Excitatory Actions of Endogenously Released GABA Contribute to Initiation of Ictal Epileptiform Activity in the Developing Hippocampus

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In the developing rat hippocampus, ictal epileptiform activity can be elicited easily *in vitro* during the first three postnatal weeks. Changes in neuronal ion transport during this time cause the effects of GABA$_A$ receptor (GABA$_A$-R) activation to shift gradually from strongly depolarizing to hyperpolarizing. It is not known whether the depolarizing effects of GABA and the propensity for ictal activity are causally linked. A key question is whether the GABA-mediated depolarization is excitatory, which we defined operationally as being sufficient to trigger action potentials. We assessed the effect of endogenous GABA on ictal activity and neuronal firing rate in hippocampal slices from postnatal day 1 (P1) to P30. In extracellular recordings, there was a strong correlation between the postnatal age at which GABA$_A$-R antagonists decreased action potential frequency (P23) and the age at which ictal activity could be induced by elevated potassium (P23). In addition, there was a strong correlation between the fraction of slices in which ictal activity was induced by elevated potassium concentrations and the fractional decrease in action potential firing when GABA$_A$-Rs were blocked in the presence of ionotropic glutamate receptor antagonists. Finally, ictal activity induced by elevated potassium was blocked by the GABA$_A$-R antagonists bicuculline and SR-95531 (gabazine) and increased in frequency and duration by GABA$_A$-R agonists isoguvacine and muscimol. Thus, the propensity of the developing hippocampus for ictal activity is highly correlated with the effect of GABA on action potential probability and reversed by GABA$_A$ antagonists, indicating that GABA-mediated excitation is causally linked to ictal activity in this developmental window.

Key words: GABA; action potential; epileptiform activity; hippocampus; CA3; development

Introduction

Despite dramatically lower synaptic connectivity (Bayer, 1980; Gomez-Di Cesare et al., 1997), the immature brain is much more susceptible to seizures than the adult brain (DeLorenzo et al., 1992; Holmes, 1997). Modeling studies clearly indicate that synchronous activity is not easily maintained when synaptic connectivity is low (Traub and Miles, 1991). Thus, there must be a compelling proconvulsant factor or factors that increase seizure propensity in the immature brain.

One likely proconvulsant factor is the paradoxical action of the neurotransmitter GABA. GABA$_A$ receptor (GABA$_A$-R) activation is inhibitory in the adult brain, both by virtue of the membrane hyperpolarization induced by chloride (Cl$^-$) influx through the GABA$_A$ ionophore and by virtue of shunting of dendritic excitatory inputs (Koch et al., 1983; Staley and Mody, 1992). In the immature brain, however, the transmembrane chloride gradient is reversed, so that GABA$_A$-R activation depolarizes the neuronal membrane (Cherubini et al., 1991; Owens et al., 1996; Ben Ari et al., 1997). GABAergic circuits develop early during development (Tyzio et al., 1999), and thus it seems reasonable that the propensity for seizure activity in the immature brain could be a consequence of additional feedback excitation

mediated through the GABAergic circuits that subserve feedback inhibition in the adult nervous system (Alger and Nicoll, 1982; Freund and Buzsaki, 1996). However, experimental evidence has not clearly supported this idea. In the developing hippocampus, although some physiological forms of synchronous activity are blocked by GABA$_A$-R antagonists (Ben Ari et al., 1989; Khalilov et al., 1999), epileptiform activity is not (Wells et al., 2000). Furthermore, epileptiform activity can be suppressed by GABA$_A$-R agonists (Wells et al., 2000).

One difficulty associated with determining the role of GABA in the developing hippocampus is the tenuous nature of the ion gradients that underlie the effects of GABA$_A$-R activation in this age range (Staley and Smith, 2001). Even in adult neurons, in which GABA is strongly hyperpolarizing, overactivation of GABA$_A$-R can overwhelm K–Cl cotransport such that the Cl$^-$ reversal potential (ECl$^-$) is driven to the resting membrane potential (RMP) (Thompson and Gahwiler, 1989a). This effect becomes more pronounced as K–Cl cotransport is compromised by increased extracellular K$^+$ ([K$^+$]$_o$) concentrations (Staley and Proctor, 1999). If Cl$^-$ gradients in developing neurons are similarly labile, application of GABA agonists might cause ECl$^-$ to move negatively toward RMP. In this case, additional GABA$_A$-R activation would not depolarize the membrane further, so that shunting inhibition would be the dominant effect of GABA$_A$-R activation. This may explain the anticonvulsant effects of exogenous agonists in the neonatal period.

To resolve this issue, we need to determine the effect of endogenously released GABA. One way to determine whether GABA is excitatory is by determining whether or not GABA$_A$-R activation

triggers action potentials. Measuring the effects of synaptic $GABA_A$-R activation on the basis of intracellular recordings can be complicated by dialysis of the cytoplasmic Cl^- by the electrode solution (Staley and Proctor, 1999) and by electrode-induced shifts in RMP and action potential threshold (Staley and Mody, 1991). Although dialysis issues can be overcome by perforated patch recordings (Owens et al., 1996), the antibiotic used to perforate the membrane can diffuse through the membrane away from the patch, decreasing the membrane resistivity and thereby increasing the amount of current necessary to reach action potential threshold. Thus, perforated patch recordings are useful for determination of the reversal potential of the $GABA_A$-R-mediated responses (EGABA) but not the relationship between EGABA and action potential generation. In this study, we circumvented these difficulties by measuring extracellular unit activity (Cohen and Miles, 2000; Dzhala et al., 2001) as an assay of the net effect of $GABA_A$-R or activation by endogenously released GABA. These actions were correlated with the effects of $GABA_A$-R activation on ictal epileptiform activity at different developmental ages.

Materials and Methods

Experimental systems. Hippocampal slices were prepared from male Wistar rats at age postnatal day 1 (P1) to P30. The first 24 hr after birth were designated P0. All animal use protocols conformed to the University of Colorado Health Sciences Center animal care and use committee and the National Institutes of Health guidelines on the use of laboratory animals. Animals were anesthetized with chloral hydrate (350 mg/kg, i.p.) and decapitated. The brain was removed rapidly and placed in an oxygenated (95% O_2–5% CO_2) ice-cold artificial CSF (aCSF) of the following composition (in mM): 126 NaCl, 3.5 KCl, 2.0 $CaCl_2$, 1.3 $MgCl_2$, 25 $NaHCO_3$, 1.2 NaH_2PO_4, and 11 glucose, at a pH of 7.4. Hippocampal transverse slices (thickness of 0.6–0.7 mm) were cut with a VT 1000S vibroslicer (Leica, Nussloch, Germany) and kept in oxygenated (95% O_2–5% CO_2) aCSF at room temperature (20–22°C) at least 1 hr before use. Unusually thick hippocampal slices were used to preserve the advantages of the intact hippocampal preparation *in vitro* (Khalilov et al., 1997). There was no correlation between extracellularly recorded action potential frequency and electrode depth, indicating that, if the oxygen tension was lower in the center of our 700-μm-thick slices, it did not affect neuronal or network excitability.

Electrophysiological recordings and data analysis. For electrophysiological recordings, individual slices were transferred to a conventional submersion-type chamber and superfused continuously with oxygenated aCSF at 32°C at a rate of 2–3 ml/min. Whole-cell and extracellular field potential recordings were performed in the CA3 pyramidal cell layer. Whole-cell recordings were made using an Axopatch 200 (Axon Instruments, Foster City, CA) amplifier. Patch electrodes were made from borosilicate glass capillaries (G150F-4; Warner Instruments, Hamden, CT) and had a resistance of 5 MΩ when filled with solution containing the following (in mM): 135 potassium gluconate, 0.1 $CaCl_2$, 2.0 $MgCl_2$, 2.0 Na_2ATP, 1.0 EGTA, and 10 HEPES, at a pH of 7.25. The liquid junction potential was measured (+11 mV), and voltages reported are corrected values.

Extracellular field potentials were recorded using tungsten microelectrodes and a multichannel amplifier (bandpass, 0.1 Hz to 4 kHz; 1000×) with enhanced electromagnetic interference noise suppression developed for neurobiology research applications (TRINITI; Troitsk, Moscow, Russia). Use of microelectrodes made from coated tungsten wire 50 μm in diameter (California Fine Wire Company, Grover Beach, CA) enables simultaneous recordings of multiple-unit activity (MUA) (400 Hz high-pass filter) and population field activity (EEG band, 1–100 Hz) from tens to hundreds of neurons near each electrode (Cohen and Miles, 2000). Root-mean-square noise level with an electrode placed in the perfusion solution was typically 4–5 μV, whereas the amplitude of action potentials recorded from the stratum pyramidale ranged from this noise level up to 200 μV. The signals were digitized using an analog-to-digital converter (DigiData 1322A; Axon Instruments). Axoscope and Clampfit (Axon Instruments), Mini Analysis Program (Synaptosoft, Decatur, GA), and Origin (Microcal Software, Northampton, MA) programs were used for the acquisition and data analysis. The MUA was determined from high-pass filtered (400 Hz) raw data with a spike detection algorithm (Mini Analysis Program) and verified visually. Spikes with amplitude greater than three times the root-mean-square noise level were accepted.

Group measures are expressed as mean ± SEM; error bars also indicate SEM. The statistical significance of differences was assessed with the Student's *t* test. The level of significance was set at $p < 0.05$.

Drugs. Drugs were purchased from Sigma (St. Louis, MO) and Tocris Cookson (Ellisville, MO), prepared as stock solutions, divided into aliquots, and stored in tightly sealed vials at recommended temperatures. During the experiments, thawed aliquots were kept on ice and protected from light until use.

Results

Extracellular unit activity and population field activity were measured in the CA3 pyramidal cell layer to circumvent potential problems associated with cell dialysis from patch electrodes. Resting membrane potential was measured using whole-cell recordings. In control conditions, the resting membrane potential of CA3 pyramidal cells gradually shifted from -73 ± 1.5 mV at P8–P12 ($n = 10$) to -80.5 ± 2.1 mV at P26–P30 ($n = 8$). Despite the use of thick (0.6–0.7 mm) hippocampal slices, no anoxia-associated network oscillations or rapid neuronal depolarization have been observed (Dzhala et al., 2001)

Contributions of synaptic activities to firing rates of hippocampal neurons

In control conditions, spontaneous action potentials were invariably detected in extracellular field potential recordings from the CA3 pyramidal cell layer in neonatal rat hippocampal slices (Fig. 1*A*). We used pharmacological tools to estimate the degree to which synaptic excitation and inhibition altered this activity. Bath application of the AMPA-receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfonyl-benzo[*f*]quinoxaline (NBQX) (10 μM) and the NMDA-receptor antagonist D-2-amino-7-phosphonovalerate (D-APV) (50 μM) reduced neuronal firing rate from $94 \pm 2.4\%$ at P1 ($n = 6$ slices; $p = 0.0045$) to $44 \pm 12\%$ at P30 ($n = 5$ slices; $p = 0.0037$). Subsequent application of the $GABA_A$-R antagonist bicuculline (10 μM) in the presence of NBQX and D-APV diminished firing rate further up to P12, indicating that endogenous activation of $GABA_A$-R increases the frequency of neuronal firing. Our experiments confirm previously reported data from cell-attached recordings in the P2–P5 hippocampal slices (Khalilov et al., 1999). After P12, bicuculline increased the neuronal firing rate when AMPA and NMDA receptors were blocked, indicating that endogenously released GABA had a net inhibitory effect (Fig. 1*B*,*C*).

Ictal epileptiform patterns can be elicited in the neonatal brain slice by increasing $[K^+]_o$ to 8.5 mM (Fig. 2*A*) (see Figs. 3–7) (Rutecki et al., 1985). This $[K^+]_o$ diminishes neuronal Cl^- extrusion by decreasing the driving force for K–Cl cotransport, thereby making the GABA reversal potential more positive (Thompson and Gahwiler, 1989b). This would make GABA more strongly depolarizing in neurons expressing the K–Cl cotransporters (Rivera et al., 1999). Elevated $[K^+]_o$ also depolarizes the neuronal membrane, decreasing the amount of depolarization needed to trigger action potentials. Thus, exogenously released GABA would be expected to elicit more action potentials in the presence of elevated $[K^+]_o$ as the depolarization induced by GABA increases and the distance between RMP and action
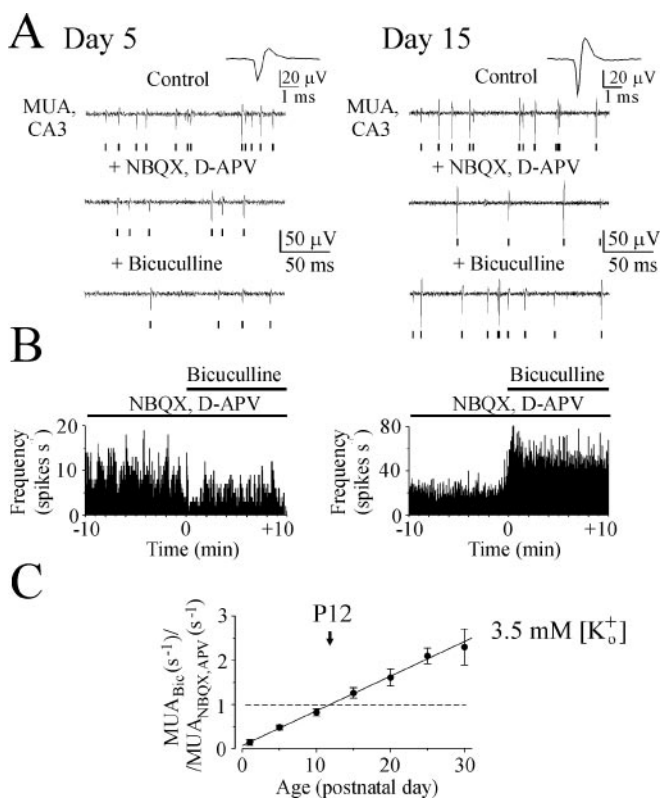
Figure 1. Effects on neuronal firing rate of suppression synaptic excitation and inhibition in control conditions. *A*, Glutamate receptor antagonists NBQX (10 μM) and D-APV (50 μM) reduced neuronal firing rate in P5 (*left traces*) and P15 (*right traces*) rat hippocampal slices. Subsequent application of the $GABA_A$-R antagonist bicuculline (10 μM), in the presence of NBQX and D-APV, diminished neuronal firing at P5 further and increased the firing rate at P15. Examples of extracellular recordings from the CA3 pyramidal cell layer filtered for the MUA (400 Hz high-pass filter). Detected spikes are marked by *vertical bars*. Insets, Averaged spikes from extracellular recordings. *B*, The frequency of MUA recorded from the CA3 pyramidal cell layer after the application of NBQX and D-APV and then after the application of bicuculline. *C*, Age dependence of the effect on MUA of suppression synaptic excitation and inhibition at 3.5 mM $[K^+]_o$.

Figure 2. Effects on neuronal firing rate and epileptiform activities of suppression synaptic excitation and inhibition at 8.5 mM $[K^+]_o$. *A*, Glutamate receptor antagonists NBQX (10 μM) and D-APV (50 μM) abolish 8.5 mM $[K^+]_o$-induced interictal and ictal epileptiform activity in P15 (*left traces*) and interictal epileptiform discharges in P30 (*right traces*) rat hippocampal slices. $GABA_A$-R antagonist bicuculline (10 μM) diminishes neuronal firing at P15 and increases the firing rate at P30. Extracellular recordings from CA3 pyramidal cell layer. *B*, The frequency of MUA (in sec $^{-1}$) recorded from the CA3 pyramidal cell layer after the application of NBQX and D-APV and then after the application of bicuculline. *C*, Age dependence of the effect on MUA of suppression synaptic excitation and inhibition at 8.5 mM $[K^+]_o$.

potential threshold decreases. In 8.5 mM $[K^+]_o$, the $GABA_A$-R antagonist bicuculline, when AMPA and NMDA receptors were blocked, diminished firing rate in the CA3 subfield by 79 ± 7% at P1 ($n = 12$ records from five slices) and 9 ± 10% at P22 ($n = 9$ records from three slices), indicating that endogenous GABA was excitatory. At P25, firing rate increased to 109 ± 8% of control ($n = 9$ records from three slices) and at P30 to 149 ± 21% of control ($n = 9$ records from three slices) (Fig. 2C). These data from immature animals are in contrast to the effects of GABA antagonists in hippocampal slices from mature rodents (Cohen and Miles, 2000), in which the rate of spike discharge increased to 177 ± 71% of its control value, indicating that endogenous GABA is strongly inhibitory.

When the $[K^+]_o$ concentration was varied, endogenously released GABA triggered progressively more action potentials at higher concentrations of $[K^+]_o$ ($n = 18$ slices) (Fig. 3A). To determine whether this effect of $[K^+]_o$ on neuronal firing rate was correlated with ictal epileptiform activity triggered by $[K^+]_o$, we perfused P10–P11 rat hippocampal slices with solutions containing elevated concentrations of extracellular potassium (Jensen and Yaari, 1988, 1997; Traynelis and Dingledine, 1988). Of the hippocampal slices from P10–P11 rats, 71.4% displayed recurrent ictal epileptiform activities at 8.5 mM $[K^+]_o$, and a threshold for the proconvulsant effect of $[K^+]_o$ was seen at 7.5
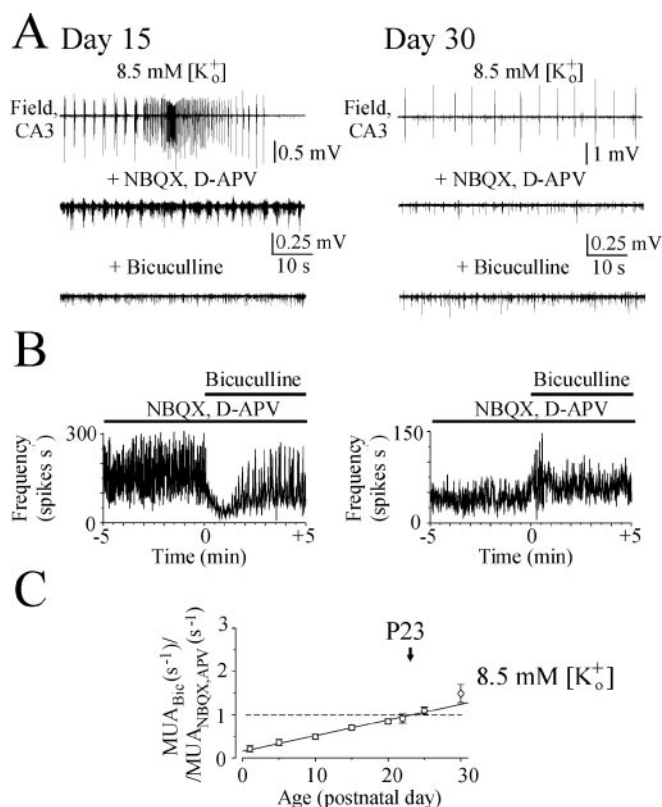
mM $[K^+]_o$ (16.7% of the hippocampal slices displayed ictal activity), which was similar to the $[K^+]_o$ effect on action potential generation (Fig. 3A,B).

Ictal epileptiform activity induced by high $[K^+]_o$ in the rat hippocampal slices

In the hippocampal slices from P5 rats, bath application of 8.5 mM K^+ induced a progressive increase in neuronal firing that developed to interictal epileptiform discharges (Pedley, 1980). Interictal epileptiform discharges (IEDs) were generated regularly with an averaged interval 1.08 ± 0.1 sec ($n = 6$ slices from P5 rats) and consisted of a large-amplitude primary population spike and a subsequent slow spike wave. IEDs were initiated in the CA3a–CA3b pyramidal cell layer and propagated bidirectionally to the distal CA1 and dentate gyrus. Before transition to ictal epileptiform activity, IEDs were followed by secondary afterdischarges with durations of 100–500 msec. High-frequency MUA (400 Hz high-pass filter) during IEDs was phase-locked to population-field activity (1–100 Hz bandpass filter) (Fig. 4B). Ictal epileptiform activity consisted of an initial sustained and subsequently intermittent pattern of population discharges (Fig. 4C). The sustained pattern lasted 4–8 sec and was characterized by rhythmic oscillations of 4–10 Hz that gradually decreased in frequency and increased in duration and amplitude. This activity was followed by a more intermittent pattern that lasted 21 ± 4 sec
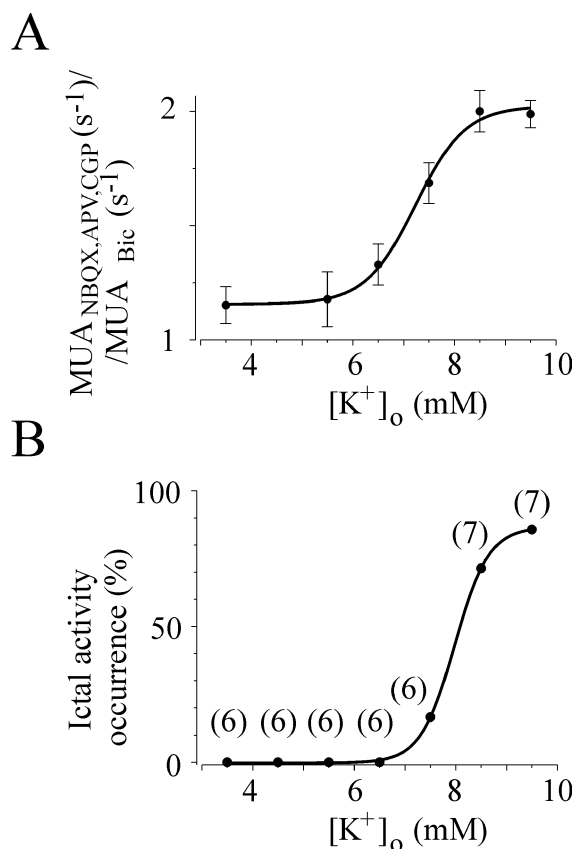
A

B

Figure 3. Effects of extracellular potassium and synaptic transmissions on neuronal firing rate and the occurrence of ictal epileptiform patterns in the P10–P11 rat hippocampal slices. *A*, Extracellular potassium concentration dependence of the effect of bicuculline (10 μM) on neuronal firing rate. *Ordinates*, MUA frequency (sec^{-1}) in NBQX (10 μM), D-APV (50 μM), and CGP55845 (*CGP*) (1 μM) normalized to MUA frequency (sec^{-1}) in bicuculline (10 μM) at various concentration of $[K^+]_o$. *B*, Occurrence of ictal epileptiform activities at different concentrations of extracellular potassium (number of slices).

(*n* = 4) and was characterized by repetitive bursts consisting of initial population spike and secondary afterdischarges. Similar field potentials also have been demonstrated in chronic seizure models *in vivo* (Bragin et al., 1999b) and in the epileptogenic region of humans with temporal lobe epilepsy (Bragin et al., 1999a). The ictal discharges were followed by a postictal depression with reduced neuronal activity. Continuous application of high K$^+$ resulted in recurrent ictal epileptiform activities with a regular interval within a given slice. The interval varied between slices from a minimum of 60 to a maximum of 180 sec (data not shown).

Age-dependent alterations in epileptiform activity
The expression pattern of high $[K^+]_o$-induced epileptiform activity was strongly age dependent. Despite increased MUA and synchrony, application of 8.5 mM $[K^+]_o$ failed to induce epileptiform activity in the P0–P1 hippocampal slices, suggesting that the synaptic connectivity of P0–P1 rats is too low to support ictal activities. At P2, 8.5 mM $[K^+]_o$ induced large-amplitude interictal epileptiform discharges (*n* = 5), followed by intermittent ictal discharges and postictal depression (*n* = 2 of 5). The intermittent ictal pattern lasted 29.5 ± 1.2 sec and consisted of epileptiform bursts that decreased in frequency from 3 to 0.5 Hz and increased in duration and amplitude (Fig. 5*A*). Starting from P4–P5, ictal activities were characterized by 4–10 Hz sustained discharges.
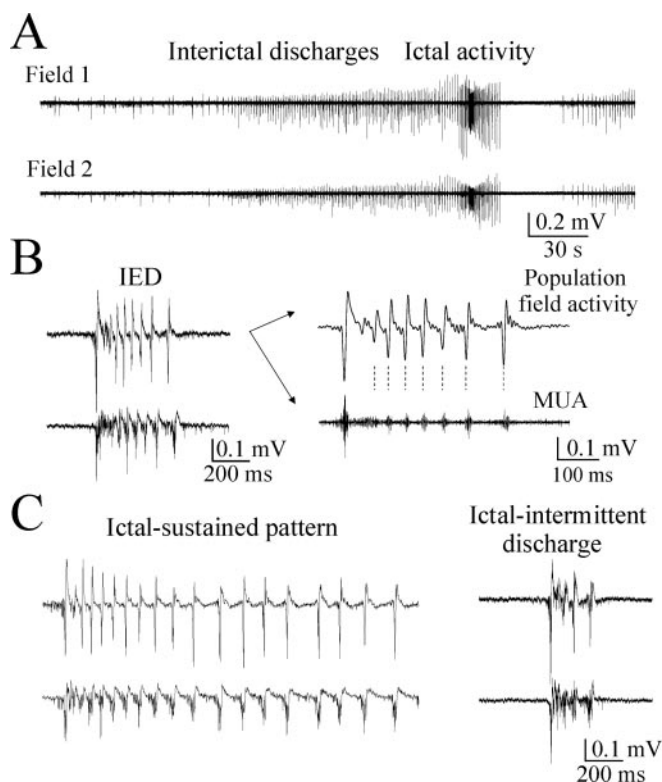
A

Figure 4. Epileptiform activities induced by 8.5 mM extracellular potassium in hippocampal slice of a P5 rat. *A*, Extracellular field potential recordings (wide band, 0.1 Hz to 10 kHz) in the pyramidal cell layer of the CA3b (*top trace*) and CA3a (*bottom trace*) subregions. Bath application of 8.5 mM $[K^+]_o$ induced interictal epileptiform discharges, ictal-sustained and -intermittent discharges, and postictal depression. *B*, Examples of interictal epileptiform discharges on an expanded time scale before filtering (*left traces*) and after filtering (*right traces*) for population field activity (1–100 Hz bandpass filter) and MUA (400 Hz high-pass filter). *C*, Hypersynchronous ictal-sustained and ictal-intermittent epileptiform discharges on an expanded time scale.

Ictal epileptiform activities with this pattern were observed in the hippocampal slices until postnatal day P16. At P15–P16, the sustained phase was characterized by 14–18 Hz oscillations lasting 8–10 sec (Fig. 5*B*). Ictus-like activities with the intermittent pattern were observed until P22 (33.3% of *n* = 6). Only brief epileptiform discharges with an interval of 2–6 sec were observed in hippocampal slices from P23-P30 rats (Fig. 5*C*), which is similar to what has been found in studies using slices from adult brain (Rutecki et al., 1985; Korn et al., 1987).

Thus, in these *in vitro* conditions, activation of the neuronal network by elevated extracellular potassium enhanced ictal epileptiform activity propensity in the hippocampal slices from P2–P23 rats (Fig. 5*D*). This developmental window corresponds closely to the window in which GABA has a net excitatory effect, as measured by extracellular multiple-unit activity counting.

Effects of GABA$_A$ receptor antagonists and agonists on ictal epileptiform activities
If the net effect of GABA$_A$-R activation in 8.5 mM $[K^+]_o$ is excitatory in slices from animals younger than P23, then, in contrast to its actions in slices from adult animals (Miles and Wong, 1987), bath application of the GABA$_A$-R antagonists should reduce epileptiform activity. Bicuculline (10 μM) reduced the frequency of interictal epileptiform discharges and blocked the ictal sustained epileptiform discharges (100%) and the ictal intermittent epileptiform discharges (90%) evoked by 8.5 mM $[K^+]_o$ in the hippocampal slices from P5–P20 rats (*n* = 10) (Fig. 6*A*). Low
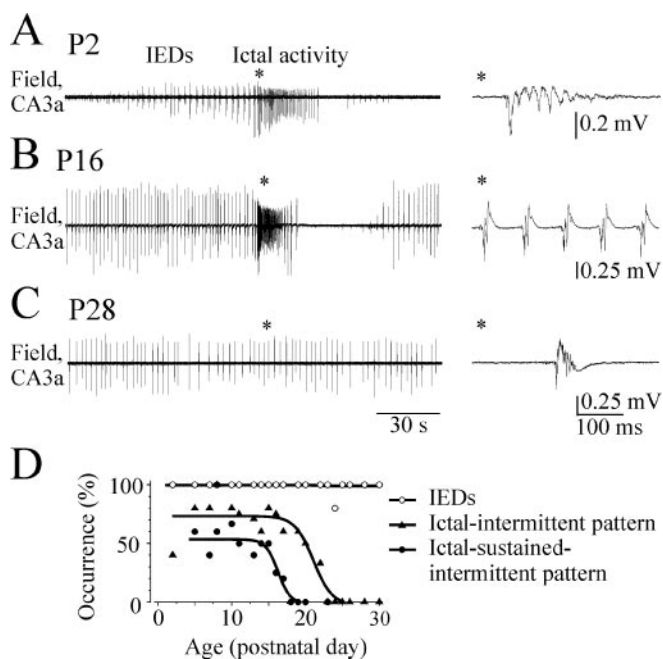
Figure 5. Age-dependent alterations of ictal epileptiform patterns induced in rat hippocampal slices by 8.5 mM extracellular potassium. *A*, Example of extracellular field recording from electrode placed in CA3a pyramidal cell layer in P2 hippocampal slice. Bath application of high K$^+$ (8.5 mM) induced interictal epileptiform discharges, followed by ictal-intermittent discharges and postictal depression. *B*, At P16, ictus-like activities were characterized by high-frequency ictal-sustained discharges. *C*, At P28, only brief epileptiform discharges were observed. The events marked by *asterisks* are shown on extended time scale. *D*, Developmental profile of 8.5 mM [K$^+$]$_o$ induced epileptiform activities in rat hippocampal slices.

Figure 6. Effects of GABA$_A$ receptor antagonists on high K$^+$-induced epileptiform activities in rat hippocampal slices. *A*, GABA$_A$ receptor antagonist bicuculline (10 μM) abolished ictal epileptiform discharges and reduced the frequency of interictal epileptiform discharges. The events marked by small letters (*a–c*) are shown on extended time scale. *B*, Low concentration (200 nM) of the GABA$_A$ receptor antagonist SR95531 [gabazine (*GBZ*)] increased ictal activity interval and decreased their duration. High concentration (10 μM) of SR95531 abolished high K$^+$-induced ictal activity in rat hippocampal slices. *A*, *B*, Extracellular field potential recordings from the CA3b pyramidal cell layer in P11 and P12 rat hippocampal slices. **At the $p = 0.05$ level, the two means are significantly different.

concentrations (200 nM) of the specific competitive GABA$_A$-R antagonist SR95531 (gabazine) reduced the frequency of interictal and ictal epileptiform discharges and decreased duration of ictal epileptiform pattern from 47.6 ± 3 to 31 ± 1.8 sec ($n = 9$; $p = 0.0002$) (Fig. 6*B*). High concentrations (10 μM) of SR95531 reduced the frequency of interictal epileptiform discharges further and abolished ictal epileptiform patterns ($n = 6$) (Fig. 6*B*).

In contrast, bath application of the GABA$_A$-R agonist muscimol (200 nM) increased the frequency of ictal epileptiform activities in hippocampal slices from P11–P14 rats ($n = 3$) (Fig. 7*A*). Low concentrations of the GABA$_A$-R agonist isoguvacine (2 μM) decreased ictal epileptiform activity intervals from 155 ± 7 to 129 ± 5 sec ($n = 9$; $p = 0.006$) and increased ictal epileptiform activity duration from 35.4 ± 3 to 46 ± 2 sec ($n = 9$; $p = 0.005$) (Fig. 7*B*). Higher concentrations of isoguvacine (10 μM) abolished ictal sustained discharges and periodically suppressed the interictal epileptiform discharges. Isoguvacine (20 μM) suppressed both ictus-like and interictus-like activities (data not shown). After washing out of isoguvacine, the ictus-like activity recovered rapidly to the control level. These results from young animals are contrary to the anticonvulsant effects of GABA$_A$-R agonists in hippocampal slices from adult rats (Korn et al., 1987).

Discussion
In this article, we describe developmental changes in the effect of endogenously released GABA, demonstrate that these effects are closely correlated with the propensity of the slice to exhibit ictal epileptiform activity, and demonstrate that GABA antagonists block this ictal epileptiform activity, whereas GABA agonists enhance it. The effects of endogenously released GABA were excitatory up to P12 in control conditions and up to P23 when [K$^+$]$_o$
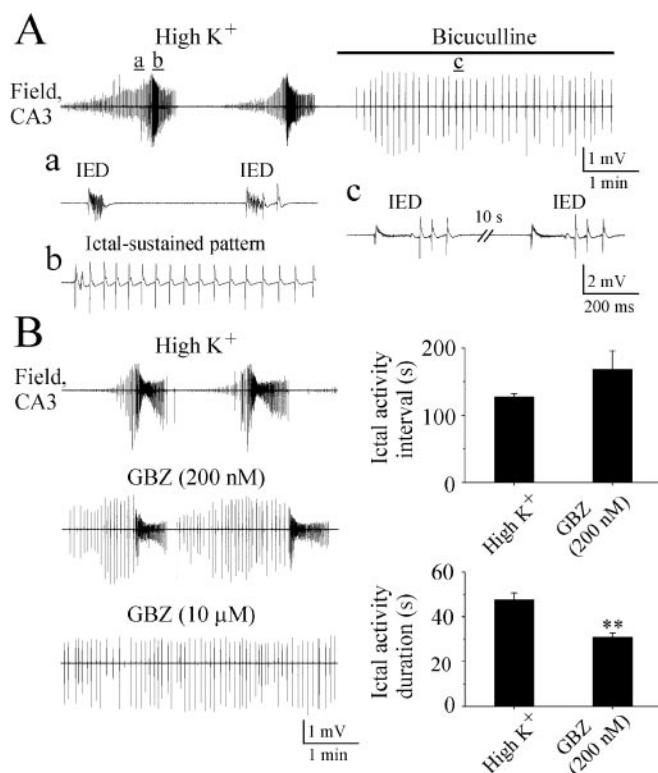
was increased to 8.5 mM. Ictal epileptiform activity could be elicited by 8.5 mM [K$^+$]$_o$ up to P23, i.e., in precisely the same age range in which GABA was excitatory. The concentration–response relationship between [K$^+$]$_o$ and the excitatory action of GABA coincided with the concentration–response relationship between [K$^+$]$_o$ and in vitro ictal epileptiform activity, providing additional evidence that the excitatory actions of endogenously released GABA are proconvulsant.

The development of hippocampal synapses are driven by early physiological patterns of synchronized neuronal activity (Garaschuk et al., 1998; Leinekugel et al., 2002). GABAergic synapses are established first (Tyzio et al., 1999) and exert an excitatory action as measured by the capacity for GABA$_A$ receptor activation to trigger action potentials in the postsynaptic cells. GABA$_A$ receptor-mediated synaptic currents with depolarizing reversal potentials are common in the embryonic and neonatal brain (Serafini et al., 1995; Chen et al., 1996; Warren and Jones, 1997) and are most likely explained by a high intracellular chloride concentration (Owens et al., 1996; Rivera et al., 1999). In addition to triggering action potentials, the depolarizing action of GABA in the neonatal brain may induce Ca^{2+} entry through voltage-dependent Ca^{2+} channels (Yuste and Katz, 1991; Lin et al., 1994; Leinekugel et al., 1995; Owens et al., 1996), contribute to removal of the Mg^{2+} block from NMDA channels, and facilitate network activity (Ben Ari et al., 1997) and may thereby trigger and modulate a wide range of developmental processes (LoTurco et al., 1995; Mitchell and Redburn, 1996).
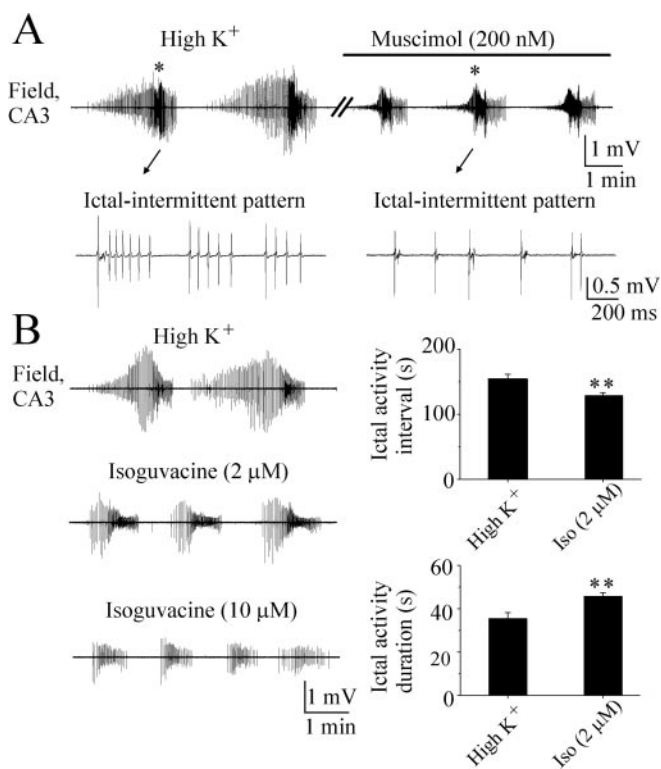
Figure 7. Effects of $GABA_A$ receptor agonists on epileptiform activities in rat hippocampal slices. *A*, $GABA_A$ receptor agonist muscimol (200 nM) decreased interval between ictus-like activities in P13 hippocampal slice. The events marked by *asterisks* are shown on extended time scale. *B*, Low concentration of the $GABA_A$ receptor agonist isoguvacine (2 μM) decreased ictal activity interval and increased ictal activity duration in P12 rat hippocampal slice. Higher concentration of isoguvacine (10 μM) abolished ictal-sustained discharges and periodically suppressed interictal epileptiform discharges. *A*, *B*, Extracellular field potential recordings from the CA3b pyramidal cell layer in rat hippocampal slices. ** At the $p = 0.05$ level, the two means are significantly different.

As development proceeds, GABA gradually becomes less depolarizing and eventually becomes hyperpolarizing. It has been most difficult to determine whether weakly depolarizing GABA responses are excitatory, because depolarizing synaptic responses can still be inhibitory if they shunt more strongly depolarizing excitatory glutamate-mediated currents (Staley and Mody, 1992). Indeed, the large conductance (~70–90 nS) of GABAergic synaptic inputs (Connors et al., 1988) and their preferential location between the excitatory inputs and the spike generation zone (Cipolloni et al., 1998) make GABA response an effective shunt for glutamate-induced postsynaptic currents.

In the early postnatal period, when GABA is most strongly excitatory, glutamatergic synaptic connectivity is too low to support ictal epileptiform activity (Traub and Miles, 1991) under normal conditions. As glutamatergic synapses develop over the first 3 postnatal weeks, the GABA response shifts from excitatory to inhibitory, which maintains the stability of the network. The developing neural network can be destabilized by increasing the strength of excitatory glutamatergic synapses, for example, by removing the magnesium block of the NMDA receptor (Wells et al., 2000). The network can also be destabilized by increasing $[K^+]_o$, as was done in this study. Increasing $[K^+]_o$ will depolarize the resting membrane potential, so that neurons are closer to action potential threshold. Increasing the extracellular potassium concentration will also decrease the rate at which Cl^- can be extruded from the neuron via K–Cl cotransport (Thompson and

Gahwiler, 1989b; Staley and Proctor, 1999), which shifts the GABA response toward excitation. The result of these effects is an increase in the age at which endogenously released GABA excites neurons (Figs. 1*C*, 2*C*). Thus, the important correlation for this study is the age at which ictal epileptiform patterns can be elicited (P23) and the age at which GABA increases action potential firing rate in elevated $[K^+]_o$ (P23).

The finding that ictal epileptiform activities can be generated by high $[K^+]_o$ in hippocampal slices during the postnatal period from P2 to P23 is earlier than reported previously in *in vitro* models (Swann and Hablitz, 2000). This may reflect continued improvements in slice preparation techniques and the thicker slices used in this study. We did not find a correlation between spike frequency and electrode depth, indicating that hypoxia in the center of our 700-μm-thick slices did not contribute to seizure propensity.

Our data suggest that endogenously released GABA is excitatory and that GABA contributes to ictal activity initiation throughout the developmental window in which $GABA_A$-R activation triggers action potentials. Previous studies have indicated that bath-applied $GABA_A$-R agonists initially increase network activity but then over several minutes inhibit network activity (Khalilov et al., 1999; Lamsa et al., 2000). This is consistent with our findings (Fig. 7*B*) and is most easily explained by a rundown in the transmembrane chloride gradient, such that ECl^- moves closer to RMP. In the neonatal hippocampus, this move toward RMP is in the negative direction and will decrease $GABA_A$-R-mediated excitation. Endogenously released GABA does not seem to be sufficient to cause this sort of rundown under the conditions studied here. Thus, the net effect of endogenously released GABA is excitatory and contributes to the decreased ictal epileptiform activity threshold in the early postnatal period.

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