from granule neurons, pure monosynaptic IPSPs were evoked by proximal stimulation of the stratum granulosum in the presence of CNQX (10 μM) and d-APV (40 μM). Disinhibition of monosynaptic IPSPs was examined using paired-pulse stimulations in the stratum granulosum. Intervals between stimulations ranged from 30 to 900 msec. Intracellular recordings were only made from cells with resting membrane potentials more hyperpolarized than ~60 mV, input resistances ~30 MΩ, and overshooting action potentials.

Data from paired-pulse experiments were compared using a one-way ANOVA. Statistical significance of intracellular measurements were determined with the Student’s t test for unpaired data.

**Histology.** Histological examination for cell loss and synaptic reorganization were performed on a subset of the rats used for electrophysiological studies (n = 5 KA treated; 5 controls). Brains were removed and bisected, with half used for paired-pulse electrophysiology as described above. The other half was prepared for histological examination using the Timm silver sulfide stain for mossy fiber terminals and thionin stain for neuronal loss.

The hemissections were immersed in a 0.4% sodium sulfide solution for 20 min followed by overnight fixation in a 1% paraformaldehyde and 1.25% glutaraldehyde solution (Tauck and Nadler, 1985), followed by 24 hr in fixation solution with 30% sucrose. Brains were frozen rapidly with methylbutane (~35°C) and cut horizontally in a cryostat (30 μm sections). The sections were developed in the dark for 45–60 min in a solution of 20% (w/v) gum arabic, 5.6% (w/v) hydroquione, and citric acid/sodium citrate buffer with a solution of 17% silver nitrate. After staining, the sections were dehydrated in alcohol. Alternate sections were hydrated and stained with thionin. The sections then were dehydrated in alcohol and examined microscopically.

**RESULTS**

**KA-induced seizures**

60-d-old rats received a single, systemic, intraperitoneal injection of KA (15 mg/kg), which produced severe tonic–clonic seizures and a high mortality rate (40%). Intermittent seizure behavior and mouth and forelimb clonus developed within 30–60 min after injection, and then progressed to continuous forelimb clonus, with rearing and falling lasting from 30 min to >1 hr. After termination of status, rats continued to express sporadic, short-duration bouts of seizure behavior for 24 hr. All rats examined histologically 2 weeks after seizures (n = 5) showed dense supragranular Timm staining (Fig. 1 A, KA, arrows), indicative of mossy fiber sprouting and aberrant synaptogenesis (Tauck and Nadler, 1985; Sperber et al., 1991), and loss of CA3 pyramidal cells with thionin staining.

**Haas et al. • Seizure-Enhanced Inhibition by Reduced GABA<sub>B</sub> Function**

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KA status epilepticus produces a persistent increase in perforant path inhibition

PP stimulation evoked a positive-going EPSP and a superimposed negative-going population action potential, or spike, in field recordings from the stratum granulosum (Fig. 2A). PP stimulation also activated inhibitory interneurons mediating feedforward inhibition, whereas granule cell discharge evoked recurrent inhibition. Once activated, both feedforward and feedback inhibition suppressed granule cell firing, producing paired-pulse inhibition.

Paired-pulse stimulation of the PP in hippocampal slices from control rats showed a triphasic profile of granule cell inhibition (Fig. 2B, open circles, each point = mean ± SEM). A rapidly activating, short-latency inhibition was present at ISIs ranging from 10 to 30 msec (Fig. 2B, Control, 10 msec). ISIs between 30 and 120 msec caused facilitation of the second population spike (Fig. 2A, Control, 50 msec), whereas longer ISIs (120 msec to 6 sec) produced a late-activating, long-lasting inhibition (Fig. 2A, Control, 200 msec). The time courses of early and late paired-pulse inhibition are consistent with those of GABAA receptor- and GABAB receptor-mediated IPSPs, respectively.

Two weeks after KA-induced seizures, slices from these rats showed a significant increase in paired-pulse inhibition over ISIs ranging from 10 to 500 msec, a duration that encompasses both early and late inhibition, as well as facilitation (Fig. 2B, closed circles, asterisks, p < 0.05, one-way ANOVA compared with controls). This apparent enhancement of inhibition completely eliminated paired-pulse facilitation in slices from KA-treated rats (Fig. 2A, KA, 50 msec). Electrophysiological responses from KA-treated rats otherwise appeared normal; in particular, multiple population spikes were not evoked either by PP or mossy fiber stimulation.

Role of GABAA and GABAB receptors in seizure-enhanced inhibition

The GABAA receptor antagonist bicuculline (10 μM) completely blocked early inhibition in slices from both control and KA-treated rats (Fig. 3). Blockade of GABAA receptor-mediated inhibition by bicuculline produced multiple spiking in response to PP stimulation and unmasked paired-pulse facilitation at short ISIs in both groups. In bicuculline, there was no longer any significant difference in paired-pulse inhibition in control and KA slices at short ISIs (Fig. 3) (ISI < 70 msec). In contrast, late GABAB receptor-mediated inhibition was unaltered by bicuculline (compare ISIs > 90 msec in Figs. 3 and 2B), and the significant difference between slices from control and KA rats was still present at longer ISIs, 70–400 msec (Fig. 3, asterisks, p < 0.05). These results indicate that enhanced early inhibition is caused by increased GABAA receptor activation, whereas KA-induced enhancement of late inhibition is mediated through enhanced GABAB receptor function. Therefore, enhanced inhibition after KA status is not attributable to the selective enhancement of activation of only one subtype of postsynaptic GABA receptor.

The GABAB receptor antagonist CGP 35348 acts as a selective blocker of presynaptic GABAB receptors

To determine the contribution of GABAB receptors to control paired-pulse inhibition and enhanced inhibition in KA-treated rats, the selective GABAB antagonist CGP 35348 was bath applied to slices. CGP 35348 (400 μM) produced a significant increase in control-slice paired-pulse inhibition at ISIs ranging from 10 to 600 msec (Fig. 4A, closed circles, asterisks, p < 0.05 compared with controls without CGP 35348, open circles). The late paired-pulse inhibitory component was not decreased by CGP 35348, as would be expected from a postsynaptic GABAB receptor antagonist. Thus, the drug-induced enhancement of both GABAA and GABAB postsynaptic responses is consistent with selective blockade of presynaptic GABAB receptors, which normally act to suppress GABA release.
The magnitude and time course of CGP 35348-induced increases in inhibition in control slices were very similar to changes produced by KA seizures (compare Fig. 2B, closed circles with Fig. 4A, closed circles). Furthermore, in marked contrast to controls, CGP 35348 was largely ineffective on slices from KA-treated rats (Fig. 4B). The GABA<sub>B</sub> antagonist produced an increase in inhibition only at short ISIs (10–30 msec) (Fig. 4B, asterisks, p < 0.05). These data suggest that CGP 35348- and KA status-mediated enhancement of inhibition might be produced through the same mechanism, a functional downregulation of presynaptic GABA<sub>B</sub> receptors.

**Measuring recurrent disinhibition in control and KA-treated rats**

Extracellular field potential measurements of paired-pulse depression of recurrent IPSPs were carried out to assess directly presynaptic GABA<sub>B</sub> receptor function. Disinhibition, the decrease in IPSP strength with repeated stimuli, has been shown to be mediated, in large part, by the activation of presynaptic GABA<sub>B</sub> receptors (Deisz and Prince, 1989; Thompson and Gahwiler, 1989; Brucato et al., 1992; Mott et al., 1993). Figure 5C illustrates the profile of disinhibition produced by paired mossy fiber stimuli in control hippocampal slices. Pairs of mossy fiber stimuli, which evoked recurrent inhibition of granule cells, increased or decreased the amount of inhibition induced by the second stimulus of the pair, depending on the ISI. This distribution can be measured by the amount of inhibition produced by the pair on a third, PP-evoked response. In control rats, at short ISIs (10–20 msec), the amount of recurrent inhibition was increased, probably because of the summation of GABA<sub>B</sub> IPSPs (Fig. 5C, open circles). ISIs between 50 msec and 3 sec produced a decrease in the amount of inhibition.
GABAB antagonists, suggesting that there are at least two components of disinhibition mediated by separate mechanisms. Similar to its effect on the orthodromic paired-pulse profile in controls (Fig. 4A), the blockade by CGP 35348 of control disinhibition looked very similar to the decrease in disinhibition seen in the absence of drug in slices from KA-treated rats. CGP 35348 still produced some additional depression of disinhibition in slices from KA-treated rats (Fig. 6B, asterisks, p < 0.05). The percent decrease was markedly less than that seen in CGP 35348-treated controls, suggesting that KA seizures already had caused a functional downregulation of the contribution of presynaptic GABA_B receptors to paired-pulse IPSP disinhibition.

Disinhibition of monosynaptic IPSPs recorded intracellularly is not reduced by KA treatment

Given the extracellular data described above, we recorded intracellularly in granule cells to examine directly disinhibition of monosynaptic IPSPs. Intracellular parameters including resting membrane potential (mean ± SEM, control −77.5 ± 1.7 mV, KA −79.6 ± 3.2 mV) and input resistance (control 78.3 ± 8.4 Ω, KA 87.5 ± 6.8 Ω) were not significantly different (Student’s t-test for unpaired measures). Monosynaptic IPSPs were readily elicited by stimulation of the stratum granulosum close to an intracellularly impaled granule cell (Fig. 7) in the presence of CNXQ (10 μM) and D-APV (40 μM). Monosynaptic IPSPs were composed of an early and a late component. The early component of these IPSPs reversed at −71.2 ± 1.4 mV (controls) and −69.7 ± 2.8 mV (KA), and was blocked completely by the GABA_A receptor antagonist bicuculline (10 μM).

Pairs of stratum granulosum stimulations proximal to the recording electrode evoked paired-pulse inhibition, or disinhibition, of the second monosynaptic IPSP (Fig. 8). Disinhibition was observed in slices from both control and KA status rats, with no significant difference between groups, indicating that the changes in inhibition induced by KA are not attributable to a functional decrease in GABA_B receptors on interneuron terminals that mediate recurrent inhibition directly on granule neurons.

**DISCUSSION**

Although others have described an increase in paired-pulse inhibition after seizures (Tuff et al., 1983; King et al., 1985; Oliver and Miller, 1985; de Jonge and Racine, 1987; Maru and Goddard, 1987; Milgram et al., 1991; Sperber et al., 1991), the locus for this change has not been determined. Up to now, it has not been clear whether enhanced granule cell inhibition is produced by a heightenened postsynaptic response to GABA or an increase in inhibitory synaptic transmission impinging on granule cells. Although extensive mossy fiber sprouting into supragranular layers is also present 2 weeks after seizures (Sperber et al., 1991), the possibility that these new collaterals drive inhibitory interneurons to produce enhanced inhibition is not supported by the time courses of these alterations. Enhanced paired-pulse inhibition arises within 24 hr after seizure termination, plateaus rapidly, and returns to prespike levels after 4–5 weeks (de Jonge and Racine, 1987; Maru and...
In contrast, aberrant supragranular Timm staining requires at least 4 days to develop, increases in intensity over several weeks, and appears to be permanent (Cavazos et al., 1991; Mello et al., 1993; Okazaki et al., 1995). The new mossy fiber collaterals appear to terminate on granule neurons, not inhibitory basket cells (Represa et al., 1993; Okazaki et al., 1995), forming new excitatory feedback inputs to granule cells (Tauck and Nadler, 1985; Cronin et al., 1992). Indeed, enhanced granule cell inhibition might function to counter this new excitation directly. Cronin et al. (1992) have observed that dentate responses in slices from KA status rats appear normal in control solutions, but exhibited abnormal hyperactivity when GABAergic inhibition was blocked. The rapid development of enhanced inhibition suggests that it may be independent of extensive anatomic alteration.

Results differ about whether seizures enhance early [GABA_A receptor-mediated inhibition (Milgram et al., 1991; Otis et al., 1994; Spiller and Racine, 1994)], late [GABA_B receptor-mediated inhibition (Oliver and Miller, 1985)], or both (Tuff et al., 1983; de Jonge and Racine, 1987; Gilbert, 1991; Sperber, 1991). Oliver and Miller (1985) found that kindling selectively enhances a Cl^{-}-independent late component of paired-pulse inhibition, consistent with GABA_B receptor enhancement, whereas Otis and Mody (1994) described a kindling-induced enhancement of postsynaptic GABA_A receptor activation, possibly attributable to an increase in GABA_A receptor number. In the present studies, we show that...
KA seizure-induced enhanced inhibition encompasses both early and late components of paired-pulse inhibition, suggesting that there is an increased postsynaptic activation of both GABA_A and GABA_B receptors.

We assessed the relative involvement of GABA_A and GABA_B inhibition and paired-pulse facilitation in enhanced inhibition by the selective removal of the early inhibitory component. Blockade of GABA_A inhibition with bicuculline unmasked paired-pulse facilitation (Steffensen and Henriksen, 1991) in both controls and KA-treated rats. Because paired-pulse modulation represents the summation of inhibition and facilitation, the apparent enhancement of inhibition also could be attributable to a decrease of paired-pulse facilitation. Facilitation, produced by accumulation of Ca^{2+} in presynaptic terminals (Zucker, 1993) or NMDA receptor activation (Joy and Albertson, 1993), has a time course consistent with the apparent enhancement of inhibition after seizures. However, our data show that paired-pulse facilitation was not different after KA seizures and, therefore, did not contribute to enhanced inhibition. Because bicuculline blocked only the early component of seizure-enhanced inhibition, while not affecting the late phase, we conclude that enhanced inhibition after KA seizures consists of increases in postsynaptic responses of both GABA_A and GABA_B receptors, and not a decrease in facilitation or granule cell excitability.

The differences in GABA_A and GABA_B receptor structure (Barnard et al., 1992; Kuriyama et al., 1993; Stephenson, 1995) and signal transduction mechanisms (Alger and Nicoll, 1982; Schofield et al., 1987; Dutar and Nicoll, 1988) make it less likely that the enhancement of both responses is attributable to a single postsynaptic modification. A simpler hypothesis is that inhibitory output of GABAergic interneurons is increased after KA seizures, producing enhanced levels of synaptic GABA release that
act indiscriminately on both GABA_A and GABA_B receptors. This hypothesis is supported by our data using the GABA_B antagonist CGP 35348. Although a blocker of postsynaptic GABA_B receptors on granule cells would be expected to remove late paired-pulse inhibition selectively, CGP 35348 increased equally both early and late inhibition. These results demonstrate that CGP 35348 blocks GABA_B receptors that normally suppress GABA release. The similarity in time course of CGP 35348- and KA seizure-induced enhancement of inhibition, as well as the relative ineffectiveness of CGP 35348 to enhance inhibition further in slices from KA-treated rats, suggests that both treatments alter inhibition through the same mechanism, i.e., a reduction in functional activation of GABA_B receptors presynaptic to granule neurons.

Presynaptic GABA_B receptors have been shown to be responsible, in part, for the suppression of IPSPs during repeated stimulation, a phenomenon called disinhibition (Deisz and Prince, 1989; Thompson and Gahwiler, 1989; Brucato et al., 1992; Mott et al., 1993; Lambert and Wilson, 1994; Olpe et al., 1994). The involvement of a population of GABA_B receptors that regulates GABA release is supported further by our finding that a CGP 35348-sensitive component of disinhibition of recurrent inhibition is decreased after KA status. Disinhibition in slices from KA status rats and control slices treated with CGP 35348 was decreased significantly at ISIs from 30 to 180 msec, whereas disinhibition at longer intervals was unaltered. The selective loss of an early component of disinhibition by the downregulation of mechanisms that suppress GABA release would lead to greater than...
normal GABA output and enhanced postsynaptic inhibition. The ability of CGP 35348 to further reduce disinhibition in slices from KA-treated rats, but to a lesser extent than controls, demonstrates that although GABAB autoreceptor function may be downregulated, it was not completely absent. The effects of KA seizures and CGP 35348 support the theory that there are at least two components to disinhibition, one seizure- and CGP 35348-sensitive, at ISIs shorter than 200 msec, and one seizure- and CGP 35348-insensitive, at intervals longer than 200 msec (Lambert and Wilson, 1994; Olpe et al., 1994). The lack of CGP 35348- or KA-induced reduction in disinhibition at ISIs of 10–20 msec may suggest that an alternative mechanism underlies enhanced inhibition at short intervals.

There are three GABAB receptor populations presynaptic to granule neurons in the polysynaptic feedback inhibitory circuit that could control disinhibition and for which downregulation would enhance inhibition: (1) autoreceptors on GABAergic terminals synapsing directly on granule cells (Davies et al., 1990; Mott et al., 1993), (2) presynaptic receptors on glutamatergic terminals, which synapse on inhibitory interneurons (Collins et al., 1982), and (3) somatic or dendritic receptors directly on inhibitory interneurons (Misgeld et al., 1989). In the hippocampus, the release of both GABA (Davies et al., 1990; Mott et al., 1993) and glutamate (Harrison et al., 1990) has been shown to be suppressed by the activation of GABAB receptors on presynaptic terminals. Our finding that paired monosynaptic IPSPs did not show a decrease in disinhibition after KA status discounts a role for autoreceptors on the presynaptic GABAAergic terminals synapsing directly on granule neurons. In contrast, Buhl et al. (1996) have reported recently that kindling did produce a decrease in disinhibition of monosynaptic GABAB-mediated IPSCs in granule cells, suggesting that altered GABAB autoreceptor function may contribute to enhanced inhibition in kindled seizures.

Although GABAB autoreceptors directly presynaptic to granule cells have been shown to be involved in disinhibition, other GABAB receptor populations in the polysynaptic inhibitory pathway also mediate frequency-dependent suppression of inhibition. For example, the GABAB receptor agonist baclofen depresses polysynaptic IPSPs to a greater extent than monosynaptically evoked IPSPs (Mott et al., 1993). GABA release from interneurons synapsing on granule cells can be modulated by GABAB receptors at excitatory and inhibitory inputs to these interneurons. In the polysynaptic recurrent inhibitory circuit, mossy fiber collaterals either directly activate inhibitory interneurons or activate glutamatergic mossy cells, which then drive the interneurons. Activation of presynaptic GABAB receptors on mossy fiber terminals or axonal terminals of mossy cells would suppress glutamate release and, therefore, decrease excitation of inhibitory interneurons (Collins et al., 1982). A decrease of these presynaptic GABAB receptors on glutamate terminals would allow more glutamate release and heightened activation of interneurons. Such an increase in excitatory activation of inhibitory interneurons has also been demonstrated after kindling (Buhl et al., 1996). However, Misgeld et al. (1989) found that the GABAB agonist baclofen did not effect mossy fiber-evoked EPSPs in hilar inhibitory interneurons, suggesting an absence of GABAB receptors on these glutamatergic synapses. Interneuron excitability also is controlled by GABAergic synapses from other inhibitory neurons (Scharfman et al., 1990). GABAB agonists hyperpolarize hilar interneurons, which mediate recurrent inhibition in granule cells (Misgeld et al., 1989). Thus, it is possible that downregulation of postsynaptic GABAB receptors on interneurons mediates the KA-induced loss of disinhibition and enhanced paired-pulse inhibition.

In conclusion, our data indicate that the mechanism for seizure-induced enhancement of dentate inhibition is, at least in part, through downregulation of GABAB receptors in the polysynaptic recurrent inhibitory circuit. These GABAB receptors normally cause suppression of GABAergic transmission, or disinhibition, with repetitive stimulation. Downregulation of a single receptor population could be induced rapidly, consistent with the time course of the appearance of enhanced inhibition, and would not require slower mechanisms such as sprouting and synaptogenesis.
Such a mechanism would be well suited to enhance both early and late inhibition during trains of inputs, as might be produced in a hyperexcitable, epileptic circuit. Because enhanced inhibition develops while other seizure-induced alterations promote excitabil-

ity elsewhere in the hippocampus, this may be an important compensatory mechanism in limiting seizure spread.

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Figure 8. Disinhibition of monosynaptic IPSPs in dentate granule cells (holding potential = −100 mV). A, Overlaid traces from 17 paired-pulse trials with ISIs ranging from 50 to 900 msec. The amplitude of the second IPSP was depressed compared with the first at all ISIs in slices from control and KA-treated rats. Seven-point smoothing was done to limit noise for overlaying traces. B, Paired-pulse profile of suppression of monosynaptic IPSPs at ISIs ranging from 30 msec to 9 sec. There was no significant difference in the monosynaptic disinhibition between controls (open circles, n = 9) and slices from KA-treated rats (closed circles, n = 13).

Such a mechanism would be well suited to enhance both early and late inhibition during trains of inputs, as might be produced in a hyperexcitable, epileptic circuit. Because enhanced inhibition develops while other seizure-induced alterations promote excitabil-


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