Presynaptic $GABA_B$ Receptors on Glutamatergic Terminals of CA1 Pyramidal Cells Decrease in Efficacy After Partial Hippocampal Kindling

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ABSTRACT We tested the hypothesis that presynaptic $GABA_B$ receptors on glutamatergic terminals ($GABA_B$ heterosynaptic receptors) decreased in efficacy after partial hippocampal kindling. Rats were implanted with chronically indwelling electrodes and 15 hippocampal afterdischarges were evoked by high-frequency electrical stimulation of hippocampal CA1. Control rats were implanted with electrodes but not given high-frequency stimulations. One to 21 days after the last afterdischarge, excitatory postsynaptic potentials (EPSPs) were recorded in CA1 of hippocampal slices in vitro, following stimulation of the stratum radiatum. Field EPSPs (fEPSPs) were recorded in CA1 stratum radiatum and intracellular EPSPs (iEPSPs) were recorded from CA1 pyramidal cells. $GABA_B$ receptor agonist ± baclofen (10 μM) in the bath suppressed the fEPSPs significantly more in control than kindled rats, at 1 or 21 days after kindling. Similarly, baclofen (10 μM) suppressed iEPSPs more in the control than the kindled group of neurons recorded at 1 day after kindling. Suppression of the fEPSPs by 1 μM N^6-cyclopentyladenosine, which acted on presynaptic A1 receptors, was not different between kindled and control rats. Activation of the $GABA_B$ heteroreceptors by a conditioning burst stimulation of CA3 afferents suppressed the iEPSPs evoked by a test pulse. The suppression of the iEPSPs at 250–500 ms condition-test interval was larger in control than kindled groups of neurons. It was concluded that the efficacy of presynaptic $GABA_B$ receptors on the glutamatergic terminals was reduced after partial hippocampal kindling. The reduction in heterosynaptic presynaptic $GABA_B$ receptor efficacy will increase glutamate release and seizure susceptibility, particularly during repeated neural activity. **Synapse 59:125–134, 2006.**

INTRODUCTION

In addition to $GABA_A$ receptors, $GABA_B$ receptors are also important in the control of neuronal excitability and stability. The importance of $GABA_B$ receptors in neuronal stability is underscored by the presence of spontaneous generalized seizures in $GABA_B$ receptor knock-out mice (Prosser et al., 2001; Schuler et al., 2001). In addition, $GABA_B$ receptor antagonist induced partial and convulsive seizures (Leung et al., 2005; Vergnes et al., 1997). Among the $GABA_B$ receptors, the presynaptic heterosynaptic $GABA_B$ receptors on glutamatergic terminals may play a particularly important role in controlling glutamate release (Isaacson et al., 1993) and seizure susceptibility.

Presynaptic $GABA_B$ receptors are found at axon terminals where they mediate presynaptic inhibition on either excitatory or inhibitory terminals (Bowery, 1993; Mott and Lewis, 1994; Thompson et al., 1993; Yamada et al., 1999; Ziakopoulos et al., 2000). Presynaptic $GABA_B$ inhibition decreases neurotransmitter release likely through a decrease in presynaptic Ca^{2+} influx (Misgeld et al., 1995; Pfrieger et al. 1994).

Kindling of the hippocampus or amygdala is an animal model of temporal lobe epilepsy (Goddard et al.,

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1969). Spaced and repeated stimulations of the brain evoke afterdischarges (ADs) that increase progressively in duration and in the accompanying clinical/behavioral symptoms. Kindling to the stage of generalized seizures is called full kindling, while delivery of repeated ADs without evoking convulsions is called partial kindling. Partial hippocampal kindling is a model of repeated temporal lobe seizures, since these seizures do not often show secondarily generalization (Engel, 1989). Progression of partial kindling can be seen in the increase in evoked AD duration (Leung et al., 1994) and in the subsequent savings in ADs to achieve full kindling (Dennison et al., 1995).

We have shown that presynaptic $GABA_B$ receptors on inhibitory terminals in hippocampal CA1 were decreased in efficacy for at least 3 weeks after partial hippocampal kindling (Wu and Leung, 1997), while the sensitivity of postsynaptic $GABA_B$ receptors was unchanged (Liu and Leung, 2003). Haas et al. (1996) inferred that kainic-acid induced seizures enhanced dentate gyrus inhibition by downregulation of $GABA_B$ receptors, which could be either the presynpatic $GABA_B$ receptors on glutamatergic terminals synapsing on GABAergic interneurons or the postsynaptic $GABA_B$ receptors on the interneurons. More specifically, full amygdala kindling decreased the sensitivity of $GABA_B$ receptors on glutamatergic terminals in the basolateral amygdala (Asprondini et al., 1992), and status epilepticus induced a loss of $GABA_B$ presynaptic receptors on glutamatergic mossy fiber terminals in CA3 (Chandler et al., 2003).

In this study, we studied the function of presynaptic $GABA_B$ receptors in the hippocampal slice in vitro by activating them with baclofen and synaptically released GABA. The release of glutamate was measured indirectly by means of excitatory postsynaptic potentials (EPSPs), recorded intra- and extracellularly. We hypothesize that $GABA_B$ presynaptic receptors on glutamatergic terminals in hippocampal CA1 area decrease in sensitivity after partial hippocampal kindling in vivo.

METHODS

The partial hippocampal kindling procedure has been described elsewhere (Leung et al., 1994). Briefly, under sodium pentobarbital (65 mg/kg i.p.) anesthesia, two chronically indwelling electrodes were implanted bilaterally across the CA1 cell layer, with the ventral electrode at stratum radiatum of dorsal hippocampal CA1 (3.5–4 mm posterior to bregma, 2.7–3 mm from the midline, with a flat skull). After at least 1 week of recovery from surgery, behaving rats were given train stimulation (1 s at 100 Hz) at the stratum radiatum electrode, and the hippocampal EEG was recorded during the stimulation. The intensity used for train stimulation was ~3 times the commissural evoked response threshold, typically ranging from 100 to 300 μA (pulse duration 0.2 ms). In the kindled group, hippocampal ADs were evoked hourly, five times a day for 3 days. Two control groups were used. In the low-frequency control group, 100 pulses of similar intensity given to the kindled group were delivered at 0.17 Hz at the same intervals as the kindled group. In the no-stimulation control group, the rats were handled, but no train stimulations were given. Recordings were performed in the hippocampal slice in vitro at 1 or 21 days after the last AD or control treatment. There was no difference in the results obtained between the control groups, so in the following, only the average of all the control rats was given.

Transverse hippocampal slices of about 450-μm-thick were prepared, as described elsewhere (Leung et al., 1994; Leung and Yim, 1991). The slices were incubated in an interface chamber, and perfused with 32°C artificial cerebrospinal fluid (aCSF), saturated with 95% O_2–5% CO_2, with: NaCl 124 mM, KCl 5 mM, $NaH_2PO_4 \cdot H_2O$ 1.25 mM, $MgSO_4 \cdot 7H_2O$ 2 mM, $CaCl_2 \cdot 6H_2O$ 2 mM, $NaHCO_3$ 26 mM and glucose 10 mM. Extracellular recordings were made using 2 M Na acetate microelectrodes of about 10 Mohms resistance, and the recordings were routinely made in stratum radiatum following stimulation of the same layer about 0.3 mm away on the CA3 side (Fig. 1A). Typically, a pair of pulses was given to stratum radiatum electrode at 100 ms interpulse interval (IPI) so as to assess paired-pulse facilitation of the responses. The field excitatory postsynaptic potentials (fEPSPs) had to be stable for ≥15 min in normal CSF before CSF containing ± baclofen (0.1–10 μM) or 1 μM N^6-cyclopentyladenosine (CPA) was perfused. Baclofen and CPA were ordered from Sigma, St. Louis, MO. Specific A1 receptor agonist CPA at 1 μM was reported to reduce fEPSPs in CA1 to 24% of the baseline (Wu and Saggau 1994). Evoked responses were digitized (typically four sweeps were averaged) and stored at 10 KHz by a 12-bit analog-to-digital converter, using custom software. Input–output curves of the fEPSPs in response to various stimulus intensities (1, 1.5, 2, and 4 times, and sometimes 6, threshold intensity) were obtained at −5 min and 20 min after the perfusion of the drug. Paired-pulse facilitation of the fEPSPs was measured by the ratio of the second EPSP slope (E2) to that of the first slope (E1) (Fig. 1B).

Intracellular recordings were made using micropipettes filled with 2 M potassium acetate of 80–200 Mohm resistance. Impalements were made at the CA1 cell layer, and only neurons with >65 mV spike height were recorded. Input resistance (Rin) and time constant were measured from the response to a long-duration (>180 ms) hyperpolarizing current of 0.1–0.2 nA (Leung and Yim, 1991). Some neurons were also impaled with micropipettes containing QX-314 (0.2 M) in the K acetate, which blocked the postsynaptic

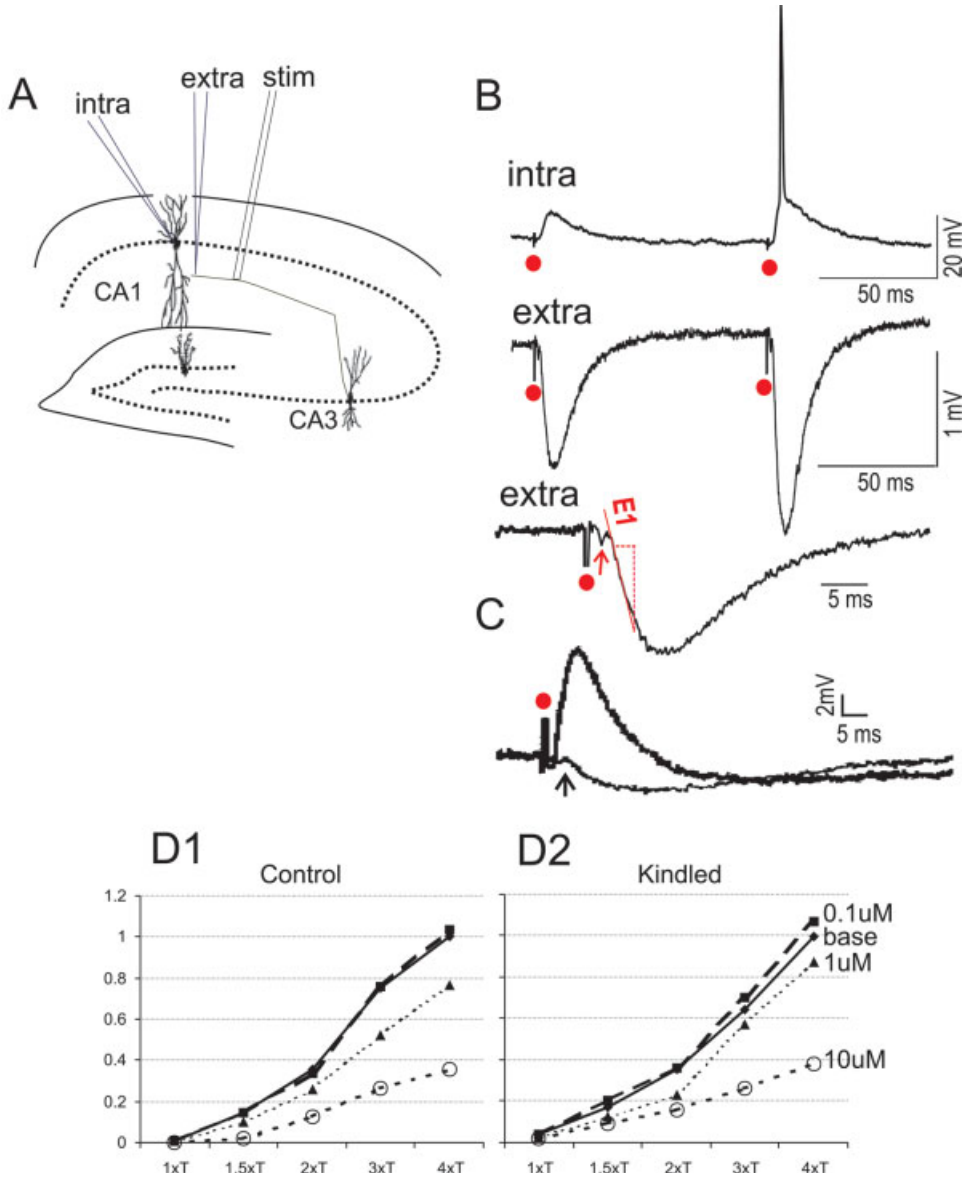

Fig. 1. Recording and measuring field and intracellular excitatory postsynaptic potentials (EPSPs) in hippocampal CA1 in vitro. **A:** Schematic diagram to illustrate the placement of stimulating and recording electrodes in CA1. Stimulating electrode (stim) in CA1 stratum radiatum near CA3 side evoked a negative field EPSP (fEPSP) at an extracellular (extra) electrode in the same layer and EPSPs recorded intracellular from a micropipette at the cell layer (intra). Shock artifacts indicated by filled (red) circles. **B:** Intracellular and extracellular EPSP recorded in a representative slice following paired pulse stimulation; lowest trace is expanded extracellular fEPSP showing presynaptic volley (red up arrow) and measurement of fEPSP slope (E1). **C:** Intracellular EPSPs recorded in a CA1 pyramidal cell before (thick dark trace) and after (thin lower trace) the perfusion of AMPA receptor antagonist CNQX (20 μM). The onset of the inhibitory postsynaptic potential (up arrow) was delayed from the onset of the EPSP by 1.6 ms. **D:** Dose response of baclofen was tested on the fEPSPs of 4 control (D1) and 3 "kindled" slices day 1 after partial kindling (D2).

$GABA_B$ mediated response (Andrade, 1991; Nathan et al., 1990) as well as voltage-dependent Na^+ channels (Leung and Yim, 1991). Other neurons were perfused with a medium with 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) added in order to block α-amino-3-hydroxyl-5-methyl-isoxazole-4-propionate (AMPA) receptor mediated EPSPs. Input-output curve of intracellular EPSPs were recorded following paired-pulse stimulation of the stratum radiatum at 100 ms IPI. The rising slope of the intracellular EPSPs (iEPSPs) was determined after the first pulse (iE1) and the second pulse (iE2). A small hyperpolarizing current (<0.15 nA) was typically injected in order to maintain the membrane potential at −70 to −75 mV. The small hyperpolarizing current served a purpose to reduce the contribution of the $GABA_A$ receptor mediated Cl^- current, which was minimal at approximately −72 mV, and also to reduce spiking and increase stability. When stable EPSPs were obtained for >10 min, baclofen (10 μM) was perfused, and Rin and EPSPs were recorded at 15–25 min. Baclofen induced a slight hyperpolarization, which was compensated by injecting an additional steady-state depolarization current after baclofen.

For the study of synaptically released GABA on $GABA_B$ heterosynaptic receptors, the procedure of Isaacson et al. (1993) was used. Bicuculline methiodide (20 μM) and ±2-amino-5-phosphonovalerate (D,L-APV; 50 μM) for blocking $GABA_A$ and *N*-methyl-D-aspartate (NMDA) receptors respectively, were added to the perfusate. Two separate stimulating electrodes (S1 and S2) were placed in the stratum radiatum, on either side of the recording electrode (Fig. 1A). The

conditioning train stimulus was given to one electrode (S2) and a test-pulse was given to the other electrode (S1) at various condition-test intervals (150, 250, 500, 1000, and 3000 ms). A steady-state hyperpolarizing current (0.1–0.2 nA) was typically applied so as to keep the test EPSP below the firing threshold, using S1 test pulses of ~1.5 times stimulus threshold (15–50 μA) and 0.1–0.2 ms duration. Rising slope and the peak of the iEPSPs were analyzed. The competitive $GABA_B$ receptor antagonist CGP35348 (500 μM), gift from Novartis Pharma, was subsequently added to the perfusate in some experiments.

Determination of EPSP slope parameters and dose of baclofen

To optimize the measurement of the rising slope of the iEPSPs, the delay of the IPSP from the onset of the iEPSP was determined from intracellular recordings of four putative pyramidal cells in CA1. Applying 1.5 times threshold stimulus intensity to stratum radiatum evoked an iEPSP with onset latency of 1.7 ± 0.2 ms ($n = 4$). Perfusion of 20 μM of CNQX abolished the iEPSP and revealed the underlying IPSP (Fig. 1B). The delay of the IPSP onset (after CNQX) from the onset of the original iEPSP was 2.6 ± 1.4 ms ($n = 4$; range 1.1–4.2 ms). As illustrated in Figure 1B, the IPSP rose slowly to a maximal hyperpolarization in ~15 ms after onset. Thus, we measured the slope of the iEPSP over an interval of 1 ms within 0.5 ms of its onset, since the evoked IPSP was negligible within this interval. The same slope measure was applied to the field EPSPs, since the extracellular and intracellular EPSPs have similar onset latencies (Fig. 1B).

The dose of baclofen in the perfusate needed to suppress fEPSPs was determined in pilot experiments in slices each from three kindled (1 day after kindling) and four control rats. At 4 times threshold stimulus intensity, the fEPSPs at 20 min after 0.1, 1, and 10 μM dose of baclofen were 102, 78, and 36% of the baseline fEPSP values in the control group (Fig. 1D1). The average suppression was slightly lower in the kindled group (Fig. 1D2), but the sample size was not sufficient to show statistically significant difference between kindled and control groups. Suppression of E1 was not different among stimulus intensities (1.5–6 times threshold). Baclofen at 0.1 and 1 μM did not significantly suppress fEPSPs in control or kindled slices. In some slices, 1 μM baclofen increased fEPSPs slightly. Thus, 10 μM baclofen was the dose used in most experiments reported below.

RESULTS

Partial hippocampal kindling

Hippocampal AD duration increased during hippocampal kindling in awake rats, indicating the progression of epileptogenesis. In 23 rats used for the following experiments, the first AD measured 23.3 ± 1.6 s in duration, and it increased to 39.5 ± 2.9 s by the 5th AD, 63.3 ± 4.6 s by the 10th AD, and 87.5 ± 4.9 s (mean ± standard error of the mean; $n = 23$ for all AD durations) by the 15th AD. Repeated measure ANOVA confirmed that the AD durations were significantly different (F (3, 66) = 81.1, $P < 0.001$) with significant differences in duration between any pairs of AD durations from the 1st, 5th, 10^{th}, and 15th AD ($P < 0.01$, Newman-Keuls post hoc test). No behavioral convulsions were observed during the 15 ADs.

Field EPSPs in CA1 of control and kindled rats and response to baclofen

At low intensity, single stimulus applied to stratum radiatum evoked a negative wave at the same layer in CA1 (Fig. 1B). The negative wave corresponded to the fEPSP that reversed to a positive wave at the cell body layer (Leung and Au, 1994). The threshold for a visually detectable response was 20 ± 1 μA in normal aCSF ($n = 68$ slices), and this threshold was not significantly different between kindled and control slices. The size of the presynaptic volley (arrow in Fig. 1B) was also not significantly different between kindled and control groups, at day 1 or 21 after partial hippocampal kindling. At 2 times threshold stimulus intensity, the fEPSP slope (E1) was 0.52 ± 0.04 mV/ms ($n = 68$). The peak of the fEPSP was ~2 mV, but since this peak was sometimes obscured by the population spike, it was not analyzed. The fEPSP slope E1 or the E2/E1 ratio, while perfused with normal aCSF, was not significantly different between kindled and control groups.

Hippocampal slices were recorded from two groups of control rats, each consisting of five rats recorded on 1 and 21 days after control treatment. For the two control groups, the stimulus threshold and the E1 response were not different ($P > 0.15$, Wilcoxon), and the E1 response to 10 μM baclofen was also not different (F (1, 35) = 0.27, $P > 0.6$). Thus, the 20 slices from day 1 control group and the 17 slices from day 21 control group were combined into a single control group.

Baclofen (10 μM) decreased the fEPSPs evoked at 2 times threshold stimulus intensity (Fig. 2A). At 2–3 min after perfusion with 10 μM baclofen, the slope of the first-pulse fEPSPs (E1) typically increased (not shown). However, by 5 min after perfusion, the slope of the fEPSPs decreased until it reached a minimum at 15–25 min. At 20 min after baclofen, control slices ($n = 37$ slices) reached a steady level of 32.5 ± 2.4% of the baseline E1 value, significantly smaller than 44.5 ± 3.5% of baseline in 24 slices from five kindled rats recorded 1 day after kindling. The presynaptic volley (Fig. 1B) was decreased after baclofen, but this

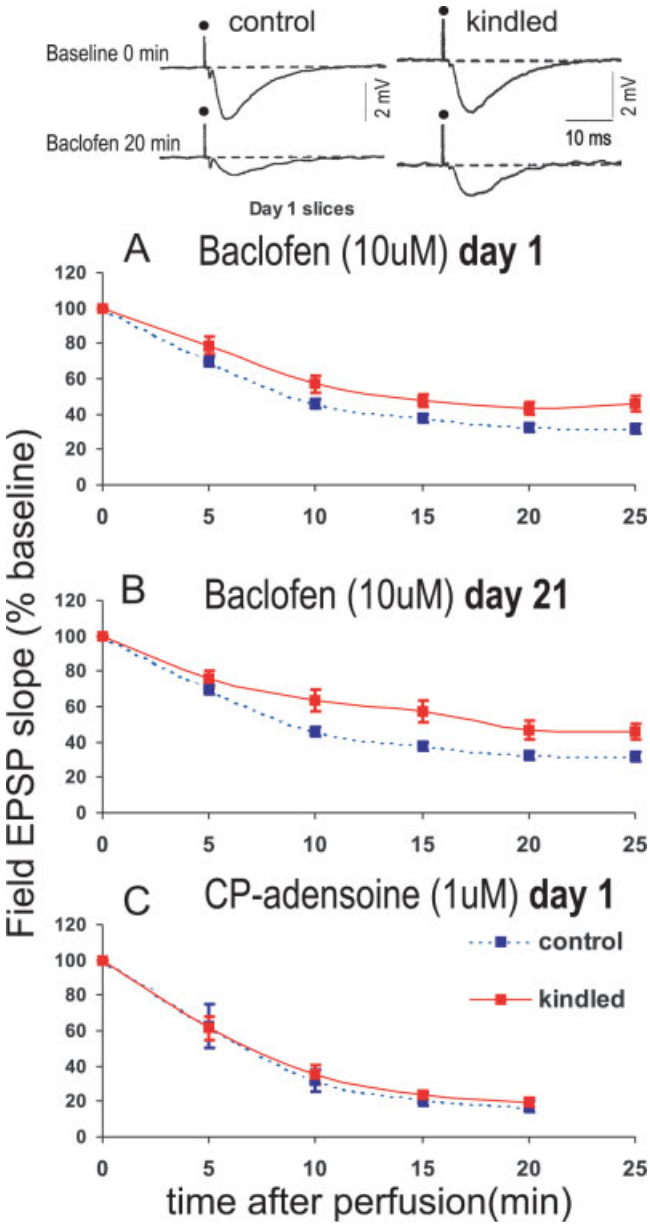

Fig. 2. Partial hippocampal kindling reduced the suppressive effect of baclofen (A, B) but not that of N^6-cyclopentyladenosine on the field excitatory postsynaptic potentials (fEPSPs) in CA1. Kindling was done by evoking 15 hippocampal afterdischarges over 3 days by high-frequency train stimulations of CA1. Inset on top, negative fEPSPs (average of four sweeps) recorded at CA1 stratum radiatum following stimulation of the same layer (see Figure 1) at 2 times the threshold intensity. Representative slices from control and kindled rats (1 day after kindling) show that baclofen (10 μM) suppressed field EPSPs more in control than 'kindled' slice. As shown by the calibrations, voltage gains were adjusted to show similar peak amplitudes between slices during baseline. Circles indicate shock artifacts. **A:** Group data show that baclofen suppressed the slope of the fEPSPs more in control (dotted trace) than kindled rats (solid trace) recorded 1 day after partial hippocampal kindling (37 control slices and 24 kindled slices). Slope of the fEPSPs was normalized to 100% for each slice before perfusion of baclofen (time 0). **B:** Same as A except for recordings 21 days after kindling or control treatment (37 control slices and 17 'kindled' slices). **C:** Same as A except 1 μM cyclopentyladenosine (CP-adensoine) was perfused, instead of baclofen, and control and kindled groups show no significant difference in response to CP-adensoine (11 control and 11 kindled slices).

decrease was not different between kindled and control rats. However, the time course of change of fEPSPs after baclofen (fEPSPs measured at 5, 10, 15 and 20 min after 10 μM baclofen) was different between control and kindled slices (main effect F (1, 59) = 7.18, $P < 0.01$; 2-factor, repeated measures ANOVA; 24 "kindled" slices and 37 control slices), with a significant time effect ($P < 0.001$) and nonsignificant group × time interaction ($P > 0.5$). Statistics using per rat as the basis (2–5 slices averaged for each rat, 5 kindled and 10 control rats) also yielded a significant group effect (F (1, 13) = 6.28, $P < 0.03$), a significant time effect and a nonsignificant group × time interaction.

Seventeen slices from five rats were recorded at 21 days after partial hippocampal kindling. The time course of the response to 10 μM baclofen was significantly different between slices from kindled and control groups (Fig. 2B; main effect F (1,52) = 10.5, $P < 0.01$; 17 kindled slices and 37 control slices). The time effect ($P < 0.001$) and group × time interactions ($P < 0.02$) were also significant. Statistics using per rat as the basis (2–4 slices per rat) yielded a similar result—baclofen had a significant group effect (F (1,13) = 8.37, $P < 0.02$) and also significant time and group × time effects. There was no statistical difference between slices from 1 day or 21 days after partial hippocampal kindling in their response to baclofen (F (1, 39) = 0.3, $P > 0.5$, per slice data).

The E2/E1 ratio was significantly increased by baclofen in both kindled and control slices ($P < 0.01$, Wilcoxon, $n > 20$ each group). Interestingly, E2/E1 ratio decreased with stimulus intensity before baclofen, but this ratio became independent of stimulus intensity after baclofen (not shown); the relation of E2/E1 ratio to stimulus intensity and the effect of baclofen on this relation was not different between kindled and control rats.

Effect of N^6-cyclopentyladenosine on the fEPSPs

As an alternate way to suppress glutamate release and EPSPs in CA1, presynaptic adenosine A1receptors were activated by perfusing 1 μM CPA in the bath (cf. Wu and Saggau, 1994). Eleven slices from three control rats and 11 slices from three kindled rats were recorded 1 day after kindling. There was no difference in the baseline response from the control and kindled group of slices; their combined E1 at 2 times threshold stimulus intensity was 0.74 ± 0.02 mV/ms ($n = 22$) during baseline before the perfusion of CPA. CPA suppressed the fEPSPs in both kindled and control slices (Fig. 2C). At 20 min after perfusion of CPA, E1 was reduced to 21 ± 3% of baseline in control slices ($n = 11$), not different from 24 ± 2% of baseline slices ($n = 11$) from kindled rats. The time

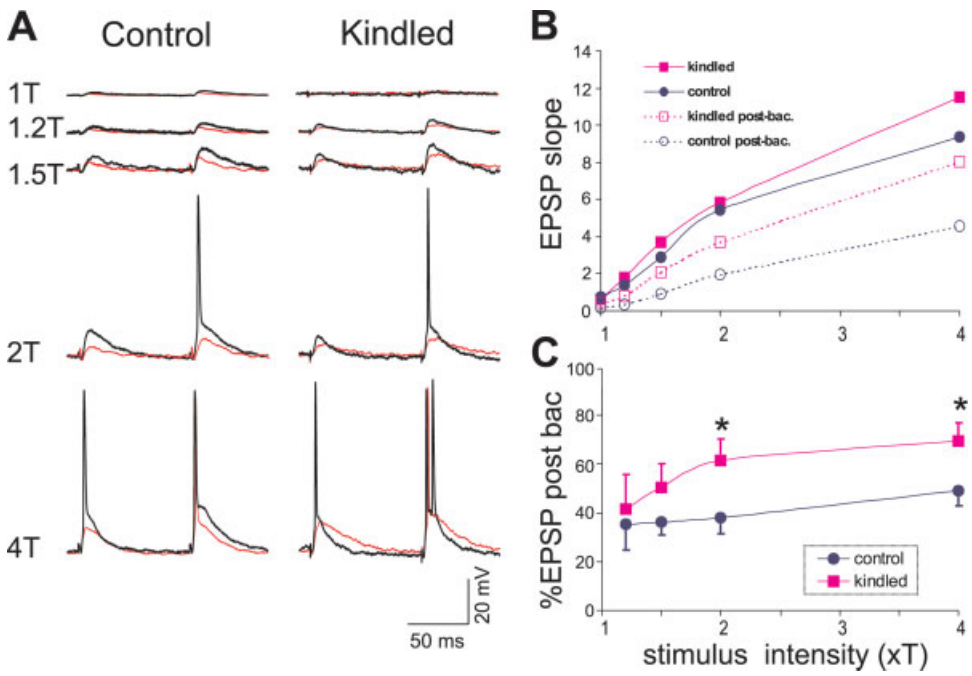

Fig. 3. Partial hippocampal kindling reduced the effect of baclofen on the intracellular recorded EPSPs in hippocampal CA1 neurons on 1 day after kindling/control treatment. **A:** EPSPs evoked by various stimulus intensities, at 1, 1.2, 1.5, 2 and 4× T (threshold stimulus intensity), are shown for a representative neuron from a control rat and another from a kindled rat. Dark lines before baclofen and thin red lines after baclofen. **B:** Average intracellular EPSP slope (in mV/ms) plotted vs. stimulus intensity for the kindled and control groups of neurons, before and 15–20 min after baclofen. **C:** Average percent of EPSP slope post baclofen (baseline normalized to 100% before baclofen) vs. stimulus intensity (10 control and 14 kindled neurons in B and C). * $P < 0.05$ post hoc Newman-Keuls test after a significant repeated measures ANOVA; also $P < 0.05$, Wilcoxon).

course of the effect of CPA on E1 was not different between kindled and control slices (F (1, 21) = 0.22, $P > 0.6$) and there was no significant group × time effect ($P > 0.9$), although the time effect was significant ($P < 0.0001$).

Intracellular EPSPs in CA1 of control and kindled rats

Resting membrane potential was −67 ± 2 mV in neurons from kindled rats (day 1 after kindling, n = 32 neurons; day 21 after kindling, n = 12 neurons), not different from neurons from matched control rats (day 1 control, n = 30 neurons; day 21 control, n = 13 neurons). The input resistance and time constant were also similar across kindled and control groups, with a grand mean of 62 ± 4 Mohm for Rin and 18 ± 2 ms for time constant. At day 1, after kindling or control treatment, intracellular EPSPs (iEPSPs) recorded in CA1 neurons showed a gradual increase with the stimulus intensity applied at stratum radiatum (Figs. 3A and 3B). At 2 times stimulus threshold, the EPSP rising slope (iE1) measured 5.4 ± 1.1 mV/ms (n = 14) in control neurons, not significantly different from iE1 of 5.8 ± 1.8 mV/ms (n = 10) in neurons from kindled animals. At 15–20 min following the perfusion of 10 μM baclofen, the resting membrane potential hyperpolarized by 2.4 ± 0.7 mV, and the input resistance decreased 16 ± 4% in either control (n = 16) or kindled (n = 11) group of neurons. The change in RMP and input resistance did not differ between kindled and control groups of neurons.

Baclofen decreased the EPSP slope in both control and kindled (1 day after kindling) groups of neurons (Fig. 3). At 15–20 min after 10 μM baclofen, iE1 at 2 times threshold stimulus intensity was 38 ± 6% of the baseline value in control neurons, significantly smaller than 61 ± 9% baseline value in the kindled neurons ($P < 0.05$, Wilcoxon; Fig. 3C). Two-factor repeated measures ANOVA showed a similar result that the decrease in iE1 was larger in the control than the kindled group for stimulus intensities of 1.5–4 times threshold (group effect F (1, 23) = 4.33, $P < 0.05$; Fig. 3C) with no significant intensity effect or group x intensity interaction. Responses at 1.2 times threshold stimulus intensity showed a large degree of variation in response to baclofen. Baclofen tended to increase iE2/iE1 ratio in most neurons, since iE1 was usually suppressed more than iE2 after baclofen (cf. Fig. 3). However, the iE2/iE1 ratio and its change by baclofen were not different between control and kindled groups.

Cellular responses to synaptic activation of $GABA_B$ heterosynaptic receptors

GABA was released synaptically by a brief conditioning train stimulation at the S2 electrode. The iEPSP evoked by test-pulse at S1 was reduced after a preceding conditioning train at 150–1000 ms conditioning-test (C-T) intervals (Fig. 4A). The nonconditioned first-pulse EPSP slope (E1) measured 1.5–2 mV/ms for various groups of neurons, and was not significantly different between control and kindled groups. Neurons from both control and kindled group of neurons showed a decrease in the test response following the conditioning train. For neurons recorded on 1 day after kindling/control treatment, the condi-

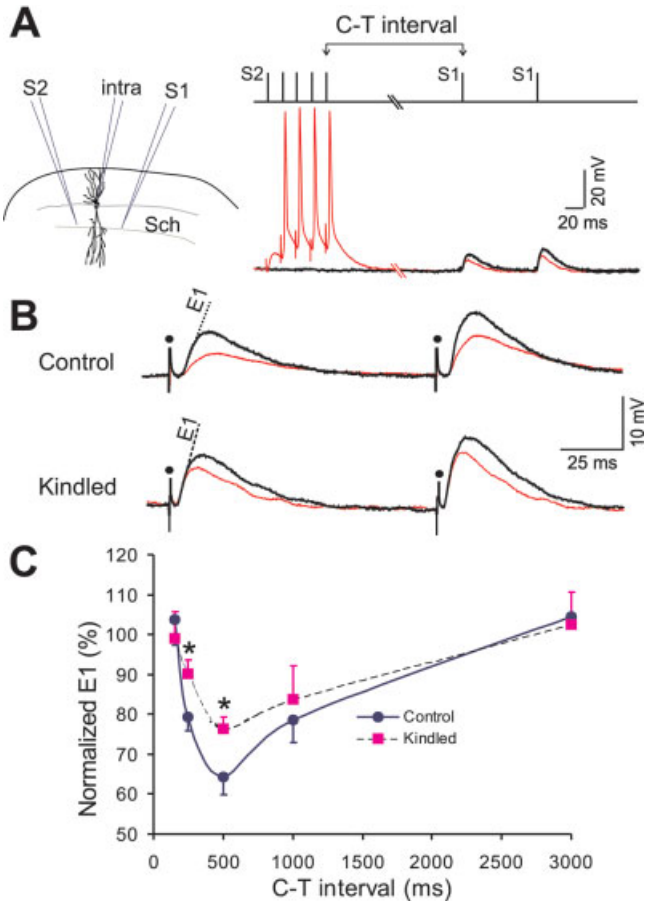

Fig. 4. Synaptically released GABA suppressed intracellularly recorded EPSPs more in control than kindled rats. **A:** Stimulus electrodes S2 and S1 were placed at different sides of the intracellular (intra) recording of CA1 neuron at the cell layer (left) to stimulate the Schaffer collaterals (Sch). A 5-pulse train of 50 Hz at S2 was used to evoke GABA release that suppressed the paired-pulse (S1-evoked) EPSPs recorded after a variable condition-test interval (C-T interval) shown as thin red traces, as compared to responses without conditioning shown as thick, black traces. **B:** Representative examples of paired responses from a control and a kindled group of neurons, showing relatively smaller depression after conditioning (thin red traces) than without conditioning (black traces). Filled circles indicate shock artifacts. **C:** Average percent suppression of the slope of the first-pulse EPSP (E1) in control neurons was larger than that in kindled group of neurons at 250–500 ms C-T intervals. Numbers of neurons (control, kindled) for different C-T intervals were 150 ms (2, 2), 250 ms (13, 19), 500 ms (6, 11), 1000 ms (5, 3) and 3000 ms (1, 2). * $P < 0.02$, Wilcoxon.

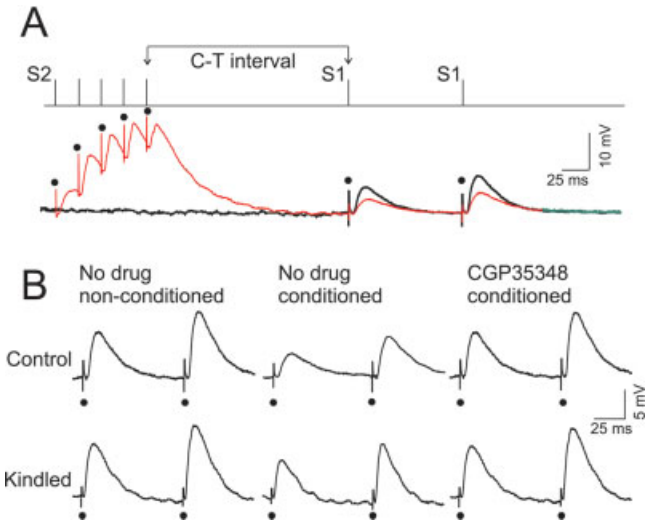

Fig. 5. Synaptically released GABA suppressed intracellulary recorded EPSPs after blockade of spikes and postsynaptic $GABA_B$ receptors in the recorded CA1 neuron. **A:** Pattern of stimulation was the same as in Figure 4A, but response was from a control neuron impaled with a micropipette containing QX-314 that blocked both spiking and postsynaptic $GABA_B$ receptors. **B:** Representative control and kindled neuron showing paired-pulse response without conditioning and after conditioning. The effect of conditioning was strongly attenuated by the addition of $GABA_B$ receptor antagonist CGP35348 (0.5 mM) to the perfusate.

tioned suppression of the EPSP slope or peak was larger in the control group than kindled group neurons at 250 and 500 ms C-T intervals, (Figs. 4B and 4C). For neurons recorded on 21 days (four cells from control and five cells from kindled rats), there was no statistical difference in the decrease in EPSP peak or slope by conditioning at 250 ms or other C-T intervals. For the day 1 group, the conditioned suppression of iE1 was 21.4 ± 3.2% of the baseline at 250 ms C-T interval in the control group ($n = 14$ neurons from eight rats), significantly larger ($P < 0.02$, Wilcoxon) than 9.7 ± 3.3% of the baseline ($n = 21$ neurons from nine rats) in the kindled group. At 500 ms C-T interval, iE1 (slope) suppression was at a maximal 35.6 ± 4.6% (6 neurons in 6 rats) in the control group, significantly larger ($P < 0.04$, Wilcoxon) than the same measure of 23.7 ± 2.9% in the kindled group (11 neurons in seven rats). Statistics using the EPSP peak measure also showed highly significant suppression for the kindled as compared to the control group at 250 ms C-T interval ($P < 0.002$, Wilcoxon) and marginally nonsignificant at 500 ms C-T interval ($P = 0.056$, Wilcoxon). At 250 ms C-T interval, paired-pulse facilitation of the EPSPs (iE2/iE1; 100 ms IPI) was not different between control and kindled groups of neurons. iE2/iE1 increased during conditioning, largely because of a decreased iE1, and the iE2/iE1 increase was larger for the control than the kindled group of neurons ($P < 0.03$, Wilcoxon). Increase in iE2/iE1 at 500 ms C-T interval was larger in control than kindled group of neurons as well, but the increase only showed a trend ($P = 0.11$, Wilcoxon).

To block postsynaptic $GABA_B$ receptors, neurons were impaled with a QX-314 containing micropipette (Methods). Input resistance in the QX-314 loaded neurons averaged 112 ± 9 Mohms ($n = 7$), significantly larger than the 57 ± 2 Mohms in the 35 neurons in which heterosynaptic suppression was studied without QX-314. QX-314 also blocked fast Na^+ action potentials (Fig. 5A), but it did not change the intracellularly recorded iEPSP or its suppression by a brief conditioning train in control neurons and "kindled"

neurons (example shown in Figs. 5A and 5B). At 250 ms C-T interval, the suppression of E1 peak was 27.9 ± 5.9% in five control neurons and 14 ± 3.5% in two kindled neurons; difference in E1 peak suppression was significant ($P < 0.05$, Wilcoxon) between control and kindled neurons loaded with QX-314. On the other hand, E1 peak suppression was not different between control neurons with or without QX-314. In the neurons with postsynaptic $GABA_B$ receptor blocked by QX-314, subsequent perfusion of $GABA_B$ antagonist CGP35348 (500 μM) in the bath blocked the conditioned suppression of iEPSPs in both control and kindled groups of neurons (Fig. 5B).

DISCUSSION

The effect of baclofen (10 μM) on suppressing fEPSPs and iEPSPs in CA1 neurons was reduced in kindled as compared to control rats. This result was found at all stimulus intensities substantially above threshold (1.5–4 times threshold), and 1 or 21 days after partial hippocampal kindling. Synaptically released GABA was found to have a similar effect in suppressing iEPSPs more strongly in control neurons as compared to those from rats 1 day after kindling. Effect of baclofen on the fEPSPs was found to extend to 21 days after partial hippocampal kindling.

Baclofen exerted both postsynaptic and presynaptic effects on the CA1 pyramidal cells. By opening postsynaptic K^+ channels, baclofen hyperpolarized the postsynaptic membrane. Somatic recordings from CA1 neurons indicated that this hyperpolarization was ~2.4 mV after 10 μM baclofen, which was not statistically different between control and kindled group of neurons. Similarly, both kindled and control groups of neurons showed a similar change in Rin after baclofen. These results are consistent with our other study using whole-cell somatic patch recordings of CA1 pyramidal cells, in which baclofen induced a $GABA_B$ receptor-mediated postsynaptic current that was not different between kindled and control groups of neurons (Liu and Leung, 2003).

Baclofen's suppression of iEPSPs likely results from activation of presynaptic $GABA_B$ receptors that suppress glutamate release from excitatory terminals (Ault and Nadler, 1982; Lanthorn and Cotman, 1981; Olpe et al. 1982). The use of a conditioning train to release GABA synaptically allows a robust demonstration of the presynaptic $GABA_B$ receptor function. In this paradigm, $GABA_A$ and NMDA receptors were blocked, thus isolating the effects of $GABA_B$ receptors on AMPA-receptor mediated potentials. In addition, QX-314 was used to block postsynaptic $GABA_B$ receptors in some neurons, with little effects on the iEPSPs or their suppression by a conditioning train (Fig. 5B). However, subsequent perfusion of $GABA_B$ receptor antagonist CGP35348 abolished the conditioned suppression of iEPSPs. Whether the amount of synaptically released GABA differed between controls and kindled slices is not known, although the time course of depression of iE1 appears similar between control and kindled groups (Fig. 4C). In kindled as compared to control neurons, the maximal effect of synaptically released GABA (iE1 slope or peak at 250–500 C-T interval) was reduced by 50–70%, similar to the magnitude of sensitivity decrease in iEPSPs (Fig. 3C) or fEPSPs (Fig. 2A) after exogenously applied baclofen (10 μM). In total, the above manipulations provide strong evidence that the suppression of iEPSPs by a conditioning train stimulus was mediated by presynaptic $GABA_B$ receptors.

$GABA_A$ receptor antagonists were not used in the study of baclofen on the iEPSPs (Fig. 3) or fEPSPs (Fig. 2). While a direct effect of baclofen on the presynaptic $GABA_B$ receptors can only be inferred in these studies, EPSPs were unlikely to be confounded by intracellular IPSPs for several reasons. First, the effect of baclofen was measured from iEPSPs at the same resting potential before and after baclofen, and the measure was independent of postsynaptic membrane potential change. Second, the effect of $GABA_A$ mediated IPSPs on shunting the iEPSPs or fEPSPs was not strong in vitro (Leung and Fu, 1994). Third, the rising slopes of the iEPSPs and fEPSPs were measured at <2 ms from onset of the EPSPs, before the onset of the evoked IPSPs (Fig. 1C). Thus, previous researchers also concluded that baclofen's main effect in suppressing the fEPSPs was presynaptic (Ault and Nadler, 1982; Olpe et al., 1982).

Presynaptic $GABA_B$ receptor inhibition may be mediated by reducing Ca^{2+} influx at the axon terminals (Pfrieger et al., 1994; Wu and Saggau, 1997), other Ca^{2+}-independent mechanisms (Thompson et al., 1993; Wu and Saggau, 1997), or by suppressing recruitment of releasable vesicles (Sakaba and Neher, 2003). We found that baclofen significantly suppressed the presynaptic volley in the field potential, suggesting a direct effect on action potentials. However, the latter effect was not different between control and kindled groups of hippocampal slices.

The kindling-induced effect was selective for the presynaptic $GABA_B$ receptors on the glutamatergic terminals, and it was not found for presynaptic adenosine A1 receptors. The A1 receptor agonist CPA decreased presynaptic Ca^{2+}(Wu and Saggau, 1994) and suppressed iEPSPs (Thompson et al., 1993). Accordingly, we showed that CPA suppressed fEPSPs (Fig. 2C), and yet CPA's effect was not different between control and kindled group of slices. After a different type of seizure induced by pentylenetetrazol, CPA was shown to decrease population spike in CA1 more in seizure than control hippocampal slices in vitro (Psarropoulou et al., 1994).

Partial hippocampal kindling also induced a decrease of the efficacy of presynaptic $GABA_B$ autore-

ceptors on the GABAergic terminals (Buhl et al., 1996; Leung and Wu, 2003; Wu and Leung, 1997), but it did not change the sensitivity of postsynaptic $GABA_B$ receptors on CA1 pyramidal cells (this study and Liu and Leung, 2003). Similarly, a decrease in presynaptic but not postsynaptic $GABA_B$ receptor function was reported by Asprondini et al. (1992) in the basolateral amygdala after amygdala full kindling. Why pre- and not postsynaptic $GABA_B$ receptors are affected by kindling is not known. Pre- and postsynaptic $GABA_B$ receptors are presumably the same molecular entities (Prosser et al., 2001; Schuler et al., 2001) although differences in pharmacological responses have been reported (Davies et al., 1991; Misgeld et al., 1995). Possibly, a neuron determines the efficacy of its own $GABA_B$ receptors. Thus, glutamatergic CA3 pyramidal cells whose axons innervate CA1 (expressing heterosynaptic $GABA_B$ receptors) and GABAergic interneurons in CA1 (expressing $GABA_B$ autoreceptors) may decrease $GABA_B$ receptor function, while CA1 pyramidal cells (expressing $GABA_B$ postsynaptic receptors) do not.

In contrast to a decrease in $GABA_B$ receptor function, increase in mRNA and immunoreactivity of $GABA_B$ receptor (subunits) were reported after hippocampal kindling (Francis et al., 1999; Kokaia and Kokaia, 2001). These expression studies did not distinguish pre- and post- synaptic receptors, or membrane vs. intracellular receptors. The relation between $GABA_B$ receptor expression and function is not necessarily direct (cf. Francis et al., 1999) in view of the signaling pathway of G-protein coupled receptors (Couve et al., 2000). The detailed mechanism of the kindling-induced decrease in efficacy of the presynaptic $GABA_B$ receptors is not known. It could be caused by a decrease in number of presynaptic $GABA_B$ receptors at the surface of the membrane or a lower efficacy in G-protein coupling (Couve et al., 2000).

Chandler et al. (2003) reported that status epilepticus induced by pilocarpine or perforant path stimulation was followed 24 h later by a loss of $GABA_B$ receptor-mediated heterosynpatic depression of mossy fiber transmission in CA3. Results here show that electrographic seizures in the hippocampus, without convulsions, were sufficient to induce a decrease in heterosynaptic $GABA_B$ receptor function in CA1 neurons. Partial hippocampal kindling also induced amygdala ADs (Leung, 1987), but whether amygdala heterosynaptic $GABA_B$ receptors (Asprondini et al. 1992) are altered after partial hippocampal kindling is not known. The functional significance of a decrease in heterosynaptic $GABA_B$ receptor efficacy is not totally clear. We speculate that it will increase seizure susceptibility in kindled as compared to control rats. Heterosynaptic $GABA_B$ receptors are suggested to be important after high neural activity such as repeated stimulation (Isaacson et al., 1993) or electrical seizures. Kindled animals are deficient in the suppression of glutamate release by GABA, which will likely result in unchecked increase in glutamate release across time and space, and an increase in seizure susceptibility.

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