Acute and Chronic Increases in Excitability in Rat Hippocampal Slices After Perinatal Hypoxia In Vivo

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Jensen, F. E., C. Wang, C. E. Stafstrom, Z. Liu, C. Geary, and M. C. Stevens. Acute and chronic increases in excitability in rat hippocampal slices after perinatal hypoxia in vivo. J. Neurophysiol. 79: 73–81, 1998. We have previously shown that hypoxia induces both acute and chronic epileptogenic effects that are age dependent. Global hypoxia (3–4% O2) induces seizure activity in the developing brain [postnatal day (P)10–12] but not at younger or older ages. Adult rats with prior seizures induced by hypoxia at P10 show increased seizure susceptibility to chemical convulsants compared with controls. In the present study, we tested the hypothesis that acute and chronic epileptogenic effects of hypoxia are demonstrable in hippocampus both in vivo and in vitro. Depth electrode recordings confirmed the presence of ictal activity within hippocampus in P10 rats during global hypoxia. Hippocampal slices prepared from P10 pups killed at 10 min after recovery from hypoxia showed evidence of increased excitability. Extracellular field recordings revealed that the amplitude and duration of long-term potentiation (LTP) was increased significantly in area CA1 of hippocampal slices removed from hypoxic pups. In addition, extracellular recordings within areas CA1 and CA3 showed significantly longer afterdischarge durations in response to kindling stimuli in slices from hypoxic pups compared with controls. To evaluate whether there were also long-term changes in hippocampal excitability, hippocampal slices were prepared from adult rats that had undergone hypoxia at P10 and compared with slices from adult litter-mate controls. A Mg2+-free medium was superfused to induce epileptiform activity within the slices. Extracellular recordings from stratum pyramidale of area CA1 showed that Mg2+-free media induced significantly more frequent ictal discharges in slices from previously hypoxic rats compared with controls. These results provide evidence that the naturally occurring stimulus of hypoxia can result in both acute and chronic changes in the excitability of the CA1 neuronal network. These results parallel our previous in vivo studies demonstrating that global hypoxia acutely increases excitability in the immature brain and that hypoxia during the age window ~P10 results in long-lasting increases in seizure susceptibility within hippocampus. Our results suggest that the age-dependent epileptogenic effects of hypoxia are in part mediated by a direct and permanent effect on neuronal excitability within hippocampal neuronal networks.

INTRODUCTION

Hypoxia is a leading cause of encephalopathy and seizures in the human newborn, occurring in a variety of conditions including birth asphyxia or respiratory distress associated with prematurity (Volpe 1989, 1994). In the neonate, seizures can be prolonged and refractory to medical therapy, whereas in the adult, hypoxia less frequently leads to severe seizures. A subset of infants with hypoxia-induced seizures develops chronic epilepsy (Bergamasco et al. 1984; Connell et al. 1989; Holmes and Kull 1990; Volpe 1989). The mechanisms underlying the heightened vulnerability of the immature brain to hypoxia-induced seizures are unknown. In addition, the relationship of the perinatal hypoxic insult and seizures to the later development of epilepsy is poorly understood.

We previously have developed an animal model of perinatal hypoxia that reproducibly demonstrates the age-dependent epileptogenicity of hypoxia. In this model, brief periods of moderate global hypoxia (3–4% O2) induce spontaneous tonic clonic seizure activity in rats aged P10–12 but not at older or younger ages (Jensen et al. 1991b). Furthermore, rat pups that are exposed to hypoxia during this time window show minimal to no histopathologic damage but exhibit increased susceptibility to convulsant-induced seizures as adults (Jensen et al. 1991a, 1992). In contrast, performance on neurobehavioral tests in later adulthood is not affected by brief global perinatal hypoxia (Jensen et al. 1992). Hence, hypoxia may selectively alter seizure susceptibility, and both the acute and chronic epileptogenic effects of hypoxia appear to be age dependent.

These in vivo observations suggest that, despite the absence of significant histopathology, moderate global hypoxia might cause acute and long-lasting increases in excitability in regions selectively vulnerable to hypoxia. In the present study, depth electrode recordings were used to determine how different brain regions are involved differentially in hypoxia-induced seizures with specific interest in the hippocampus. The hippocampus is highly susceptible to hypoxic injury, particularly area CA1 or Ammon’s horn (Crair et al. 1988; Petito et al. 1987; Pulsinelli et al. 1982). To determine whether hypoxia causes acute changes in hippocampal function, hippocampal slices were prepared from immature rats immediately after hypoxia-induced seizure activity to assess synaptic function, long-term potentiation (LTP), and seizure susceptibility. This area was chosen because preliminary results had demonstrated prolonged field excitatory postsynaptic potentials (EPSPs) in area CA1 of hippocampal slices acutely prepared from hypoxic pups (Jensen and Stafstrom 1992). Finally, to determine if neonatal hypoxia produces long-lasting changes in intrinsic seizure susceptibility of the hippocampus, extracellular electrophysiologic recordings were performed in hippocampal slices from adult rats 8–9 wk after exposure to hypoxia at P10–11.

METHODS

Male Long Evans hooded rats from Charles River (Wilmington, MA) were rendered hypoxic at P10 and then permitted to survive
for the different protocols used in this study. Pups were housed with their dam, and adult rats housed together in cages in the animal facility with a 12 h light/dark cycle. All experimental protocols were performed with the review and approval of the Institutional Animal Care and Use Committee. The care and use of the animals conformed to institutional policies and guidelines.

In vivo depth electroencephalographic recording

Animals in each treatment group underwent placement of depth electrodes before hypoxia. Rats were anesthetized with chloral hydrate (400 mg/kg ip), and bipolar electrodes (Plastics One, Roanoke, VA) were implanted stereotaxically in the same rats in two locations with the combination of locations varying between rats. The stereotaxic locations relative to the interaural line were determined from an atlas of the developing rat brain (Sherwood and Timiras 1970) as follows (in mm): hippocampus (AP +0.8, L 4.8, V +0.6), neocortex (AP +1.2, L 2.5, V +9.4), amygdala (AP –3.2, L 4.5, V –0.8), and piriform (AP 2.0, L 6, V +0.8). Electrodes were fixed to the skull with stainless steel retaining screws using cyanoacrylate and dental cement, and pups were allowed to recover for 48 h before exposure to hypoxia. The electroencephalograph (EEG) was monitored continuously throughout hypoxia.

Exposure to hypoxia in vivo and depth EEG recordings

At P10–11, animals were removed from the litter and placed in an airtight chamber with temperature maintained at 32–33°C. Electrocardiac (ECG) recordings were made with two needle electrodes placed subcutaneously on the trunk. Exposure to hypoxia was achieved by infusion of nitrogen until the internal oxygen concentration of the chamber was 3–4%. EEG and ECG activity were recorded continuously throughout hypoxia as described previously (Jensen et al. 1991b). Seizure onset and occurrence was measured by an observer blind to electrode location. Seizure activity produced by this model consists of a period of myoclonic jerks followed by tonic-clonic activity of the trunk and neck. Pups were maintained in the chamber until the onset of apnea or bradycardia to <20% of the baseline heart rate so that the endpoint of hypoxia for each animal was dependent on the same systemic responses. Using these parameters, the duration of hypoxia ranged between 15 and 20 min for most rats. Acute EEG and behavioral responses during hypoxia were recorded on a polygraph (Grass Instruments) and videotape, respectively. The latency to seizures and number of myoclonic jerks and tonic-clonic episodes were counted for each animal. Only rats exhibiting at least one tonic clonic seizure during hypoxia were used in this study.

Hippocampal slice preparation and electrophysiology

Hippocampal slices were prepared at one of two time points: 10 min after recovery from hypoxia or at 70–80 days of age (~8–9 wk after hypoxia). Control slices were prepared from litter mate rats that had not been exposed to hypoxia at P10. After decapitation, the brains were dissected rapidly from the skull and placed in cold artificial cerebrospinal fluid (ACSF) at 6°C. The formula of the ACSF varied between test paradigms as described below. The hippocampus was removed and sliced at 400-μm thickness with a manual tissue chopper (Stoelting). Slices were prepared from the middle third of the hippocampus in a plane perpendicular to the septotemporal axis. Slices were collected and transferred to a flow-through interface chamber perfused with ACSF at 38 ml/h. The ACSF and the air interface were bubbled with a 95%O2-5%CO2 gas mixture. To retain maturational differences in temperature seen in vivo, pup slices were maintained at 33.5°C and adult slices were maintained at 37°C. Long Evans rat pups removed from their dam at P10 have rectal temperatures ranging between 32.5–34°C, whereas the baseline temperature for adult rats is 37°C. Slices were permitted to incubate for 60–90 min before electrophysiological recording. EPSPs and population spikes were recorded in stratum radiatum and s. pyramidale of area CA1 and, in some cases, CA3. Recordings were made of spontaneous activity and responses to stimulation of Schaffer collaterals. A glass microelectrode filled with ACSF (1–2 MΩ) was used for recording, and the stimulating electrode was a bipolar tungsten electrode (Fred Haer). An AC amplifier (AM Systems) and storage oscilloscope (Tektronix) were used to view the response, and computerized data analysis was performed using an AD board (RC Electronics) and data acquisition software (gift of Dr. Gregory Rose, University of Utah, now available as Neuropro from RC Electronics). For evoked responses, the recording electrode was placed in s. radiatum at a depth of 75–100 μm from the slice surface and 800–1,000 μm from the stimulating electrode.

Comparison of electrophysiological responses in hippocampal slices from hypoxic versus control pups

Rat pups were killed at 10 min after onset of the recovery period. Slices from nonhypoxic litter mate pups were used as controls and coincubated with the slices removed from the hypoxic pups. Hippocampal slices were incubated in ACSF [which contained (in mM) 116 NaCl, 1.02 NaH2PO4, 26.19 NaHCO3, 5.37 KCl, 1.8 MgSO4, 3.2 CaCl2, and 10 mg/dl glucose, pH 7.4, at 33°C] for 1 h before recordings began. Before induction of LTP in both groups of slices, input/output (I/O) relationships and responses to paired pulse facilitation were measured. The EPSP in s. radiatum of CA1 was evoked by stimulation with a 0.1-ms square wave pulse delivered to the Schaffer collaterals, and an I/O curve was performed to determine the stimulus intensity required to produce a half-maximal response. All subsequent stimuli were delivered at this intensity. Next, paired-pulse stimuli were delivered at interstimulus intervals ranging from 6 to 200 ms, and the degree of paired-pulse inhibition or facilitation was calculated for each interval. EPSP slope values were used in the paired-pulse testing paradigm as only one electrode placement was made per slice to avoid injury to the slice. For paired-pulse analysis, a ratio of the second response amplitude to that of the first was used. At a given interstimulus interval, a ratio >1 indicated paired-pulse facilitation and a ratio <1 indicated paired-pulse inhibition. Finally, baseline responses were recorded every 30 s for 10–20 min. After a stable baseline of ≥10 min, tetanic stimulation was applied to the slice (2 stimuli at 100-Hz, 1-s duration, repeated 20 s apart). After tetanus, single half-maximal stimuli were applied every 30 s for 60–90 min in all slices and, in some cases, longer. The percent change in EPSP was calculated after tetanus compared with pretetanus baseline using a two-way repeated measures analysis of variance (ANOVA).

Comparison of response to in vitro kindling stimuli in hippocampal slices from hypoxic versus control pups

Rat pups used for the kindling experiments were killed at 10 min after onset of the recovery period. Slices from nonhypoxic litter mate pups were used as controls and coincubated with the slices removed from the hypoxic pups. After 1 h of incubation in ACSF [which contained (in mM) 124 NaCl, 3.3 mM KCl, 1.25 NaH2PO4, 26 NaHCO3, 1.5 MgCl2, 1.8 CaCl2, and 10 mg/dl glucose], a bipolar stimulating electrode was placed in the s. radiatum of CA3 near the timbara. Population spikes were recorded in s. pyramidale of CA3 and CA1. The stimulating intensity that induced maximal evoked response was determined by a series of single stimuli (0.1-ms duration) delivered every 30 s. A modification of the kindling paradigm described by Stasheff et al. (1985) was used
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terns of interictal or ictal epileptiform activity. Recordings were made for 60 min after exchange to the Mg$_2^+$-free medium. The presence of epileptiform activity was compared between slices from previously hypoxic and control adult rats using a Fisher’s exact test.

RESULTS

Depth electrode recordings during perinatal hypoxia

In rats with electrodes in the neocortex and hippocampus ($n = 10$), seizures were seen in both structures during hypoxia. In some rats cortex and hippocampus were activated simultaneously (Fig. 1A), but in the majority (8 of 10) cortical epileptiform activity preceded hippocampal seizures by $\approx 1-2$ s (Fig. 1B). Hypoxia induced ictal activity in pyriform cortex in 6 of 6 rat pups with pyriform electrodes. In contrast, there was no ictal activity during hypoxia in the amygdala of pups with electrodes implanted in that location ($n = 4$).

Comparison of LTP in immature hippocampal slices from hypoxic and control rats

A total of 14 P10 rats exposed to 3-4% hypoxia and 10 control P10 rats were used in this study. One slice per rat was used with the exception of two slices from a single rat in the control group, and two slices from each of two rats in the hypoxic group. A breakdown of animals used in the LTP studies is shown in Table 1. Failure to exhibit a tetanus-induced potentiation occurred in 1 of 12 control slices and 2 of 16 slices from hypoxic rats. Seizures were observed from two slices in the hypoxic group, and these slices were omitted from further analysis. No seizures were observed in the control slices. Hence, the magnitude and duration of LTP was measured from 10 control rat pup slices and 12 hypoxic rat pup slices.

The EPSP response for 10 min before and 80 min after tetanic stimulation was analyzed in 10 slices from control pups and 12 slices from hypoxic pups. Consistent with previous reports describing the developmental profile of LTP (Harris and Teyler 1984), tetanus induced only moderate EPSP potentiation in slices from P10 control rats (Fig. 2). In the control group, the average maximal EPSP change within the first 20 min posttetanus was $34.0 \pm 4.7\%$ (mean $\pm$ SE), and this typically declined over the

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FIG. 3. Representative extracellular recordings in stratum pyramidale of CA1 and CA3 after kindling stimuli in hippocampal slices from P10 rat pups in the control group (A) and from P10 rat pups after hypoxia-induced seizures in vivo (B). Afterdischarges evoked by tetanic stimuli are shown from representative slices in each group. In both CA1 and CA3, the afterdischarge duration was significantly longer in slices removed from previously hypoxic pups.

Comparison of response to kindling stimuli in hippocampal slices from hypoxic versus control pups

Six hypoxic rats and six litter-mate control rats were used in the kindling experiment. One slice was chosen from each animal for recordings. Electrographic seizures were seen after one to three trains of stimuli. The seizures consisted of high-frequency (tonic) and low-frequency (clonic) phases (Fig. 3). The high-frequency phase became constant after three to five trains of stimuli. The duration of the tonic phase was significantly longer in hypoxic slices (10.82 ± 1.17 s) than in control slices (8.46 ± 1.99 s; \( P < 0.03 \); Fig. 4). The duration of clonic phase varied among individual slices and was not significantly different between the two groups.

I/O curves and response to paired-pulse stimulation in slices from hypoxic versus control pups

I/O relationships and responses to paired-pulse stimulation were recorded in area CA1 of all slices before LTP or kindling in vitro. An analysis of I/O curves and paired pulse testing before LTP was performed for all slices that subsequently exhibited ≥25% potentiation after tetanic stimulation. Values between threshold and maximum EPSP slope were plotted against stimulus intensity for each group. The mean slope of the I/O curve for percent maximal EPSP response versus percent maximal stimulus intensity revealed
no significant differences between slices from hypoxic and control pups (mean slopes were +0.80 and +0.75 for hypoxic and control data, respectively).

Pretetanus EPSP responses were measured with paired pulse stimulation at intervals varying from 6 to 200 ms. There were no significant differences in the paired-pulse response between the hypoxic versus control groups within the range of interstimulus intervals tested. Inhibition of the second response was evident at intervals of ≈25 ms and facilitation occurred at greater interstimulus intervals in both groups.

**Incidences of seizure activity induced by Mg^{2+}-free ACSF in adult slices**

In normal ACSF, no seizure activity was recorded in area CA1 of slices from control or previously hypoxic adult rats. Spontaneous activity after exchange of the ACSF to Mg^{2+}-free ACSF was recorded for 15 slices from previously hypoxic rats and 9 slices from control adults. After the ACSF was exchanged with Mg^{2+}-free medium, all of the control and hypoxic slices exhibited interictal spikes. This interictal activity developed within 30 min after changing to the Mg^{2+}-free medium and continued throughout the 60 min exposure. In the control group, only one slice developed paroxysmal brief episodes of high-frequency spikes. In contrast, sustained ictal discharges (continuous high-frequency spike discharges) were present in 40% of the slices from adult rats with previous perinatal hypoxia (P < 0.02, Fig. 5). When all ictal-like activity was compared (paroxysmal and sustained ictal discharges), the incidence was even higher in the slices from previously hypoxic rats (67%) compared with controls (11%; P < 0.004, Table 2). Furthermore, in the slices from adult rats with previous perinatal hypoxia, intermittent ictal discharges persisted throughout the exposure to Mg^{2+}-free medium.

**I/O curves and response to paired-pulse stimulation in slices from previously hypoxic versus control adult rats**

Despite differences in response to Mg^{2+}-free medium, the I/O curves were not different between slices from adult previously hypoxic and control rats (I/O slopes were 1.29 ± 0.36 and 1.26 ± 0.35 for hypoxic and control rats, respectively). In addition, there were no differences in paired-pulse responses for the EPSP or population spike in slices from control adults versus adults with previous perinatal hypoxia-induced seizures.

**Discussion**

These studies demonstrate that a brief exposure to hypoxia during the second postnatal week in the rat is associated with both acute and chronic changes in hippocampal excitability. Depth electrode recordings confirmed the presence of ictal activity in hippocampus during hypoxia. In our model, hypoxic seizures do not result in histological changes in the hippocampus at the light microscopic level (Jensen et al. 1991b), consistent with recently reported findings (Owens et al. 1997), which also showed no dramatic acute or chronic morphological changes using a similar model. Despite the lack of overt structural changes, we report here that significant increases in excitability occurred within the hippocampal network both in response to hypoxia acutely and as a long-term sequel. Slices acutely removed from the hippocampus showed enhanced LTP and susceptibility to in vitro kindling. A long-lasting increase in susceptibility to seizures was evident in hippocampal slices removed from adult rats 70–80 days after hypoxia. These acute and chronic increases in excitability are consistent with the results from the whole animal model in which hypoxia induces acute seizures and persistently increased seizure susceptibility later in adulthood.

These results suggest that hypoxia, a naturally occurring form of perinatal injury, alters the intrinsic excitability of the hippocampus acutely and chronically. Area CA1 of hippocampus is selectively vulnerable to hypoxia/ischemia, and we now show that this region is also vulnerable to the epileptogenic effects of moderate global hypoxia. The selective vulnerability to hypoxic ischemic injury is thought to be at least in part related to the extremely high-density of glutamate receptors on these hippocampal pyramidal neurons (McDonald and Johnston 1990). In our global hypoxia model, we have shown previously that the acute and chronic...
epileptogenic effects can be blocked by 6-nitro-7-sulfamoylbenzo(f)quinoxaline-2,3-dione (NBQX), an antagonist to the non-N-methyl-D-aspartate (NMDA) subtype of glutamate receptors (Jensen et al. 1995). Hence altertions in excitatory transmission may play a critical role in the epileptogenic effects of perinatal hypoxia.

**Enhanced LTP and kindling after in vivo hypoxia in P10 rats**

Enhanced LTP was observed in slices removed after 10 min of recovery from hypoxia-induced seizures. These slices showed robust LTP, which is atypical for slices from rats at age P10. The development of LTP in area CA1 of hippocampus in Long Evans rats has been characterized by Harris and Teyler (1984); LTP first can be elicited late in the first week of life but is of very low amplitude and duration. At P10, LTP is usually <40% above baseline and is not enduring (Harris and Teyler 1984; Jackson et al. 1993; K. M. Harris, personal communication), similar to the control rats in this study. The hypoxia rats exhibited LTP that was of magnitude and duration comparable with adult animals and was never seen in control P10 hippocampal slices. Hence, hypoxia appears to “prime” the immature hippocampus for LTP. Similarly, in vitro kindling was enhanced in the slices prepared from P10 rats after hypoxia-induced seizures. Taken together, these observations from immature slices suggest that moderate hypoxia may acutely enhance excitatory synaptic transmission in the immature brain. Notably, LTP and in vitro kindling both depend on activation of excitatory amino acid (EAA) receptors (Bliss and Collingridge 1993; Harris et al. 1984; Stasheff et al. 1993a,b).

In addition to the increased amplitude and duration, the pattern of potentiation after tetanus in slices from hypoxic P10 rats appeared different from controls. Many of the slices from the hypoxic rats responded to tetanus with not only an immediate increase in amplitude but an additional slowly developing potentiation during the course of 30–40 min post tetanus. The slowly developing potentiation was similar to the tetanus-induced activation of voltage-dependent calcium channels (VDCC) (Grover and Teyler 1990). Interestingly, this slower potentiation also has been observed in hippocampal slices removed from rats with prenatal cocaine exposure (Rosen and Agha 1986). This phenomenon, termed anoxic potentiation, is thought to be secondary to anoxia-induced increase in glutamate concentration and anoxia-induced reduction of the NMDA redox site, which results in an increase in the opening frequency of the NMDA channel (Aizenman et al. 1989; Crepel et al. 1993a,b). Hence the enhanced LTP may involve hypoxia-induced enhancement of VDCCs and/or postsynaptic glutamate receptors. Paired-pulse facilitation/inhibition was not altered by hypoxia-induced seizures. Given that paired-pulse facilitation of the second of a pair of stimuli is thought to be due to residual presynaptic Ca$^{2+}$ from the first stimulus (Kamiya and Zucker 1994), the lack of change in paired-pulse responses would suggest that the priming for LTP or kindling is not primarily mediated presynaptically.

**Increased seizure susceptibility in slices from adult rats after hypoxia at P10**

Recordings from area CA1 of hippocampal slices prepared from adult rats previously rendered hypoxic exhibited increased network excitability. Ictal activity was induced more frequently by Mg$^{2+}$-free media in slices removed from adult rats that survived for ~2 mo after hypoxia at P10. This is the first evidence of a permanent alteration in hippocampal excitability after a brief hypoxic insult early in life. The lack of difference in paired-pulse responses in hypoxic versus control adults suggests that the hyperexcitability also may be due to a postsynaptic rather than presynaptic change. The age for testing was chosen at 70–80 days as this was the age at which our previous studies revealed increased susceptibility to pentylentetrazol and flurothyl seizures (Jensen et al. 1991a, 1992). An original intent of the study was to compare LTP in slices from control adults and those with previous hypoxia as was done in the immature slices. However, LTP is robust and long lasting in control adults, unlike control pups, so that in pilot studies with adult slices, it became difficult to detect any differences between the control versus previously hypoxic rats. We next chose the Mg$^{2+}$-free in vitro seizure model with which we had experience as a tool to detect differences between the two groups. We have shown previously that the response to Mg$^{2+}$-free ACSF is itself age dependent, with adult CA1 exhibiting interictal activity only, whereas P10–14 pup slices predominately showed ictal discharges (Wang and Jensen 1996). Given that ictal seizures are a rare event in normal adult slices, the abnormal presence of ictal discharges in slices from previously hypoxic rats provided evidence of increased seizure susceptibility. The pattern of ictal activity in the slices from previously hypoxic adults resembled the sustained ictal bursts seen in normal P10 pups, suggesting the possibility that hypoxia may have resulted in a dysmature state.

**Maturational phenomena coincident with the developmental window of susceptibility to hypoxia-induced seizures and hyperexcitability**

Clinically, the immature brain is more susceptible to seizures with the highest incidence of seizures from all causes occurring in the first year of life (Aicardi and Chevrie 1970). A number of seizure models, such as the kainate and pilocarpine models (Liu et al. 1994; Stafstrom et al. 1992), are characterized by the lower seizure threshold of the immature brain but a relative resistance to seizure-induced neuronal death compared with the adult. Similarly, there is a developmental window of susceptibility to hypoxia-induced seizures during which significant neuronal death does not occur. The increased susceptibility to hypoxia-induced seizures is likely due to a number of factors that are present during the second postnatal week that cause excitation to predominate over inhibition. Both NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptors are undergoing a transient overshoot in density before decreasing to adult level (Insel et al. 1990; McDonald and Johnston 1990). This overshoot occurs at a developmental stage when maturation of synaptic enzymes and receptor number for the
The effects of hypoxia is not only occurring during a time of peak hypoxic pups. In addition, BDNF has been postulated to produce seizures at P10 (Jensen et al. 1995). The developmental window of susceptibility to these epileptogenic effects of hypoxia is not only occurring during a time of peak density of AMPA receptors in hippocampus and cortex but also during a period of enhanced sensitivity to AMPA toxicity (McDonald et al. 1992). Hence the efficacy of NBQX in suppressing hypoxia-induced seizures in P10 rats may be related to the maturational profile of the AMPA receptor.

The subunit composition of EAA receptors around P10 also enhances excitation. The NMDAR2c subunit, which confers lower Mg$^{2+}$ sensitivity, is expressed to a greater degree in hippocampus during this developmental window than in adulthood (Pollard 1993). The AMPA receptor is transiently permeable to Ca$^{2+}$ because of a relative lack of expression of the GluR2 subunit compared with that in adulthood (Monyer et al. 1994), rendering the cell more excitable (Bowe and Nadler 1990). During the same time period, it has been reported that levels of the Ca$^{2+}$-binding protein calbindin D$_{28K}$ are lower than in adulthood (Wastlerain et al. 1991).

Despite increased seizure susceptibility, the immature brain is relatively resistant not only to seizure-induced neuronal damage but also to hypoxia-induced neuronal death (Duffy et al. 1975; Glass et al. 1944; Himwich et al. 1941). In vitro, anoxia produces smaller changes in excitatory synaptic transmission, membrane potential, and input resistance in CA1 of immature hippocampal slices compared with those from adult rats. Excitatory synapses of immature neurons are perhaps selectively resistant to attenuation by hypoxia while inhibitory synapses in immature slices are just as sensitive to hypoxia-induced decreases as those of the adult (Cherubini et al. 1989). In fact, Cherubini et al. (1989) observed hyperexcitable responses in immature CA1 in the postanoxic period with a relative lack of the postanoxic hyperpolarization commonly seen in adult tissue (Dunwiddie 1981; Fujiwara et al. 1987).

Possible mechanisms underlying the hyperexcitable phenomena observed in hippocampal slices after hypoxia-induced perinatal seizures

Hypoxia has been shown to disrupt Ca$^{2+}$ homeostasis and mobilize intracellular Ca$^{2+}$ stores in hippocampal neurons. Disordered Ca$^{2+}$ homeostasis could be exaggerated in the immature hippocampus where Ca$^{2+}$ binding proteins are less than adult levels. Hence increased intracellular Ca$^{2+}$ may provide a mechanism for the enhanced LTP and increased kindling seen in P10 hippocampus after hypoxia-induced seizures in vivo. Hypoxia also may enhance subsequent LTP via hypoxia- or seizure-induced increases in neurotrophic factors. The immature brain has significantly higher levels of many neurotrophic factors compared with the adult. Certain neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) can be induced within hours after hypoxia/ischemia episode or seizures in vivo (Tsubokawa et al. 1992) and has been shown to be induced by depolarization in vitro (Ghosh et al. 1994). BDNF application induces a slowly developing potentiation in mature hippocampal slices in vitro (Kang and Schuman 1995) and has been shown to enhance potentiation in immature tissue in a manner similar to that observed in these experiments (Figurov et al. 1996).

Hypoxia-induced increases in BDNF could contribute to the enhanced LTP seen in hippocampal slices removed from hypoxic pups. In addition, BDNF has been postulated to have a role in maintaining Ca$^{2+}$ homeostasis (Ghosh et al. 1994); this may afford some protection from neuronal death in immature neurons where its constitutive expression is high.

The mechanism whereby perinatal hypoxia-induced seizures permanently alter seizure susceptibility is unknown. There is abundant evidence that, in more severe models of hypoxia/ischemia, the majority of events responsible for neuronal death occur during 12-24 h with a cascade of Ca$^{2+}$-mediated events (for review see Choi 1992). In the present model of perinatal hypoxia, Ca$^{2+}$-mediated events might trigger an upregulation of excitability rather than death (Ghosh and Greenberg 1995). Hypoxia-induced alteration in EAA receptor function may be a candidate mechanism for the chronic enhancement of excitability. The functional effect on EAA receptors could be due to structural alterations such as receptor phosphorylation or a change in subunit composition, as these previously have been reported after seizures or hypoxia/ischemic injury (Friedman et al. 1994; Pellegrini-Giampietro et al. 1992; Perez-Velazquez and Zhang 1994).

In summary, the second postnatal week is a period of heightened excitability and susceptibility to epileptogenic stimuli, which is associated with a high rate of synaptogenesis and plasticity. Concurrently, the immature brain at this stage is relatively resistant to seizure-induced neuronal injury. Our data suggest that hypoxia-induced seizures lead to acute and long-lasting hyperexcitability within hippocampal neuronal networks. There are numerous maturational factors that may be contributing to this clinically relevant form of age-dependent seizure susceptibility. These in vitro observations parallel our previous in vivo results and suggest that hippocampal hyperexcitability in part underlies the acute and chronic epileptogenic effects of perinatal hypoxia in our rat model. The present model of perinatal hypoxia suggests that a single brief episode of hypoxia-induced seizures can prime neuronal networks within minutes to hours for subsequent EAA receptor-mediated processes such as LTP and kindling. Furthermore, brief hypoxia results in permanent increases in seizure susceptibility within these neuronal networks. These results suggest that the age-dependent epileptogenic effects of hypoxia in part are mediated by a direct and permanent effect on neuronal excitability within hippocampus. Future use of this model should provide insight into the mechanism underlying the age-dependent susceptibility of the immature brain to the epileptogenic effects of hypoxia and to the cellular events resulting in a chronically seizure-susceptible state.

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