Reduced Excitatory Drive in Interneurons in an Animal Model of Cortical Dysplasia

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Xiang, Hui, Huan-Xin Chen, Xin-Xin Yu, Michael A. King, and Steven N. Roper. Reduced excitatory drive in interneurons in an animal model of cortical dysplasia. J Neurophysiol 96: 569–578, 2006. First published April 26, 2006; doi:10.1152/jn.01133.2005. Cortical dysplasia (CD) is strongly associated with epilepsy. Enhanced excitability in dysplastic neuronal networks is believed to contribute to epileptogenesis, but the underlying mechanisms for the hyperexcitability are poorly understood. Cortical GABAergic interneurons provide the principal inhibition in the neuronal networks by forming inhibitory synapses on excitatory neurons. The aim of the present study was to determine if the function of interneurons in CD is compromised. In a rat model of CD, in utero irradiation, we studied spontaneous and miniature excitatory postsynaptic currents (sEPSCs and mEPSCs) in cortical interneurons using whole cell recording techniques. Two types of interneurons, type I and type II, were identified based on their distinctive spike patterns and short-term synaptic plasticity. We found that the frequencies of sEPSCs and mEPSCs were significantly decreased in both types of interneurons in CD. However, the amplitude and kinetics of sEPSCs and mEPSCs were not different. Five-pulse, 20-Hz stimulation produced short-term depression in type I interneurons in both CD and control tissue. Type II interneurons showed a robust short-term facilitation in both CD and control tissue. Morphological analysis of biocytin-filled neurons revealed that dendritic trees of both types of interneurons were not altered in CD. Our results demonstrate that the excitatory drive, namely sEPSCs and mEPSCs, in two main types of interneuron is largely attenuated in CD, probably due to a reduction in the number of excitatory synapses on both types of interneurons in CD.

INTRODUCTION

Epilepsy is a debilitating neurological condition that affects ~2.5 million people in the United States with an estimated economic burden of $12.5 billion annually (Begley et al. 2000). Cortical dysplasia (CD) is a common pathological substrate of intractable epilepsy (Roper and Yachnis 2002; Taylor et al. 1971) and is found in 39–64% of children who undergo surgery for this problem (Farrell et al. 1992; Porter et al. 2003). Results from surgical resection of these lesions suggest a causal relationship (Palmini et al. 1991, 1995). However, mechanisms of epileptogenesis in CD are still poorly understood. This laboratory has adopted an animal model of CD, in utero irradiated rats, to better understand CD and epilepsy. Rats exposed to radiation in utero demonstrate microcephaly, diffuse CD, heterotopic neurons in the cerebrum and hippocampi, and hypoplasia of the corpus callosum (Roper et al. 1995). These animals have spontaneous seizures as adults (Kellinghaus et al. 2004; Kondo et al. 2001). Prior studies in irradiated rats have shown enhanced excitability in neocortical slices (Roper et al. 1997), reduced density of certain interneurons in dysplastic cortex (Roper et al. 1999), and reduced spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs) in pyramidal neurons in areas of CD (Zhu and Roper 2000) and subcortical heterotopic gray matter (Chen and Roper 2003). Recent studies from humans with focal CD and epilepsy (Calcagnotto et al. 2005) have reported a reduction in the frequency of sIPSCs and mIPSCs, similar to findings in irradiated rats (Chen and Roper 2003; Zhu and Roper 2000).

Most theories of epileptogenesis involve some imbalance of excitation and inhibition in the local circuitry of the involved cortical structure. Several epilepsy syndromes and models have shown impairment of inhibition at some level, ranging from loss of interneurons (de Lanerolle et al. 1989; Kobayashi and Buckmaster 2003; Roper et al. 1999) to alterations in GABA receptors (Brooks-Kayal et al. 1998; DeFazio and Hablitz 1999) to alterations in synaptic plasticity in inhibitory terminals (Hirsh et al. 1999). Alterations in excitatory drive in interneurons provide another potential mechanism for impaired inhibition. This has been articulated in limbic epilepsy as the “dormant basket cell” hypothesis (Bekenstein and Lothman 1993; Doherty and Dingledine 2001; Sloviter 1991; Sloviter et al. 2003; Zappone and Sloviter 2004).

The purpose of the current study was to better understand synaptic connections in cortical inhibitory interneurons in radiation-induced CD. We found a reduction of sEPSCs and mEPSCs in two types of cortical interneurons. Short-term plasticity of EPSCs was not altered. Morphological analysis did not find a significant alteration in gross dendritic arborization properties for either type of interneuron in CD. This suggests a reduction in the number and density of excitatory connections in cortical interneurons in this model. This adds another dimension to the impaired inhibition that has been described in this model and could contribute to the epileptic potential of the dysplastic cortex. We propose that similar mechanisms may be operational in human forms of injury-based CD and epilepsy.

METHODS

Animals and irradiation

Pregnant rats with known insemination times were obtained from Harlan Sprague Dawley (Indianapolis, IN). The day of insemination...
was designated embryonic day 0 (E0). Irradiation was performed on E17. Pregnant rats were placed in a well-ventilated Plexiglass box and exposed to 225 cGy of external γ-irradiation from a linear accelerator source. Control litters were obtained and housed in an identical fashion but not exposed to radiation. Offspring were used for experiments after weaning on postnatal day 21 (P21). All animals were maintained on 12 h light/dark cycles and were provided food and water ad libitum. All procedures used in the study adhered to guidelines approved by the Institutional Animal Care and Use Committee at the University of Florida.

Brain slice preparation

Coronal brain slices were obtained from 21- to 28-day-old rats using procedures described previously (Roper et al. 1997). Animals were anesthetized by the inhalation of isoflurane and decapitated, and the brain was rapidly removed. Coronal brain slices (400 μm thick) were cut at the rostrocaudal level of the anterior commissure using a Vibratome (Technical Products International, St. Louis, MO). Slices were incubated on cell culture inserts (8 μm pore diameter, Becton Dickinson, Franklin Lakes, NJ) covered by a thin layer of artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 26 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 1 CaCl2, 6 MgCl2, and 10 D-glucose and surrounded by a humidified 95% O2-5% CO2 atmosphere at room temperature (22°C). After ≥1 h incubation, the slice was transferred to a submerged recording chamber with continuous flow (2 ml/min) of ACSF as described in the preceding text except for 2 mM CaCl2 and 2 mM MgCl2 and gassed with 95% O2-5% CO2 giving pH 7.4. All experiments were carried out at room temperature (22°C).

Electrophysiological recording

Whole cell recordings were made from interneurons in dysplastic somatosensory cortex at the rostrocaudal level of the anterior commissure of animals with CD and layer IV–V of controls using infrared differential interference contrast microscopy and an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). In the coronal plane, we recorded from neurons in the dorsomedial cortex overlying the lateral ventricle as this is the region with the most pronounced dysplastic changes in the irradiated animals (Roper et al. 1995; for example, see Fig. 1 in Zhu and Roper 2000). Although heterotopic cortex occurs in this model, no recordings were performed in heterotopic cortex for this study. Patch electrodes had a resistance of 3–5 MΩ when filled with intracellular solution containing (in mM) 125 K-glucuronate, 8 NaCl, 10 HEPES, 4 MgATP, 0.3 Na3GTP, 0.2 EGTA, and 0.1% biocytin (pH 7.3 with KOH, osmolarity: 290–300 mosM). Two types of interneurons were identified at first by their morphology, then spiking pattern to a suprathreshold current pulse, and short-term plasticity evoked by 5-pulse (20 Hz) train stimulation. sEPSCs were recorded at a holding potential of −65 mV. mEPSCs were recorded by adding TTX (1 μM), Sigma) to the bath solution. Series resistance was 16–25 MΩ, and cells were rejected if it changed >10% throughout the recording session. All EPSCs (spontaneous, miniature, and evoked) were recorded with the GABA_A receptor antagonist, picrotoxin (50 μM, Sigma) in the bath solution and an Axopatch 1D amplifier.

To evoke monosynaptic EPSCs, a glass electrode (3–5 MΩ) filled with ACSF was placed 50–100 μm away from the soma of the recorded cell. Five-pulse trains at 20 Hz were used to elicit stimulus-evoked EPSCs as a measure of short-term plasticity (STP). We chose a five-pulse paradigm rather than a paired-pulse to better indicate progressive changes in STP (Varela et al., 1999). The interval between trains was 10 s. Twenty to 30 trains were given to each cell, and the responses were averaged. Monosynaptic currents were identified by their constant latency and a single peak for all five responses.

Selection and characterization of interneurons

Cortical interneurons show a great diversity with regard to their morphology, intrinsic spiking properties, STP, and proteins that they express (Maccarelli and Lacaille 2003; Markram et al. 2004). Two types of interneurons have been well characterized in a number of prior studies. One type, often termed fast spiking (FS), demonstrates high-frequency firing without adaptation in response to a depolarizing current pulse, a multipolar morphology, and is usually parvalbumin (PA) immunopositive (Amatii et al. 2002; Kawaguchi et al. 1987; McCormick et al. 1985). Another type shows lower frequency firing with adaptation both in frequency and amplitude and bursts of spikes that occur after a hyperpolarizing pulse (low-threshold spikes, LTS). These interneurons often demonstrate a bipolar morphology and are often somatostatin (SS) immunopositive (Amatii et al. 2002; Gibson et al. 1999; Kawaguchi 1995; Kubota and Kawaguchi 2000; Markram et al. 2004; Reyes et al. 1998; Xiang et al. 1998). In addition, excitatory synapses in these two types of interneurons display differences in release probability, short-term plasticity, and composition of AMPA receptor subunits (Ali et al. 1998; Beierlein et al. 2003; Reyes et al. 1998; Rozov and Burnashev 1999; Thomson and Deuchars 1997; Toth and McBay 2000) with multipolar, PA-containing cells demonstrating short-term depression and biutfuted, SS-containing cells showing short-term facilitation of EPSCs (Reyes et al. 1998).

In the present study, we studied two types of interneuron based on electrophysiological and morphological characteristics (Fig. 1). We selected cells by their morphology to record under IR-DIC microscopy. After establishing whole cell recordings in the targeted cell, we tested its firing pattern in current-clamp mode by injecting supra-threshold current (300 pA, 300 ms). Then we switched to voltage-clamp mode, and a stimulating electrode was placed in the dendritic region, 50–100 μm from the soma. STP was tested by applying 5-pulse (20 Hz) train stimulation at −65 mV. CD slices did not show clear lamination, but most disoriented pyramidal cells are located in the intermediate depths of the slices. Thus we targeted interneurons in the area where pyramidal cells are dense. The soma of the interneurons were often adjacent to the soma of pyramidal cells. In control slices, we recorded from layers IV–V. In some experiments, we recorded from layer V pyramidal cells, and these were identified based on morphology using IR-DIC microscopy.

Two methods were used for post hoc morphological characterization of the interneurons: fluorescent-tagged avidin and 3,3’-diaminobenzidine tetrahydrochloride (DAB)-based histochemistry. All slices containing biocytin-filled cells were kept in 4% paraformaldehyde overnight at 4°C, rinsed in PBS (0.1 M) two times for 30 min, and then treated with one of the following two procedures. For fluorescent labeling, slices were rinsed in PBS (0.02M) three times and then treated with 3% H2O2 in methanol for 5 min. They were incubated in 1% BSA in PBS (0.1M) for an hour and rinsed with PBS three times. They were then incubated with ExtrAvidin-FITC conjugate (1:400; Sigma) in 0.1 M PBS for 2 h in the dark at room temperature. The slices were then rinsed with 0.02 M PBS three times, coverslipped, and stored at 4°C. For DAB-based imaging, the slices were incubated in 0.1% ExtraAvidin peroxidase (Sigma) in PBS solution overnight. Slices were then treated with 3% H2O2 for 5 min, rinsed with PBS and acetate buffer, and then reacted for 10 min with a solution of 0.5 mg/ml DAB (Sigma), 4% Ni-DAB chromogen, and 0.2 μl/ml 30% H2O2 in sodium acetate buffer. Slices were then rinsed in sodium acetate buffer, dehydrated, mounted onto slides, and coverslipped. The morphology of fluorescently stained neurons was examined using fluorescence microscopy and some cells were reconstructed using confocal microscopy. Light microscopy was used to examine DAB-treated cells, and camera lucida drawings were made of these cells for morphological analysis.
Data acquisition and analysis

Data were acquired using pClamp 8 software. The recordings were started 5–10 min after accessing the cell to allow for stabilization of spontaneous synaptic activity. The recordings were analyzed only when there was no significant change in the frequency or the amplitude of spontaneous responses or in the series resistance (change <10%) during the 5-min recording. Firing rates were calculated from the number of action potentials (APs) during a 300 ms current injection (300 pA) in current-clamp configuration. Analysis of s- and mEPSCs was based on 5-min continuous recordings from each cell. Events were detected using the Mini Analysis Program (Synaptosoft, Leonia, NJ) with parameters optimized for each cell and then visually confirmed prior to analysis. The RMS noise level was 2.1 ± 0.2 pA. The initial detection threshold for s- and mEPSCs was set at 10 pA or five times the RMS noise level. Then the entire recording was visually inspected. EPSCs <10 pA were selected visually, and electrical artifacts were excluded. Overlapping events were also excluded. The peak amplitude was measured from each event and then averaged. Ten to 90% rise time and the decay time constant were measured based on the average of all events aligned by rise phase. All results are reported as means ± SE. The unpaired Student’s two-tailed t-test was used to compare group results, unless otherwise indicated. Short-term plasticity of evoked EPSCs was evaluated using a repeated-measures ANOVA to look for differences between controls and CD.

For a quantitative analysis of dendritic morphology, camera lucida drawings were performed on DAB-stained cells, and the number of intersections at concentric circles (20 μm apart) was counted for each cell (Sholl 1953). The number of primary dendrites and total dendritic branch points was also determined for each cell. The total length of dendrites for each cell was estimated by multiplying total segments within each concentric circle by 20 μm. Comparison of the number of intersections between control and CD was performed by two-way ANOVA. The difference of the number of primary dendrites, total dendritic branch points and estimated total dendritic length between control and CD were analyzed by the Student’s t-test. Statistical significance was defined as P < 0.05.

RESULTS

Identification of two types of interneurons

The structural abnormalities of CD in the irradiated rat have been detailed previously (Marin-Padilla et al. 2003; Roper 1998). In this study, all slices demonstrated CD and subcortical neuronal heterotopia. CD was distinguished from control neocortex by a thinner cortex without clear lamination, disoriented pyramidal cells, and the presence of heterotopic gray matter in the subcortical white matter.

In spite of great variability in morphological and physiological properties of cortical interneurons (Maccatheri and Lacaille 2003; Markram et al. 2004), two types of interneurons have been well characterized. FS interneurons demonstrate high-frequency firing without adaptation in response to a depolarizing current pulse, multipolar morphology, are PA immunopositive, and show short-term depression of EPSCs (Amitai et al. 2002; Gibson et al. 1999; Kawaguchi et al. 1987; McCormick et al. 1985). These are the most prevalent subtype of cortical interneurons (Gonchar and Burkhalter 1997) and play a role in feed-forward inhibition (Beierlein et al. 2003; Gibson et al. 1999). Another type interneurons show lower-frequency firing with adaptation. These interneurons often demonstrate a bipolar morphology, are SS immunopositive (Amitai et al. 2002; Gibson et al. 1999; Kawaguchi 1995; Kubota and Kawaguchi 2000; Markram et al. 2004; Xiang et al. 1998), and show short-term facilitation of EPSCs (Beierlein et al. 2003; Reyes et al. 1998).

As illustrated in Fig. 1, we identified the two types of interneurons based on firing pattern, STP, and morphology. We arbitrarily assigned the name, type I, to a group of interneurons showing fast-spiking without adaptation and short-term depression (Fig. 1A). We morphologically characterized 15 type I cells from controls and 16 cells from irradiated animals with fluorescent labeling and 5 cells from controls and 5 cells from irradiated animals using DAB immunohistochemistry. All of these cells had a multipolar morphology (Figs. 1A and 2A). In contrast, type II interneurons demonstrated spiking with frequency adaptation and robust short-term facilitation (Fig. 1B). We imaged 23 type II cells from controls and 30 type II cells from irradiated animals using fluorescent labeling and 5 cells from controls and 7 cells from irradiated animals using DAB immunohistochemistry. All type II cells had a bipolar morphology (Figs. 1B and 3A).

FIG. 1. Functional and morphological characteristics of two types of interneurons. A: a representative type I interneuron shows fast spiking without adaptation to suprathreshold current injection (300 pA, 300 ms, left), progressive depression of excitatory postsynaptic currents (EPSCs) evoked by 5-pulse (20 Hz) train stimulation (middle) and a multipolar morphology (right). Middle: trace is an average of 10 consecutive traces (in both A and B). B: a representative type II interneuron shows a moderately fast-spiking frequency with adaptation to suprathreshold current injection (300 pA, 300 ms, left), progressive facilitation of EPSCs evoked by train stimulation (middle) and a bipolar morphology (right).

observe significant alterations in membrane properties or AP properties between CD and control in either type of interneuron. Pyramidal cells were easily distinguished from both types of interneurons based on morphology, a higher membrane capacitance, a slower firing rate, and absence of large, fast AHPs indicated by arrows. In both CD and control slices, we encountered some neurons that were multipolar but displayed regular spiking patterns and STP similar to pyramidal cells (i.e., short-term depression). These cells were considered putative excitatory spiny stellate neurons and were excluded from analysis. Therefore it was possible to exclude excitatory cells from our recordings based on physiological profiles.

**Frequency of sEPSCs and mEPSCs in type I interneurons is decreased in CD**

We made successful whole cell recordings from 16 type I interneurons in CD and 15 in control slices. As shown in Fig. 2, the frequency of sEPSCs was significantly lower in CD compared with controls. On average, the frequency was 1.76 ± 0.36 Hz in CD and 4.38 ± 0.74 Hz in controls ($P < 0.05$). However, the median amplitude (CD = 12.58 ± 0.46 pA; control = 12.84 ± 0.85 pA), median rise time (CD = 0.71 ± 0.03 ms; control = 0.92 ± 0.09 ms), and average decay constant (CD = 3.66 ± 0.48 ms; control = 3.04 ± 0.34 ms) of sEPSCs did not show any significant differences.

The synaptic events described as sEPSCs are principally generated by two different mechanisms. Some sEPSCs are driven by presynaptic spontaneous action potentials and depend on Ca$^{2+}$ influx into the presynaptic terminal. Other events, mEPSCs, are independent of action potentials in the presynaptic cells and caused by random releases of glutamate in the presynaptic vesicles. We wanted to determine the relative contribution of mEPSCs on the reduction in frequency of sEPSCs that we found in CD. TTX (1 μM) was added to the perfusion after the cell was identified to block the action potential-driven events. In control tissue, we found that a large portion of sEPSCs (64%) in type I interneurons was driven by action potentials. As illustrated in Fig. 4, the frequency of mEPSCs was found to be significantly reduced in CD. The average mEPSC frequency in type I interneurons was $0.91 ± 0.14$ Hz in CD ($n = 11$) and $1.59 ± 0.26$ Hz in controls ($n = 11$; $P < 0.05$). Thus mEPSC frequency was reduced by 43% in dyplastic type I interneurons. We did not detect any significant difference in mEPSC median amplitude (CD = 11.61 ± 0.69 pA; control = 11.82 ± 0.89 pA, Fig. 4C).
We recorded 30 type II interneurons in CD and 23 in controls. As illustrated in Fig. 3, the frequency of sEPSCs in CD was significantly reduced by 52%. On average, the frequency was 1.29 ± 0.19 Hz in CD and 2.66 ± 0.37 Hz in control (*P < 0.05). Again, we did not notice any change in the amplitude or kinetics of sEPSCs between CD and controls. On average, the median amplitude, median rise time, and average decay constant were 12.78 ± 0.89 pA, 1.17 ± 0.12 ms, and 5.55 ± 0.45 ms in CD and 13.27 ± 0.92 pA, 0.96 ± 0.05 ms, and 4.93 ± 0.33 ms in controls, respectively.

We found the average mEPSC frequency was also significantly reduced in type II interneurons. As shown in Fig. 4, we found a 68% reduction of mEPSC frequency in CD compared with controls. mEPSC frequency was 0.56 ± 0.16 Hz in CD (n = 10) and 1.73 ± 0.58 Hz in controls (n = 9; *P < 0.05).

### TABLE 1. Intrinsic membrane properties of interneurons and pyramidal cells

<table>
<thead>
<tr>
<th>Type I</th>
<th>Type II</th>
<th>Pyramidal Cells</th>
</tr>
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<tbody>
<tr>
<td>CD (16)</td>
<td>Control (15)</td>
<td>CD (30)</td>
</tr>
<tr>
<td>C_m, pF</td>
<td>40 ± 2</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>−60 ± 2</td>
<td>−57 ± 3</td>
</tr>
<tr>
<td>R_in, MΩ</td>
<td>190 ± 16</td>
<td>166 ± 11</td>
</tr>
<tr>
<td>AP, Hz</td>
<td>97 ± 6</td>
<td>−17 ± 2</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>65 ± 4</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>Half width, ms</td>
<td>0.91 ± 0.2</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
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Values are means ± SE. The numbers of tested neurons are in parentheses. C_m, membrane capacitance; RMP, resting member potential; R_in, input resistance; AP, action potential; FR, firing rate.
Dendritic trees were not altered for either type of interneuron in CD

It is possible that a reduced number of excitatory connections could simply result from damage to the irradiated inter-neurons in CD animals resulting in less extensive dendritic arborization in these cells. To test this hypothesis, we quantified the dendritic trees from camera lucida drawings of biocy-tin-filled cells by counting the intersections of dendritic branches at concentric circles around the soma (Sholl analysis), and the number of primary dendrites and total dendritic branch points and by estimating total length of dendrites. Using Sholl analysis (Fig. 6, A, B, D, and E), we did not observe any significant difference of dendritic arborization between control and CD for either type of interneuron (type I, control: n = 10, CD: n = 16, P = 0.95; type II, control: n = 13, CD: n = 14, P = 0.97). We also found no difference between control and CD interneurons in the number of primary dendritic branches (type I, control: n = 10, CD: n = 16, P = 0.5; type II, control: n = 13, CD: n = 14, P = 0.4) or total number of dendritic branch points (type I, P = 0.7, type II, P = 0.8). Estimated
total dendritic length was not different between control and CD interneurons. For type I interneurons, the average total length of dendrites in control and CD was 644 ± 93 and 696 ± 93 μm, respectively (control: n = 10, CD: n = 16, P = 0.7). For type II cells, it was 630 ± 75 μm in controls and 660 ± 93 μm in CD (control: n = 13, CD: n = 14, P = 0.9). These morphological comparisons indicated that the dendritic trees of both types of interneurons were not significantly altered in CD.

**DISCUSSION**

This study demonstrated a reduction of spontaneous and miniature EPSCs in two types of cortical interneurons in an animal model of CD. First, the frequency of sEPSCs and mEPSCs was reduced, but the amplitude and kinetics of the currents were not altered in CD. This suggests a presynaptic locus for the impaired excitatory activity. Presynaptic alterations of mEPSCs could result from a decreased number of terminals on each interneuron or a reduction in release probability in those terminals. Short-term plasticity has been used as an indirect measure of release probability (Beierlein et al. 2003; Chen and Roper 2003, 2004; Dobrunz and Stevens 1997; Manabe et al. 1993; Trombley and Westbrook 1990) and, in this study, there was no difference in STP of EPSCs in the two types of interneurons. This leaves a reduction in the number of presynaptic terminals and in the density of excitatory synapses as the most likely explanation for our findings. However, because it was not tested directly using cell-attached recordings, an alteration in release probability cannot be fully excluded by this study. Because the extent of dendritic arborization was not altered in CD interneurons, the reduced number of excitatory terminals would not be simply due to a reduced area for potential synapses to form. Rather, it suggests a reduced density of excitatory connections on a morphologically normal dendritic tree in CD interneurons.

Although many advances have been made (Somogyi and Klausberger 2005), the relationship among morphology, immunohistochemical profile, and physiological function in cortical interneurons is still not very clear (Amitai et al. 2002; Cauli et al. 1997; Gibson et al. 1999; Maccaferrri and Lacaille 2003; Markram et al. 2004). As our primary determinant for classification was physiological, we chose to simply describe the two types of interneurons as type I and type II. Type I cells were multipolar cells that showed fast-spiking without adaptation when depolarized and short-term depression. Type I cells correspond to FS interneurons that have been described by numerous other investigators (Amitai et al. 2002; Beierlein et al. 2003; Gonchar and Burkhalter 1997; Kawaguchi and Kubota 1998; Kawaguchi et al. 1987; McCormick et al. 1985; Gibson et al. 1999). Type II cells were bipolar cells that demonstrated a slightly slower initial firing frequency when depolarized (although still much faster than pyramidal cells), frequency adaptation, and short-term facilitation. These cells probably correspond to regular spiking nonpyramidal cells (RSNPs) (Kawaguchi and Kubota 1997) and low-threshold spiking cells (McCormick et al. 1985). Based on morphology and short-term facilitation, this group probably includes the SS-containing (SS) cells described by previous authors (Kawaguchi and Kondo 2002; Reyes et al. 1998). Irrespective of classification, both types of interneuron showed reduced excitatory drive in CD in the current study.

The alteration is specific for interneurons because prior studies have shown unchanged or increased frequency of mEPSCs and sEPSCs in pyramidal neurons in dysplastic cortex and heterotopic gray matter of irradiated rats (Chen and Roper 2003; Zhu and Roper 2000). Because there is no difference in overall neuronal density between control and irradiated animals (Roper et al. 1999), loss of cortical excitatory neurons would not be a good explanation. The source of reduced excitatory synapses is not known from the current data. Potential candidates include nearby cortical pyramidal cells, ascending afferents from the thalamus, and callosal afferents from the contralateral hemisphere. Beierlein et al. (2003) have shown that thalamocortical afferents strongly activate layer IV FS interneurons but not layer IV LTS interneurons, whereas local axon collaterals from layer VI pyramidal cells activate both FS and LTS cells in layer IV. The fact that both type I (corresponding to FS cells) and type II interneurons (including some LTS cells) were affected in the current study would suggest...
that excitatory connections from local pyramidal cells were probably involved but does not tell us whether thalamocortical afferents were involved. Because the corpus callosum is essentially absent in irradiated rats (Roper et al. 1995), this represents another potential explanation for reduced excitatory contacts in dysplastic cortex.

Considering the current results, the in utero irradiation model of CD-associated epilepsy has shown two major alterations in cortical inhibition. The first finding was a major reduction in the number of cortical interneurons (Deukmedjian et al. 2004; Roper et al. 1999) and an associated reduction of mIPSCs in dysplastic pyramidal cells (Chen and Roper 2003; Zhiu and Roper 2000). The current findings show that the surviving interneurons have a reduction in excitatory drive. We are not proposing that reduced excitatory drive is the primary explanation for epilepsy in irradiated rats because they also demonstrate an ∼50% loss of cortical interneurons. But the combined effect of loss of neurons and reduced excitatory drive in the surviving interneurons should produce a major functional impairment of inhibition both at rest and during periods of increased neuronal activity. Recently, Traub and colleagues (2005) have demonstrated the importance of excitatory activation of interneurons in the generation of epileptiform bursts using experimental and modeling data.

Other models of CD have shown different physiological alterations. The perinatal freeze-lesion model produces a focal area of dysplasia (the microgyrus) with hyperexcitability in the adjacent paramicrogyral zone (PMG). There is a reduction in PA-expressing interneurons in the PMG of these animals (Rosen et al. 1998). However, in contrast to irradiated rats, e- and siPSCs are increased in amplitude in pyramidal cells of the PMG (Jacobs and Prince 2005; Jacobs et al. 1999). Additional studies have shown that this is due to an increase in excitatory drive onto interneurons of the PMG that is outweighed by an increase in excitatory drive onto pyramidal cells of the PMG (Jacobs and Prince 2005). The freeze-lesion model differs from in utero irradiation because the insult is delivered later in development, P0 or P1, and it produces a focal malformation. Indeed many of the abnormalities in synaptic organization seen in the PMG of the freeze-lesion model may occur in response to a loss of normal targets for ascending excitatory connections in layer IV of the microgyrus (Jacobs and Prince 2005; Jacobs et al. 1999). Because the in utero irradiation model produces a diffuse malformation, the potential for redirection of afferent connections into nearby, unaffected regions of the cortex does not exist.

The MAM-treated rat is another model of diffuse CD that results from exposure to the DNA alkylating agent, methyla

zoxymethanol, and produces diffuse CD, subcortical heterotopia, and heterotopic neurons in the hippocampus (Baraban and Schwartzkroin 1995; Chevassus-au-Louis et al. 1988; Singh 1977). A number of abnormalities are present in heterotopic hippocampal neurons in area CA1 of MAM-treated rats (Baraban and Schwartzkroin 1995; Castro et al. 2001; Calcagnotto and Baraban 2005; Pentney et al. 2002). Similar to irradiated rats, s- and mIPSCs have a reduced frequency in heterotopic hippocampal neurons in this model (Calcagnotto et al. 2002). But, in contrast to irradiated rats, s- and eIPSCs are prolonged in heterotopic pyramidal hippocampal neurons in this model, apparently due to alterations in GABA transporters (Calcagnotto et al. 2002). Yet another model of diffuse CD, in utero exposure to BCNU, produces cortical pyramidal cells that show a decreased sensitivity to exogenously applied GABA (Benardete and Kriegstein 2002). Recent studies in tish rat, a spontaneous mutant that has seizures and a diffuse double cortex malformation (Lee et al. 1997), have shown alterations in GABA_A subunit composition in the affected cortex (Trotter et al. 2005). These disparate results suggest that early insults may produce a variety of abnormalities dependent on timing and type of injury and that epilepsy may represent a common endpoint for several different pathological alterations.

The relationship between inhibition and seizure activity is still poorly understood. In fact, GABAergic activity is clearly necessary for some forms of normal synchronous, oscillatory activity (Szabadi et al. 2001; Tamas et al. 2000; Traub et al. 2001) and some types of epileptiform activity (D’Antuano et al. 2004; Khalilov et al. 2003; Lopantsiev and Avoli 1998). Conversely, a number of epilepsy models and syndromes have been associated with various types of impaired inhibition. These include loss of cortical interneurons in human and experimental CD (Ferrer et al. 1994; Powell et al. 2003; Roper et al. 1999; Sarkisian et al. 2001; Spreatfico et al. 1998, 2000) and reduction of s- and mIPSCs in pyramidal cells from human CD (Calcagnotto et al. 2005). Loss of certain types of hippocampal interneurons also occurs in human and experimental limbic epilepsy (Cossart et al. 2001; de Lanerolle et al. 1989; Kobayashi and Buckmaster 2003; Obenaus et al. 1993; Slvotier 1989). Alterations in GABA-receptor subunit composition and pharmacology have been demonstrated in human and experimental CD (Crino et al. 2001; DeFazio and Hablitz 1999; Redecker et al. 2000) and in the dentate gyrus of pilocarpine-treated rats (Cohen et al. 2003). Hirsch et al. (1999) showed, in kainate- and pilocarpine-treated rats, that mIPSC frequency was reduced in CA1 pyramidal cells due to a reduction in presynaptic release probability. Using the pilocarpine model, Doherty and Dingeldine (2001) demonstrated enhanced short-term depression of evoked EPSCs in hilar border interneurons, suggesting an activity-dependent impairment of excitatory drive. It is clear that describing any of the epilepsies simply as a “lack of inhibition” is overly simplistic (Cossart et al. 2005). Therefore the true significance of the alterations in inhibition that have been demonstrated in irradiated rats will ultimately require a better understanding of the role of cortical interneurons in normal function as well as epileptic states.

As mentioned in the preceding text, various epilepsy models have demonstrated different types of impairment of inhibition in the hippocampus and neocortex. But the combined findings of loss of cortical interneurons with reduction in excitatory drive in the surviving interneurons have been reported only in the irradiated rat. This underscores the unique qualities of epilepsy associated with CD and in utero injury. As acquired CD has been documented in children that have survived perinatal cerebral insults (Marin-Padilla 1999), it will be important to see if similar changes are found in those types of human epilepsy. If so, these findings may ultimately provide novel avenues of therapy for epilepsy associated with CD.

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Reduced Excitation in Dysplastic Interneurons

GRANTS

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REFERENCES


