Prolonged NMDA-Mediated Responses, Altered Ifenprodil Sensitivity, and Epileptiform-Like Events in the Malformed Hippocampus of Methylazoxymethanol Exposed Rats

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Calcagnotto, Maria Elisa and Scott C. Baraban. Prolonged NMDA-mediated responses, altered ifenprodil sensitivity, and epileptiform-like events in the malformed hippocampus of methylazoxymethanol exposed rats. J Neurophysiol 94: 153–162, 2005. First published March 16, 2005; doi:10.1152/jn.01155.2004. Cortical malformations are often associated with refractory epilepsy and cognitive deficit. Clinical and experimental studies have demonstrated an important role for glutamate-mediated synaptic transmission in these conditions. Using whole cell voltage-clamp techniques, we examined evoked glutamate-mediated excitatory postsynaptic currents (eEPSCs) and responses to exogenously applied glutamate on hippocampal heterotopic cells in an animal model of malformation i.e., rats exposed to methylazoxymethanol (MAM) in utero. Analysis revealed that the late N-methyl-D-aspartate (NMDA) receptor-mediated eEPSC component was significantly increased on heterotopic cells compared with age-matched normotopic pyramidal cells. At a holding potential of +40 mV, heterotopic cells also exhibited eEPSCs with a slower decay-time constant. No differences in the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) component of eEPSCs were detected. In 23% of heterotopic pyramidal cells, electrical stimulation evoked prolonged burst-like responses. Focal application of glutamate (10 mM) targeted to different sites near the heterotopia also evoked epileptiform-like bursts on heterotopic cells. Ifenprodil (10 μM), an NR2B subunit antagonist, only slightly reduced the NMDA receptor (NMDAR)-mediated component and amplitude of eEPSCs on heterotopic cells (MAM) but significantly decreased the NMDAR component and peak amplitude of eEPSCs in normotopic cells (control). Our data demonstrate a functional alteration in the NMDA-mediated component of excitatory synaptic transmission in heterotopic cells and suggest that this alteration may be attributable, at least in part, to changes in composition and function of the NMDAR subunit. Changes in NMDAR function may directly contribute to the hyperexcitability and cognitive deficits reported in animal models and patients with brain malformations.

INTRODUCTION

Injection of methylazoxymethanol (MAM) into pregnant rats produces offspring with diffuse cortical dysplasia, microcephaly, loss of lamination in cortical and hippocampal structures, and heterotopic cell clusters in the hippocampus. These animals are highly seizure susceptible (Baraban and Schwartzkroin 1996; Chevassus-au-Louis et al. 1998a; de Feo et al. 1995; Germano et al. 1996) and exhibit significant cognitive impairment (Balduini et al. 1986; Gourevitch et al. 2004; Ramakers et al. 1993). Dysplastic pyramidal neurons immunoreactive for SMI 311 and MAP2 are observed both in the brains of MAM-exposed rats and in humans with cortical dysplasia (Baraban et al. 2000; Colacitti et al. 1999; Spreatico et al. 1998). Moreover, the morphology of dysplastic neurons in MAM-exposed animals (Castro et al. 2002; Colacitti et al. 1999; Singh 1977, 1978) is strikingly similar to that of cells found in human periventricular nodular heterotopias. All of these features indicate that MAM-exposed animals can be used as an experimental model of human brain malformations. Clinically, neurological impairment and intractable seizure disorders are often associated with brain malformations (Palmini et al. 1994), and analysis of an appropriate animal model could provide significant insights into how a malformed brain functions.

Electrophysiological studies of the MAM model have indicated that regions of abnormal hippocampal organization are capable of independent epileptiform activity generation (Baraban et al. 2000), possibly attributable to “excessively bursting” heterotopic neurons lacking an A-type potassium channel (Castro et al. 2001; Sancini et al. 1998). That MAM animals are seizure susceptible but do not exhibit a robust spontaneous seizure phenotype could be explained by enhanced GABA-mediated synaptic inhibition in and around heterotopic cell regions (Calcagnotto et al. 2002). Using molecular approaches, studies have shown alterations in targeting and proper assembly of the N-methyl-D-aspartate (NMDA) glutamate receptor subunits NR2A and NR2B on the postsynaptic membrane of heterotopic neurons (Gardoni et al. 2003), a decrease in CaMKII-dependent phosphorylation of NR2A/B subunits (Caputi et al. 1999), and abnormalities in the expression of glutamate receptor subtypes in cortical and hippocampal heterotopic areas (Rafiki et al. 1998) in the brains of MAM-exposed rats. Although it is likely that these factors interact to contribute to hyperexcitability of heterotopic hippocampal neurons in the MAM model, relatively little is known about overall excitatory synaptic function within heterotopic regions; reduced sensitivity to an NMDA receptor antagonist and biochemical similarities between hippocampal heterotopic and neocortical Layer II/III pyramidal cells were noted in a prior study (Pentney et al. 2002). In epilepsy associated with a brain malformation, the NMDA subtypes of glutamate receptors are of particular interest as they are involved in neuronal circuit formation, synaptogenesis, synaptic plasticity, and hyperexcitability (Carroll and Zukin 2002; Gardoni et al. 2003; Komuro and Rakic 1998). Here we provide a more complete examina-

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tion of glutamate receptor-mediated components of synaptic transmission in the MAM model at early stages of development.

The NMDA-receptor (NMDAR) complex has attracted considerable attention in the last decade for its possible involvement in the pathogenesis of neurological disorders, including epilepsy (Mathern et al. 1998; Meldrum 1992; Mody and Heinemann 1987; Mody and MacDonald 1995). NMDARs are heteromeric structures primarily concentrated at postsynaptic sites (Chen and Diamond 2002; Diamond 2001; Isaacson 1999; Kullmann et al. 1999; Tovar and Westbrook 1999), although some appear to be presynaptic (Liu et al. 1994; Paquet and Smith 2000; Woodhall et al. 2001) or extrasynaptic (Lozovaya et al. 2004a,b). They consist of subunits from distinct classes termed NR1, NR2 (Kutsuwada et al. 1992; Monaghan et al. 1998; Monyer et al. 1992; Moriyoshi et al. 1991) and, as recently reported, NR3 (A and B) (Das et al. 1998; Eriksson et al. 2002; Nishi et al. 2001). NR2 subunits confer variability in the functionality of NMDARs and are composed of four homologous subunits: NR2A, NR2B, NR2C, and NR2D (Ishii et al. 1993; Monyer et al. 1992). Co-expression of NMDAR1 with one or more of the NR2 subunits generates receptors with distinct functional, pharmacological, and kinetic properties. (Ikeda et al. 1992; Ishii et al. 1993; Kutsuwada et al. 1992; Monyer et al. 1992, 1994; Vicini et al. 1998). During neocortical development, there is a shift from predominately NR2B to both NR2A and NR2B receptor subunit expression (Sheng et al. 1994; Williams et al. 1993), with a concomitant decrease in the decay time constant of excitatory postsynaptic currents (Flint et al. 1997; Roberts and Ramoa 1999; Stocca and Vicini 1998).

Alterations in NMDARs in other animal models (e.g., the freeze-lesion model) and in humans with cortical malformations and epilepsy have been reported. In the freeze-lesion model, NMDARs appear to be important in the initiation and/or propagation of epileptiform activity, and the NR2B subunit is functionally increased (Swann and Hablitz 2000). Studies examining NMDAR proteins and mRNA in humans report an increase in the expression and/or assembly of NR1 and NR2 subunits in dysplastic neurons (Babb et al. 1998; Crino et al. 2001; Ying et al. 1998, 1999). Cellular density and the distribution of increased NR2A and NR2B subunit expression were shown to correlate with the presence of intrinsically epileptogenic cortical dysplasia (Najm et al. 2000). In contrast, a decrease in NR2B subunit expression (Andre et al. 2004; Battaglia et al. 2002) in dysplastic neurons has also been reported. At present, the functional consequences of NMDAR subunit expression in the malformed brain are not completely understood.

To study NMDAR-mediated responses within a malformed brain, we examined the functional and pharmacological properties of evoked excitatory postsynaptic currents (eEPSCs) in individual heterotopic cells from the MAM model, and the physiological responses to exogenously applied glutamate. Our experiments utilized infrared differential interference contrast (IR-DIC) microscopy to visualize dysplastic neurons and whole cell patch-clamp recordings to analyze synaptic currents. We present evidence that functional alterations in NMDA receptor subunits may contribute to the hyperexcitability observed in hippocampal heterotopias of MAM-exposed rats.

**METHODS**

**Prenatal methylazoxymethanol injection**

Hippocampal malformations in MAM-exposed rats have been described in detail previously (Baraban et al. 2000; Chevassus-au-Louis 1998b; Colacitti et al. 1999; Singh 1977). Pregnant Sprague Dawley rat pups were injected with 25 mg/kg MAM. MAM was purchased from the NCI Chemical Carcinogen Reference Standard Repository (Kansas City, MO). Intraperitoneal injections (0.3 ml, 15% DMSO) were made on embryonic day 15. All animal care and use conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals and was approved by the University of California, San Francisco Institutional Animal Care and Use Committee.

**Hippocampal slice preparation**

Tissue slices were prepared from male and female Sprague Dawley rat pups [postnatal day 10 (P10) to P20 (50 pups total)]. Briefly, the rats were decapitated and the brains were rapidly removed in 4°C oxygenated (95% O2-5% CO2) slicing medium, an artificial cerebrospinal fluid (ACSF) consisting of (in mM) 220 sucrose, 3 KCl, 1.25 NaH2PO4, 2 MgSO4, 26 NaHCO3, 2 CaCl2, and 10 dextrose (295–305 mosM). The hemisphere of the brain containing the hippocampus was blocked and glued using cyanoacrylic adhesive to the stage of a vibroslicer model VT1000S (Leica, Nussloch, Germany). Parasagittal hippocampal slices (300 μm thick) were cut in 4°C oxygenated slicing medium. The resulting slices were immediately transferred to a holding chamber, in which they remained submerged in oxygenated recording medium (ACSF) consisting of (in mM) 124 NaCl, 3 KCl, 1.25 NaH2PO4, 1.2 MgSO4, 26 NaHCO3, 2 CaCl2, and 10 dextrose (295–305 mosM). Slices were heated to 37°C, held at 37°C for 45 min, and then cooled to room temperature. For each experiment, an individual slice was gently transferred to a submersion-type recording chamber, in which it was continuously perfused with oxygenated recording medium at 33–35°C.

**Whole cell recording**

Whole cell voltage-clamp pipette recordings were obtained from neurons visually identified using an IR-DIC video microscopy system (Stuart et al. 1993). Conventional whole cell patch pipette recordings were obtained from identified neurons within 75 μm of the slice surface. Patch electrodes (3–7 MΩ) were pulled from 1.5 mm OD borosilicate glass capillary tubing (World Precision Instruments, Sarasota, CA) using a micropipette puller (P-87; Sutter Instruments, Novato, CA), coated with silicone elastomer (Sylgard, Dow Corning, Midland, MI), and fire polished. Intracellular patch pipette solution for whole cell voltage-clamp recordings contained (in mM) 135 CsCl2, 10 NaCl, 2 MgCl2, 10 HEPES, 10 EGTA, 2 Na2ATP, 0.2 Na2GTP, and 1.25 QX-314, adjusted to pH 7.2 with CsOH (285–290 mosM). Intracellular patch pipette solution for whole cell current-clamp recordings contained (in mM) 135 CsCl2, 10 NaCl, 2 MgCl2, 10 HEPES, 10 EGTA, 2 Na2ATP, 0.2 Na2GTP, and 1.25 QX-314, adjusted to pH 7.2 with CsOH (285–290 mosM). eEPSCs were recorded in age-matched heterotopic and normotopic CA1 pyramidal (control) cells; in some cases, normotopic cells were sampled in tissue slices with no clear evidence of malformation. The mean age for heterotopic cells (14 days old; P = 0.2). eEPSCs were evoked at 0.1 Hz using a bipolar electrode placed in sites adjacent to the heterotopia or in the Schaffer collaterals. Low-frequency (0.1 Hz) 100-μs pulses were applied, and their intensity was increased until the threshold for eliciting a detectable monosynaptic eEPSC was reached. Stimulus intensity was then increased to two times the threshold and maintained at this intensity for the entire experiment. Voltage and current were recorded with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA) and monitored on an
oscilloscope. Whole cell voltage-clamp data were low-pass filtered at 1 kHz (3 dB, 8-pole Bessel filter), digitally sampled at 2 kHz, and monitored with pClamp software (Axon Instruments) running on a personal Pentium computer (Dell Computer, Round Rock, TX). Whole cell access resistance was carefully monitored throughout the recording, and cells were excluded from analysis if values changed by >15% or exceeded 20 MΩ; only recordings with stable series resistance of <20 MΩ were used for eEPSC analysis. Cells were held at −70 and +40 mV, and the data were discarded if the holding current required to maintain this membrane potential changed by >15%. Alpha- amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-mediated eEPSCs were measured as peak inward current at −70 mV; the NMDA-mediated component was measured as the late component (80–85 ms after stimulus) of the outward current at +40 mV when the AMPA EPSC had fully decayed (Ehrlich and Malinow 2004).

Pharmacological agents were bath applied during voltage-clamp experiments. All eEPSCs were recorded in the presence of 10 μM bicuculline to block the postsynaptic inhibitory currents caused by activation of GABA<sub>A</sub> receptors. Kynurenate application (100 μM) at the conclusion of each recording eliminated eEPSCs, indicating that these currents were glutamatergic. The non-NMDAR antagonists 6-cyano-7-nitroquinoline-2,3-dione (CNQX; 20 μM) and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μM) were used to block the AMPA receptor-mediated eEPSCs. The noncompetitive antagonist dixocipine maleate (MK801; 50 μM used) blocks open NMDA channels (Huetten and Bean 1988; McDonald et al. 1987). D-(-)-2-amino-5-phosphonovaleric acid (D-APV; 50 μM used), an NMDAR antagonist, was also applied in several experiments. This compound shows a tendency toward selectivity for NMDAR complexes containing NR2A and NR2B subunits, with a higher affinity for NR1 and NR2A complexes and the lowest affinity for NR1 and NR2D complexes at a depolarized membrane potential (~40 mV) (Buller and Monaghan 1997). The competitive NMDA antagonist R-(-)-3-(2-carboxyipiperazine-4-yl)-propyl-1-phosphonic acid (R-CPP; 20 μM used) has higher affinity for NR2A subunits than for NR2B subunits and the lowest affinity for NR2D subunits (Beaton et al. 1992; Buller et al. 1994). The noncompetitive polyamine site antagonist ifenprodil (10 μM) and glutamate uptake blockers: dihydokainate (DHK; 10 mM) and threo-3-hydroxy-aspartate (THA; 10 mM) and threo-3-hydroxy-aspartate (THA; 0.5 mM) (Isrhot et al. 1999; Johnston et al. 1980; Morishita and Alger 1999) were also applied. A picospritzer (Parker Hannifin, Cleveland, OH) was used for 1999; Johnston et al. 1980; Morishita and Alger 1999) were also pressure pulses (10 ms; 20 psi) were used to eject glutamate from the visualized by using an IR-DIC video microscopy system. Brief positive-

**RESULTS**

All hippocampal slices used for in vitro studies contained IR-DIC-identifiable clusters of displaced pyramidal cells (heterotopia). In preliminary studies, we recorded spontaneous and miniature glutamate-mediated excitatory postsynaptic currents (EPSCs) in heterotopic and age-matched normotopic pyramidal neurons located in CA1. Analysis of spontaneous and miniature EPSC amplitude, decay time constant, rise time, and frequency revealed no differences between the two cell types (Table 1).

**NMDAR and AMPA receptor-mediated components of evoked EPSCs in MAM hippocampal heterotopias**

In voltage-clamp recordings, we found that the late NMDAR-mediated eEPSC component (most prominent at a holding potential of ±40 mV) was significantly increased in heterotopic cells compared with age-matched normotopic pyramidal cells (Fig. 1, A and B; I and 2; het: 55.6 ± 3.3 pA, n = 90; normo: 40.3 ± 3.6 pA, n = 60). We did not detect a difference in the AMPA component of synaptic transmission, most prominent at a holding potential of −70 mV (Fig. 1, A and B3; het: 172.2 ± 10.3 pA; normo: 186.2 ± 12.2 pA). Evoked EPSC events on heterotopic cells also exhibited a slower decay time constant at +40 mV as compared with normotopic cells (het: 110.1 ± 6.9 ms; normo: 78.4 ± 6.1 ms; Fig. 1C). If the long EPSCs observed resulted from altered transporter-mediated glutamate re-uptake, we would expect no (or little) change in amplitude or decay time constant for heterotopic neurons when these transporters are blocked. To control for this possibility, we examined the kinetic properties of eEPSCs in the presence of saturating concentrations of glutamate uptake blockers: DHK and THA. Bath application of DHK (1 mM) or THA (0.5 mM) produced a similar prolongation of the decay time constant for eEPSCs recorded on both cell types (P > 0.1; n = 5; Fig. 2, A and B).

Both NMDA and AMPA eEPSC components were confirmed by using appropriate receptor antagonists. The effect of D-APV, a selective NMDAR blocker, is illustrated in Fig. 3A. At a depolarized membrane potential (+40 mV), D-APV completely abolished the slow eEPSC component in both types of cells recorded at different ages from P10 to P20 (het: n = 7; normo: n = 7); this effect was fully reversible on drug washout

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**TABLE 1. Characteristics of spontaneous and miniature EPSCs in normotopic and heterotopic cells**

<table>
<thead>
<tr>
<th></th>
<th>Amplitude, pA</th>
<th>Decay Time Constant, ms</th>
<th>Rise Time (10–90), ms</th>
<th>Frequency, Hz</th>
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<tr>
<td>sEPSCs</td>
<td></td>
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<tr>
<td>Normotopic (n = 15)</td>
<td>41.4 ± 7.5</td>
<td>3.0 ± 0.3</td>
<td>2.6 ± 0.1</td>
<td>1.9 ± 0.7</td>
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<tr>
<td>Heterotopic (n = 20)</td>
<td>35.5 ± 6.6</td>
<td>2.7 ± 0.2</td>
<td>2.8 ± 0.3</td>
<td>1.8 ± 0.7</td>
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<tr>
<td>mEPSCs</td>
<td></td>
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<tr>
<td>Normotopic (n = 5)</td>
<td>13.8 ± 0.9</td>
<td>2.6 ± 0.1</td>
<td>3.6 ± 0.5</td>
<td>0.8 ± 0.06</td>
</tr>
<tr>
<td>Heterotopic (n = 5)</td>
<td>11.0 ± 0.7</td>
<td>2.8 ± 0.2</td>
<td>3.8 ± 0.4</td>
<td>0.8 ± 0.04</td>
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sEPSCs, spontaneous excitatory postsynaptic currents; mEPSCs, miniature EPSCs

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**Data analysis**

Evoked EPSCs were analyzed using Clampfit (Axon Instruments). ≥10 eEPSCs for each cell were averaged. Decay constants for eEPSCs at +40 mV were fit using a single-exponential equation. Results are presented as the means ± SE. Data obtained before and after drug application were analyzed using a Student’s t-test on the SigmaStat program (Jandel Scientific, Corte Madera, CA). To compare the results between different cell types, we used a one-way ANOVA. Significance level was set as P < 0.05.
consistent with previous findings (Hestrin et al. 1990). D-CPP, a competitive NMDAR antagonist, abolished the slow eEPSC component at +40 mV in heterotopic (n = 7) and normotopic cells (n = 7; Fig. 3B). MK-801 similarly abolished the NMDA-mediated component of eEPSCs in both cell types (het: n = 7; normo: n = 7; Fig. 3C). An example of CNQX application is illustrated in Fig. 4. CNQX or DNQX, AMPA receptor antagonists, essentially abolished eEPSCs at hyperpolarized potentials in both cell types (het: n = 10; normo: n = 7) as expected because no NMDA component is present at these potentials (Fig. 4B) (Hestrin et al. 1990). The slow eEPSC component was unaffected by either compound (Fig. 4A). Synaptic current remaining in the presence of CNQX or DNQX was abolished by adding 50 µM APV (Fig. 4A), indicating that it is mediated by an NMDAR. No differences in AMPA receptor-mediated function were found between normotopic and heterotopic cells. Taken together, these data suggest a functional increase in the NMDA-mediated component of excitatory synaptic transmission on heterotopic cells.
Evoked burst responses

In 23% of heterotopic pyramidal cells (21 of 90), we observed prolonged burst-like responses evoked by the same stimulus intensity applied for all experiments (Fig. 5, A and B), suggesting increased excitability in the heterotopic region. Burst-like events were 200–500 ms in duration and were evoked at an average stimulus intensity of two times the threshold for detection as described (see METHODS). Bath application of D-APV (50 \(\mu\)M) reversibly blocked these burst-like eEPSCs, demonstrating a requirement for postsynaptic NMDARs (Fig. 5 B, 2 and 3). To control for the possibility that differences represent a “boundary effect,” some heterotopic cells were recorded near the edge of the malformation. Focal application of glutamate within the heterotopia evoked the same burst-like responses as described in the preceding text for cells not at the heterotopia/normotopia boundary.

Co-application of 50 \(\mu\)M D-APV in the perfusion medium completely blocked glutamate-evoked synaptic responses in all experiments, confirming the involvement of an NMDA receptor subtype (Fig. 6B4). Responses were reproducible during repeated local application of glutamate at the same sites (Fig. 6A2), and they did not occur when ACSF alone was applied as a control (data not shown). Although we believe space-clamp issues to be minimal for heterotopic cells (as these cells were shown to have relatively small dendritic arborizations and short dendritic shafts) (Singh 1980), we considered the possibility that burst responses could be attributed to inadequate voltage clamp. Additional experiments were performed in current-clamp mode. Focal application of glutamate (10 mM) evoked a prolonged depolarization with multiple action potentials riding on top (i.e., a burst-like response) that lasted \(\approx 600\) ms (Fig. 6C1) or 2 s (Fig. 6C4), for all heterotopic cells tested (n = 5). Burst-like responses were not observed on CA1 normotopic pyramidal neurons (not shown; n = 4).

From these data, we conclude that NMDA receptors are modified in a relatively restricted area at the site of the heterotopia.

Sensitivity to ifenprodil

Because we found alterations in the NMDAR-mediated component of eEPSCs in heterotopic cells, we wanted to test...
the hypothesis that changes in NMDAR subunit function contributes to increased NMDA current. As we found a slow decay time constant for eEPSCs in heterotopic cells, we examined whether the NR2B subunit, which displays slow decay kinetics (Chen et al. 1999, 2001), possessed altered functionality in those cells. Voltage-clamp recordings were obtained from heterotopic neurons and EPSCs were evoked by stimulation of the Schaffer collaterals. Ifenprodil effectively inhibits NR1/NR2B channels with an EC50 of 0.34 μM, whereas NR1/NR2A channels are inhibited at an ~400-fold lower affinity (EC50 of 146 μM) (Williams 1993); 10 μM ifenprodil only slightly reduced the NMDAR-mediated component and amplitude of eEPSCs on heterotopic cells (30.4 ± 5.5%; 31.8 ± 5.8%, n = 10) but significantly decreased the late current component and the peak amplitude of eEPSCs from normotopic cells (86.3 ± 4.4%; 60.2 ± 5.9%, n = 8; Fig. 7, A and B).

FIG. 5. Evoked bursting in heterotopic cells. A and B1: representative evoked bursting recordings obtained in hippocampal slices from heterotopic cells held at –70 mV. B: application of d-APV (50 μM) abolished the bursting responses (B2). The effect of d-APV was reversible (A, gray trace, and B3).

FIG. 6. Glutamate-evoked responses in heterotopic neurons. A1: frame-grabber image of an acute hippocampal slice showing the relative location of the recording electrode in the heterotopia and the sites of glutamate picospritzer application (1–4). Glutamate application was repeated in triplicate at the sites labeled in the diagram. Closed circles represent sites where bursts were evoked; open circles represent sites where bursts were not evoked. A2. B and C: sample voltage-and current-clamp recordings from a heterotopic neuron during focal glutamate application at the sites indicated in A. No response was obtained when the puffer pipette was loaded with vehicle alone (not shown). The burst responses were blocked with bath application of d-APV (50 μM) (B4).

DISCUSSION

Principal findings of our experiments are as follows. No differences in spontaneous and miniature EPSCs were detected between normo- and heterotopic hippocampal neurons in the MAM model. Hippocampal heterotopic pyramidal neurons exhibit large NMDA-mediated evoked excitatory synaptic responses with a slow decay-time constant, burst-like responses evoked by electrical stimulation and exogenous applied glutamate, and decreased sensitivity of NMDAR-mediated eEPSCs to an NR2B-subunit antagonist.

Increase of NMDA-mediated eEPSCs in heterotopic cells

A key finding in our studies is that a brief single stimulus evokes a prolonged excitatory synaptic response on hippocampal heterotopic cells. In normal CA1 pyramidal cells (or normotopic neurons), a single stimulus evokes a prominent and fast AMPA receptor-mediated eEPSC (Atluri and Regehr 1998; Barbour et al. 1994) and a much smaller and slow NMDAR-mediated eEPSC (Glitsch and Marty 1999). The brief AMPA-mediated eEPSC is a consequence of a short-lived transient release of glutamate into the synaptic cleft (Atluri and Regehr 1998; Chen and Regehr 1999) and the rapid deactivation kinetics of the AMPA receptor subunit (Barbour et al. 1994). The small, long-lived NMDA-mediated eEPSC is also consistent with a brief glutamate signal activating a small number of NMDARs with stereotypically slow kinetics (Edmonds et al. 1995; Lester et al. 1990). In contrast to what we observed for normotopically organized CA1 pyramidal neurons, heterotopic cells featured synaptic responses with a larger and slower NMDAR-mediated component; no change in the AMPA receptor-mediated response was noted. In addition, no decreased functionality of the glutamate transporters was found in regions of malformation, suggesting that the long EPSCs observed did not resulted from altered transporter-mediated
glutamate re-uptake. That these findings are consistent with a condition of increased excitation that potentially contributes to epileptogenesis is further supported by observations of stimulation evoked burst-like responses in some heterotopic neurons. An increase in NMDA-mediated excitatory current onto heterotopic neurons, in combination with the increased firing frequency (Castro et al. 2002; Sancini et al. 1998) and direct connectivity with neocortex (Chevassus-au-Louis et al. 1998b) characteristic of these displaced neurons would establish a condition where hyperexcitability can be quickly transformed into generalized seizure discharge. Indeed, the burst-like epileptiform responses observed here may be a common feature of synaptic function in the epileptic brain: an increase in whole cell NMDA-mediated conductance has been reported in the kindling (Mody and Heinemann 1987), kainate (Wheal et al. 1991), and pilocarpine (Isokawa and Mello 1991) models of epilepsy. Whether a more complete understanding of this circuitry will lead to the design of novel antiepileptic drugs explicitly designed to combat hyperexcitability in a malformed brain remains to be determined.

**Burst-like responses in heterotopic cells**

Evoked burst-like responses observed in heterotopic cells appear to be mediated, at least in part, by activation of NMDARs. This would parallel the “normal” brain, where it has been shown that glutamatergic synaptic excitation is mediated predominantly by NMDARs (Davies and Watkins 1983; Fleidervish et al. 1998; Fox et al. 1989, 1990). In our present experiments, bath application of N-AVP (50 μM) reversibly blocked not only the recurrent component of eEPSCs but also the bursts evoked by exogenous applied glutamate (Fig. 6). Previous studies demonstrated that NMDAR antagonists either block the late recurrent component of evoked epileptiform discharges, (Luhmann et al. 1998) or abolish the discharges entirely (Jacobs et al. 1999). NMDAR antagonists can also raise the threshold for generation of epileptiform discharges, indicating that NMDARs play an important role in the initiation and/or propagation of epileptiform discharges (DeFazio and Hablitz 2000). It is important to point out that local application of glutamate did not evoke burst-like events in normotopic cells and only evoked NMDA-mediated epileptiform events in heterotopic neurons. From these data, we conclude that NMDAR function is modified in a relatively restricted area at the site of the heterotopia. This hypothesis is supported by the results of Jacobs et al. (1996), who demonstrated in a freeze-lesion malformation model that APV-sensitive epileptiform activity could be elicited in only a very small area surrounding the microgyrus and not by identical stimulation of remote areas. Our findings may also suggest that local excitatory connections in the heterotopic region are increased during epileptogenesis, as observed previously in the kindling model (Shao and Dudek 2004).

**Potential modification of the NMDAR subunit composition in heterotopic cells**

NMDAR subtype distribution and composition are likely to mediate not only prolonged excitatory synaptic responses but also alterations in sensitivity to NMDAR subunit antagonists in heterotopic cells. Here, we used a pharmacological approach to analyze NMDAR function and found that the NR2B receptor antagonist ifenprodil did not have a significant inhibitory effect on NMDAR-mediated eEPSCs onto heterotopic cells. Altered sensitivity to ifenprodil was previously described in human cortical dysplasia (Andre et al. 2004), in the kindling (unpublished data from Dalby and Mody 2003), and in the freeze-lesion (DeFazio and Hablitz 2000) models of epilepsy. In contrast to the MAM model findings, an increase in ifenprodil sensitivity was reported in cells outside of the microgyrus in the freeze-lesion model. This discrepancy may be due to differences in when the malformation-inducing insult was
administered (prenatal for MAM vs. postnatal for freeze-lesion) or be consistent with the many functional and anatomical differences already reported between these two distinct models. Interestingly, the decrease in ifenprodil sensitivity reported for cells sampled from pediatric patients with focal cortical dysplasia (Andre et al. 2004) is similar to that observed here. Although neither of these findings fits with immunohistochemical evidence suggesting an increase in NR2 NMDA subunits on dysplastic neurons (Ying et al. 1998, 1999), they may reflect an important difference in how receptor subunits are assembled (and trafficked to their appropriate dendritic site) to make functional receptors in regions of dysplasia.

It has generally been assumed that at glutamatergic synapses, abundance in NR2B subunits generate EPSCs characterized by sensitivity to NR2B-selective antagonists and slow decay kinetics (Tovar and Westbrook 1999; Vicini et al. 1998). However, we found that NMDA-eEPSCs on normotopic cells are significantly more affected by the NR2B-selective antagonist ifenprodil when compared with NMDA-eEPSCs on heterotopic cells. This result was unexpected but is not unique. Dissociation between NR2B-selective antagonist sensitivity and slow decay kinetics has previously been reported in the literature (Barth and Malenka 2001). Faster NMDA-EPSC kinetics is not always associated with a replacement of NR2B by NR2A subunits or vice versa (Flint et al. 1997; Shi et al. 1997). This suggests that a rearrangement of NMDA heteromers would form NMDARs with different kinetics (Cull-Candy et al. 2001; Flint et al. 1997) and different sensitivity to NR2B-selective antagonists (Hawkins et al. 1999; Kew et al. 1998). Interestingly, these trimERIC assemblies have also been reported to lose their sensitivity to activity-dependent antagonists (Brimecombe et al. 1997). Based on the pharmacological properties of the responses in each pathway and considering that in the adult hippocampus NR2A and NR2B mRNAs predominate in normotopic pyramidal cells (Monyer et al. 1994), we propose that an unusual contribution of NR1/NR2A/NR2B heteromers to the heterotopic synaptic NMDARs may explain the observed dissociation between ifenprodil sensitivity and slow decay kinetics.

**Conclusions**

Our findings suggest that NMDAR-mediated synaptic responses are abnormal in regions of hippocampal malformation. These findings could provide the basis for explaining cognitive deficits, developmental delay, and generation of seizures in the brains of patients with cortical malformations. It is hoped that further investigation of the precise mechanisms leading to development of these hyperexcitable synaptic responses could provide therapeutic targets for these types of disorders.

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