GABA_A receptor-dependent synchronization leads to ictogenesis in the human dysplastic cortex

M. D’Antuono,1,2 J. Louvel,4 R. Köhling,5 D. Mattia,3 A. Bernasconi,6 A. Olivier,6 B. Turak,4 A. Devaux,4 R. Pumain4 and M. Avoli 1,2,6

1 Dipartimento di Fisiologia Umana e Farmacologia ‘V. Erspamer’, Università degli Studi di Roma ‘La Sapienza’, Roma, Italy, 2 IRCCS Fondazione Santa Lucia, Roma, Italy, 3 IRCCS Neuromed, Pozzilli (Is) and 4 INSERM U 109, Centre de Recherche P. Broca and Hôpital Sainte-Anne, Paris, France, 5 Department of Epileptology, University of Bonn, Bonn, Germany and 6 Montreal Neurological Institute and Department of Neurology and Neurosurgery, McGill University, Montréal, Québec, Canada

Correspondence to: M. Avoli, MD, PhD, 3801 University Street, Montreal, QC, H3A 2B4 Canada
E-mail: massimo.avoli@mcgill.ca

Summary

Patients with Taylor’s type focal cortical dysplasia (FCD) present with seizures that are often medically intractable. Here, we attempted to identify the cellular and pharmacological mechanisms responsible for this epileptogenic state by using field potential and K+-selective recordings in neocortical slices obtained from epileptic patients with FCD and, for purposes of comparison, with mesial temporal lobe epilepsy (MTLE), an epileptic disorder that, at least in the neocortex, is not characterized by any obvious structural aberration of neuronal networks. Spontaneous epileptiform activity was induced in vitro by applying 4-aminopyridine (4AP)-containing medium. Under these conditions, we could identify in FCD slices a close temporal relationship between ictal activity onset and the occurrence of slow interictal-like events that were mainly contributed by GABA_A receptor activation. We also found that in FCD slices, pharmacological procedures capable of decreasing or increasing GABA_A receptor function abolished or potentiated ictal discharges, respectively. In addition, the initiation of ictal events in FCD tissue coincided with the occurrence of GABA_A receptor-dependent interictal events leading to [K+]o elevations that were larger than those seen during the interictal period. Finally, by testing the effects induced by baclofen on epileptiform events generated by FCD and MTLE slices, we discovered that the function of GABA_B receptors (presumably located at presynaptic inhibitory terminals) was markedly decreased in FCD tissue. Thus, epileptiform synchronization leading to in vitro ictal activity in the human FCD tissue is initiated by a synchronizing mechanism that paradoxically relies on GABA_A receptor activation causing sizeable increases in [K+]o. This mechanism may be facilitated by the decreased ability of GABA_B receptors to control GABA release from interneuron terminals.

Keywords: baclofen; epileptiform synchronization; focal cortical dysplasia; GABA receptors; [K+]o homeostasis

Abbreviations: ACSF = artificial cerebrospinal fluid; 4AP = 4-aminopyridine; BMI = bicuculline methiodide; CGP 35348 = P3-amino-propyl-P-diethoxymethyl-phosphonic acid; CNQX = 6-cyano-7-nitro-quinoxaline-2,3-dione; CPP = 3-(2-carboxy-piperazine-4-yl)-propyl-l-phosphonate; DAGO = (d-Ala2-N-Me-Phe,Gly-ol)enkephalin; FCD = focal cortical dysplasia; MTLE = mesial temporal lobe epilepsy; NMDA = N-methyl-d-aspartate


Introduction

Taylor’s type focal cortical dysplasia (FCD) corresponds to a localized disruption of the normal cortical lamination with an excess of large, dysmorphic neurons (Taylor et al., 1971). Thanks to the use of MRI investigations, FCD has been identified more and more frequently in patients presenting with epileptic disorders that previously were classified as
cryptogenic (Bronen et al., 1997; Guerrini et al., 1999; Bernasconi et al., 2001; Tassi et al., 2001, 2002). Seizures in FCD patients are often medically intractable, which makes them candidates for neurological interventions aimed at resecting the epileptogenic area that in most cases overlaps with the FCD tissue.

There exist several animal models of cortical dysgenesis (Baraban and Schwartzkroin, 1996; Jacobs et al., 1996; Luhmann and Raabe, 1996; Castro et al., 2001; Roper et al., 1997; Luhmann et al., 1998; DeFazio and Habilitz, 2000; Benardete and Kriegstein, 2002; Gabel and Lo Turco, 2002; Aronica and Gorler, 2003; Hagemann et al., 2003). However, none of them successfully replicate Taylor’s type FCD. To circumvent this problem, we have used in the past few years surgically resected human FCD tissue to identify the histochemical and functional characteristics of FCD neuronal networks. These studies have revealed that human FCD tissue has an abnormal distribution of N-methyl-D-aspartate (NMDA) receptors and a decreased number of presumptive interneurons that, however, provide increased GABAergic innervation to principal cells (Spreaco et al., 1998). Later, these findings were confirmed in other studies (Garbelli et al., 1999; Tassi et al., 2001, 2002). In addition, by employing electrophysiological recordings in an in vitro slice preparation, we have found that FCD tissue, when treated with 4-aminopyridine (4AP), generates NMDA receptor-mediated ictal discharges along with isolated, interictal-like synchronous potentials that are contributed by GABA receptor-mediated discharges along with glutamatergic mechanisms (Mattia et al., 1995; Avoli et al., 1999). Comparative studies performed with a similar 4AP treatment in human neocortical tissue presenting with no obvious structural abnormality have revealed that these slices can only generate periodic, synchronous, interictal-like events that are also due, to a large extent, to GABA receptor-mediated conductances (Avoli et al., 1994).

During 4AP treatment, the onset of ictal discharges in rodent entorhinal cortex (Avoli et al., 1996a; Lopantsev and Avoli, 1998; Barbarosie et al., 2002) or in immature rat hippocampal slices (Avoli et al., 1993, 1996b) is often associated with GABA receptor-mediated potentials resembling those seen in human FCD slices. Moreover, this mechanism probably relies on transient increases in [K+]o, that accompany the GABA receptor-mediated events. Here, we attempted to identify in human FCD tissue a similar causative relationship between GABA receptor-mediated synchronization and ictogenesis. In particular, we used field potential and K+-selective recordings in neocortical slices obtained during surgery for medically intractable seizures associated with FCD and, for purposes of comparison, from patients with mesial temporal lobe epilepsy (MTLE); this type of neocortex does not show any obvious structural aberration, including abnormal laminacion (Avoli et al., 1991, 1994, 1995; Spreaco et al., 1998; Louvel et al., 2001). Slices were superfused with 4AP-containing medium to increase neuronal excitability, and thus to elicit spontaneous epileptiform activity in vitro.

Specifically, we tested four hypotheses. First, we postulated that ictal discharge onset in FCD slices should be associated with field potential activity sharing electrographic and pharmacological properties that are typical of 4AP-induced GABA receptor-mediated synchronization. Secondly, we anticipated that if GABA receptor-mediated synchronization is necessary for ictogenesis, then ictal discharges should disappear or increase during pharmacological procedures that antagonize or enhance GABA receptor-mediated mechanisms, respectively. Thirdly, we presumed that GABA receptor-mediated events associated with ictal activity onset should be accompanied by [K+]o elevations that are larger than those seen during the interictal period. Finally, we expected that during application of 4AP and glutamatergic receptor antagonists (a procedure that abolishes epileptiform activity, but only reduces the duration of the GABA receptor-mediated potentials), GABA receptor-dependent [K+]o increases should be larger in FCD than in MTLE cortical slices.

**Methods**

Neocortical samples were obtained during neurosurgery for medically intractable seizures associated with MTLE (n = 7 patients aged 26–40 years) and with FCD (n = 11 patients aged 9–38 years). The tissue samples used for the in vitro electrophysiological investigations were always part of a block of brain tissue removed for strict therapeutic reasons. Informed consent was obtained according to the guidelines of the ethics committees of our institutions which approved the study. All patients have been maintained on a variety of antiepileptic drugs that were discontinued or reduced during the weeks preceding surgery. Surgery was carried out under general anaesthesia with halogenated compounds. During the resecting procedure, care was taken by the neurosurgeon to provide cortical samples that contained the FCD tissue. Neuropathological analysis of the removed brain tissue revealed in MTLE patients a mild to moderate degree of gliosis, while cortical lamination was always preserved. In contrast, structural disruption along with the presence of large, aberrant neurons was seen in the tissue obtained from FCD patients (see Spreaco et al., 1998). Table 1 summarizes the MRI and neurophysiological findings obtained in FCD patients.

**Preparation and maintenance of the slices**

Details on the techniques used to prepare and to maintain the slices in vitro have been described in previous studies from our laboratories (Avoli et al., 1994, 1999; Louvel et al., 2001). In brief, neocortical slices (500 μm thick) were cut with a McIlwain tissue chopper and were transferred to a recording chamber where they were maintained at 33.0 ± 1.0°C at an interface between humidified gas (95% O2–5% CO2) and oxygenated artificial cerebrospinal fluid (ACSF; pH = 7.4). Efforts were made to
The ability of 4AP treatment to disclose patterns of synchronous activity that differ in human cortical tissue obtained from MTLE or FCD patients has already been reported by us (Avoli et al., 1994, 1999; Mattia et al., 1995; Louvel et al., 2001). Here, we confirmed that bath application of 4AP to MTLE slices (n = 21) induced spontaneous, monophasic negative or biphasic negative–positive field potentials (amplitude = 0.3–4 mV; intervals of occurrence = 4–19 s) that were associated with transient elevations in [K+]o with peak values up to 4.3 mM (3.8 ± 0.3 mM, n = 46 events) from a baseline of 3.2 mM (Fig. 1A). When this 4AP-induced activity was recorded at different depths along an axis normal to the pial surface, we found that the synchronous field potentials had maximal negative amplitudes at depths of 1100–1400 μm (not shown; but see Avoli et al., 1994). Similar field potential events and [K+]o elevations were induced by extracellular focal electrical stimuli (Fig. 1A, filled triangle). Ictal discharges (defined as synchronous field potential activity organized in a continuous pattern with duration >4 s) were never seen in MTLE slices treated with 4AP.

In contrast, bathing application of 4AP to FCD slices (n = 22 out of 39 from 11 patients) caused the appearance of (i) ictal discharges that lasted up to 130 s and occurred at intervals of 1–8 min; and (ii) isolated field potentials that were similar to those recorded in MTLE slices (lines and asterisks in Fig. 1B, a and c, respectively). Only slow interictal activity could be recorded in 17 FCD slices. As reported in previous studies (Mattia et al., 1995; Avoli et al., 1999), ictal discharges were characterized by prolonged negative shifts with superimposed oscillations with frequencies up to 25 Hz (presumably representing synchronous action potential firing). As shown in the two experiments of Fig. 1B, ictal discharge onset was associated with the occurrence of a negative field potential that resembled those recorded in isolation during the interictal period, but was of larger amplitude (Fig. 1B, a and c, arrow) and often followed by a slow (duration up to 2.5 s; arrowhead in Fig. 1B, a and c) negative event from which the ictal discharge initiated. The different amplitude and shape characteristic of the negative field events occurring in isolation and at ictal discharge onset are illustrated in detail in Fig. 1B, b (panels 1 and 2, respectively). The temporal

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**Table 1** Demographic, radiological and electrophysiological data of patients with FCD

<table>
<thead>
<tr>
<th>Patient sex/age (years)</th>
<th>MRI</th>
<th>EEG interictal epileptic</th>
<th>Ictal</th>
<th>ECoG</th>
</tr>
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<tbody>
<tr>
<td>F/14</td>
<td>FCD, frontal, R</td>
<td>Fronto-central, R</td>
<td>Bilateral fronto-central, R&gt;&gt;L</td>
<td>CRSs</td>
</tr>
<tr>
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<td>FCD, frontal, R</td>
<td>Frontal, R</td>
<td>None</td>
<td>CRSs</td>
</tr>
<tr>
<td>F/38</td>
<td>FCD, frontal, R</td>
<td>Fronto-central, R</td>
<td>Bilateral fronto-central</td>
<td>CRSs</td>
</tr>
<tr>
<td>M/16</td>
<td>FCD, frontal, L</td>
<td>Bi-frontal, L&gt;R</td>
<td>None</td>
<td>RESs</td>
</tr>
<tr>
<td>F/18</td>
<td>FCD, frontal, R</td>
<td>Fronto-temporal, L</td>
<td>Bilateral fronto-central</td>
<td>CRSs</td>
</tr>
<tr>
<td>F/9</td>
<td>FCD, frontal, L</td>
<td>Frontal, R</td>
<td>Frontal, R</td>
<td>RESs</td>
</tr>
<tr>
<td>M/15</td>
<td>FCD, fronto-centro-parietal, R</td>
<td>Frontal, R</td>
<td>Frontal, R</td>
<td>RESs</td>
</tr>
<tr>
<td>M/30</td>
<td>FCD, temporal, L</td>
<td>Temporal, L</td>
<td>Temporal, L</td>
<td>CRSs</td>
</tr>
<tr>
<td>F/21</td>
<td>FCD, fronto-central, R</td>
<td>Fronto-temporal, R&gt;&gt;L</td>
<td>Fronto-temporal, R</td>
<td>RESs</td>
</tr>
<tr>
<td>M/11</td>
<td>FCD, occipital, R</td>
<td>Parieto-occipital, L&gt;R</td>
<td>Occipital, L</td>
<td>CRSs</td>
</tr>
<tr>
<td>F/13</td>
<td>FCD, occipital, L</td>
<td>Parieto-occipital, L</td>
<td>Occipital, L</td>
<td>CRSs</td>
</tr>
</tbody>
</table>

**ECoG** = intraoperative electrocorticography; **R/L** = right/left hemisphere; **CRSs** = continuous or quasi-continuous rhythmic spiking; **RESs** = repetitive electrographic seizures.

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**Electrophysiological recordings**

Field potential recordings were obtained with pipettes filled with ACSF. Field potentials were also recorded through the reference channel of the ion-selective electrodes. Signals were fed to DC high-impedance amplifiers with filters that were usually set to record between DC and 1 kHz. Measurements of [K+]o were obtained by using double-barrelled ion-selective electrodes (tip diameters = 2–4 μm) based on the valinomycin ion exchanger Fluka 60398. The reference channel was backfilled with 150 mM NaCl and the ion-selective channel with 100 mM KCl. Details concerning the [K+]o measurements can be found in our previous publications (Avoli et al., 1995; Louvel et al., 2001). Signals were displayed on an oscilloscope and on a Gould pen recorder. In some experiments, field potential and [K+]o recordings were also stored on a personal computer through a 1401 interface (C.E.D., Cambridge, UK).

**Statistical analysis**

Measurements were made and averaged over periods longer than 10 consecutive minutes and are expressed as mean ± SEM, while n indicates the number of slices analysed. Statistical comparisons were made with one-way analysis of variance (ANOVA), and differences were considered significant if P < 0.05.

**Results**

**Field potential and [K+]o recordings during 4-aminopyridine application**

The ability of 4AP treatment to disclose patterns of synchronous activity that differ in human cortical tissue

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minimize the time elapsed between surgical resection and slicing (usually <5 min). ACSF composition was (in mM): 124 NaCl, 2 KCl, 2 MgSO4, 2 CaCl2, 1.25 KH2PO4, 26 NaHCO3 and 10 glucose. The following drugs were bath applied: 4AP (50 μM, Sigma), 4-amino-3-[4-chloro-phenyl]-butanonic acid (baclofen; 2–50 μM, Sigma), 3-[2-carboxy-piperazine-4-yl]-propyl-1-phosphonate (CPP; 20 μM, Tocris Cookson), 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; 10–20 μM, Tocris Cookson), bicuculline methiodide (BMI; 20 μM, Sigma), picrotoxin (50 μM, Sigma), (D-Ala2-N-Me-Phe,Gly-ol)enkephalin (DAGO; 10 μM, Sigma), naloxone (5 μM, Sigma), phenobarbital (20–100 μM, Sigma) and P3-aminopropyl-P-diethoxymethyl-phosphonic acid (CGP 35348; 1 mM, kindly donated by Novartis, Basel, Switzerland).
Fig. 1 Spontaneous synchronous activity induced by 4AP in slices obtained from patients with MTLE (i.e. presenting with no architectural anomaly) and FCD. (A) Synchronous, isolated field potentials occur spontaneously in an MTLE cortical slice analysed with field potential and [K+]o, recordings at 1000 µm from the pia. Note that each field potential event is associated with a transient increase in [K+]o. (B) Spontaneous field potential discharges recorded in two slices (a and c) that were obtained from two FCD patients. Note that in both cases, the activity is characterized by isolated interictal field potentials (asterisks) and sustained epileptiform events resembling ictal discharges (continuous lines). Note also that the onset of the ictal event is associated with the occurrence of a negative field potential (arrow) followed by a slow negative event (arrowhead) leading to ictal discharge oscillations. The different characteristics of the isolated negative field events (1) and of the ictal discharge onset (2) are shown in b for 3–4 graphically superimposed samples obtained from the experiment in a; note that the isolated interictal events are of lower amplitude compared with those leading to ictogenesis. (C) Temporal relationship between the occurrence of slow interictal events and ictal discharge onset during 4AP application. In a is shown a histogram of the probability of occurrence of the interictal activity over a period of 50 s before the ictal onset normalized to an epoch 5 s prior to the ictal event; data were obtained from 64 epochs that were recorded in 11 FCD slices. One of these epochs (recorded with a high-pass filtered set at 0.1 Hz) is shown in b. The arrow highlights the time 0, while the asterisks point at the slow interictal events.
relationship between slow interictal events and ictal discharges is shown further in the histogram of Fig. 1C, where the probability of occurrence of the interictal activity over a 50 s period before ictal onset was obtained from 64 epochs recorded in 11 FCD slices.

Simultaneous recordings performed with field potential and K+-selective electrodes in 11 FCD slices obtained from three patients demonstrated that the isolated negative-going events were associated with transient elevations in [K+]o that reached peak values ~5.0 mM from a baseline of ~3.2 mM (Fig. 2A, asterisk). In contrast, the negative-going events occurring at the onset of the ictal discharge were accompanied by [K+]o increases up to 8 mM (Fig. 2A, small arrow). The histogram of Fig. 2B summarizes the peak values of the [K+]o elevations associated with the slow negative field potentials occurring in isolation between ictal discharges and with the negative event occurring shortly before ictal discharge onset.

\[ [K^+]_o \] recorded during the ictal event reached values in excess of 12 mM; these elevations in [K+]o were maximal throughout the initial ‘tonic’ phase of the discharge, and slowly recovered to baseline levels during the ‘clonic’ phase. Small (<500 \( \mu \)m) displacements of the recording electrodes along either the vertical or horizontal axis of the FCD slice could cause conspicuous changes in the characteristics of the field potentials and of the [K+]o elevations induced by 4AP application (control panels in Fig. 7A).

**GABA_A receptor function modulates 4-aminopyridine-induced ictal discharges in FCD slices**

Application of the GABA_A receptor antagonist BMI (\( n = 6 \)) abolished the slow negative field events along
with ictal discharges induced by 4AP, and disclosed a pattern of short-lasting (<1 s) recurrent epileptiform bursting (Fig. 3A). The activity recorded during application of 4AP + BMI was reversibly reduced in duration by the NMDA receptor antagonist CPP (10 μM) reduces the duration of most of the interictal discharges. Note in the expanded traces the occurrence of field oscillations at ~20 Hz during the afterdischarge that accompanies some of the interictal events recorded during concomitant application of 4AP + BMI + CPP. (B) Effects similar to those induced by BMI are obtained with the µ-opioid receptor agonist DAGO (10 μM). Note that DAGO reduces the amplitude of the isolated negative field events (arrows in sample a). Traces identified as a and b represent a continuous recording that was started 2 min after the onset of DAGO application.

Fig. 3 The occurrence of ictal discharges induced by 4AP in FCD slices depends on the function of GABA_A receptors. (A) Bath application of the GABA_A receptor antagonist BMI (10 μM) transforms ictal activity into regular, interictal discharges and abolishes the isolated negative field potentials. Further addition of the NMDA receptor antagonist CPP (10 μM) reduces the duration of most of the interictal discharges. Note in the expanded traces the occurrence of field oscillations at ~20 Hz during the afterdischarge that accompanies some of the interictal events recorded during concomitant application of 4AP + BMI + CPP. (B) Effects similar to those induced by BMI are obtained with the µ-opioid receptor agonist DAGO (10 μM). Note that DAGO reduces the amplitude of the isolated negative field events (arrows in sample a). Traces identified as a and b represent a continuous recording that was started 2 min after the onset of DAGO application.

with ictal discharges induced by 4AP, and disclosed a pattern of short-lasting (<1 s) recurrent epileptiform bursting (Fig. 3A). The activity recorded during application of 4AP + BMI was reversibly reduced in duration by the NMDA receptor antagonist CPP (n = 3) (Fig. 3A). Such a procedure abolished ictal synchronization in the presence of 4AP only (not shown; but see Avoli et al., 1999). Ictal discharges were also blocked by applying the µ-opioid receptor agonist DAGO (n = 3; Fig. 3B). This pharmacological treatment is known to hyperpolarize interneurons and thus decreases GABA release from interneurons (Madison and Nicoll, 1988; Capogna et al., 1993). DAGO-induced effects were characterized by a progressive reduction in both rate of occurrence and amplitude of the negative field events (Fig. 3B, a, arrows), and by the appearance of recurrent interictal bursting similar to that seen during BMI treatment. Thus, these experiments indicate that in FCD tissue, the generation of prolonged epileptiform events, resembling ictal discharges, depends on GABA_A receptor function.

Next, we analysed the effects induced by enhancing GABA_A receptor-mediated inhibition by applying phenobarbital. In FCD slices treated with 4AP-containing medium, low concentrations of phenobarbital (20 μM, n = 3/4) increased the duration and amplitude of both interictal and ictal discharges, while prolonging their interval of occurrence (Fig. 4A–C). The effects of phenobarbital on the ictal event were often accompanied by an increase in the amplitude of the field oscillations at ~20 Hz that were seen at discharge onset (inserts in Fig. 4A). Further increasing the phenobarbital dose to 40 μM abolished ictal activity in all experiments and reduced the duration and the rate of occurrence of the
interictal events (Fig. 4A). Finally, all activities disappeared with phenobarbital concentrations of 80 μM (not illustrated; but see plots in Fig. 4B and C). Moreover, in two FCD slices that generated only isolated interictal activity in the presence of 4AP, 20 μM phenobarbital made ictal events appear (Fig. 4D); epileptiform activities in these experiments were also abolished by increasing the phenobarbital doses to 60 μM (not illustrated).

**GABA<sub>B</sub> receptor activation abolishes 4-aminopyridine-induced epileptiform activity in FCD slices**

Next, we tested the effects induced by the GABA<sub>B</sub> receptor agonist baclofen on the 4AP-induced ictal discharges generated by slices obtained from FCD tissue samples (n = 4). As illustrated in Fig. 5A, baclofen abolished the ictal events recorded in these slices at concentrations as low as 2 μM. However, isolated interictal events continued to occur under these experimental conditions (asterisks in Fig. 5A). This type of activity disappeared during application of baclofen doses >40 μM (see below). The effects induced by baclofen on ictal discharges were fully reversed by additional application of the GABA<sub>B</sub> receptor antagonist CGP 35348 (1 mM) (Fig. 5A).

We also analysed the effects induced by blocking (CGP 35348, 1 mM) or activating (baclofen, 10–50 μM) GABA<sub>B</sub> receptor function on the isolated interictal discharges that represented the only epileptiform activity generated by five FCD slices during 4AP application. CGP 35348 reversibly increased interictal discharge duration by 30–65% (n = 3; Fig. 5B), while baclofen depressed them in a dose-dependent manner (Fig. 5C–F).
GABAB receptor activation and pharmacologically isolated GABA receptor events in MTLE and FCD slices

We have reported that GABA-B receptor activation reduces and eventually abolishes spontaneous GABA receptor-mediated field potentials recorded in the presence of 4AP and excitatory amino acid receptor antagonists in human neocortical tissue with no structural abnormality (Louvel et al., 2001). Here, we further compared the effects induced by baclofen on these events in MTLE and FCD slices ($n=4$ and 6, respectively). As illustrated in Fig. 6A and B, increasing concentrations of baclofen reduced the amplitude and the rate of occurrence of the epileptiform activity induced by 4AP. Note that the different vertical calibration in the 10 and 40 μM samples. (D–F) Dose–response curves of the effects induced by baclofen on the duration, rate of occurrence and amplitude of the interictal events generated by FCD slices during bath application of 4AP. Data normalized to control values obtained over a period of 10 min immediately prior to drug application.

**GABA$_B$ receptor activation and pharmacologically isolated GABA receptor events in MTLE and FCD slices**

We have reported that GABA$_B$ receptor activation reduces and eventually abolishes spontaneous GABA receptor-mediated field potentials recorded in the presence of 4AP and excitatory amino acid receptor antagonists in human neocortical tissue with no structural abnormality (Louvel et al., 2001). Here, we further compared the effects induced by baclofen on these events in MTLE and FCD slices ($n=4$ and 6, respectively). As illustrated in Fig. 6A and B, increasing concentrations of baclofen reduced the amplitude and the rate of occurrence of these presumptive GABA receptor-mediated events in both MTLE and FCD slices. However, the doses of baclofen required to exert similar depressant effects were consistently larger in FCD slices ($P<0.05$ for the data obtained with baclofen doses higher than 10 μM). Accordingly, the normalized histograms of the baclofen effects suggested an extrapolated IC$_{50}$ of ~20 and 40 μM in MTLE and FCD slices, respectively (Fig. 6C and D).
[K+]o recordings during application of 4-aminopyridine and glutamatergic receptor antagonists

In keeping with early findings obtained from human cortical slices with no structural abnormality (Louvel et al., 2001), the synchronous isolated events recorded during application of 4AP + glutamatergic receptor antagonists (i.e. CNQX + CPP, 10 μM each) from MTLE slices (n = 21) were accompanied by small [K+]o elevations (up to 4.0 mM from a background level of 3.2 mM) (Fig. 7A and B). We also confirmed (see Louvel et al., 2001) that the [K+]o increases recorded at different depths along an axis normal to the pial surface displayed a consistent pattern of distribution characterized by [K+]o increases that were largest and fastest at depths of ~1000 μm below the pial surface (Fig. 7B and C). This location should correspond to the middle layers, a site that includes the apical dendritic arborizations originating from pyramidal cells with somas localized in the deep layers. Moreover, this was the depth at which the negative field potentials were of maximal amplitude.

Next, we analysed the increases in [K+]o associated with the glutamatergic-independent synchronous events generated by FCD slices (n = 5). As shown in Fig. 7D, application of CNQX + CPP to slices pre-treated with 4AP abolished the ictal discharges and the concomitant [K+]o elevations, while the isolated field events continued to occur. It is also possible to appreciate in this experiment that under control conditions, both field potentials and [K+]o elevations associated with the ictal activity were markedly reduced by changing the position of the recording electrode along the horizontal axis of the FCD slice (cf. traces obtained from sites 2 and 6 in Fig. 7D, control). Interestingly, the sites in the slices from which ictal discharges had larger amplitude under control conditions were also those displaying the largest field potentials and [K+]o elevations during the presumptive GABA receptor-mediated event recorded during blockade of glutamatergic transmission (Fig. 7D, + CNQX + CPP). Therefore, in contrast to what was observed in MTLE slices, the distribution of the [K+]o increases (and of the field potential amplitude) in FCD slices superfused with 4AP + CNQX + CPP...
was not restricted to any particular depth from the pia. For instance, as shown in the experiment of Fig. 7E, the largest 
\([K^+],[n]) increases were found at 1200 and 2400 m (corresponding to positions 2 and 8, respectively). This is summarized for the four experiments performed under these conditions in the normalized histogram of Fig. 7E.

**Discussion**

The present findings indicate that epileptiform synchronization leading to in vitro ictal activity in the human FCD tissue is initiated (and presumably maintained) by a synchronizing mechanism that paradoxically relies on the activation of GABA_A receptors. This conclusion rests on: (i) the temporal relationship seen between the slow interictal-like events (which are mainly contributed by GABA_A receptor activation) and ictal activity onset; and (ii) the evidence obtained by modifying GABA_A receptor function through the use of pharmacological agents. We have also provided evidence that supports the potential role of GABA_A receptor-dependent increases in 
\([K^+],[n]) in exerting such a pro-epileptogenic action in FCD tissue. Finally, we have found that this new mechanism may rely on a decreased function of GABA_B receptors presumably located at the presynaptic inhibitory terminals.
Temporal relationship between presumptive GABA receptor-mediated events and ictal discharge onset in FCD tissue

We have confirmed here that 4AP application to neocortical slices obtained from FCD patients makes prolonged (so-called ictal) and short-lasting (termed interictal) epileptiform discharges appear (Avoli et al., 1999). In contrast, only the latter type of activity was seen under similar experimental conditions in MTLE slices (which presumably did not present with any obvious structural abnormality) (Avoli et al., 1994; Louvel et al., 2001). It is unlikely that this difference was due to the fact that our control tissue originated from the temporal cortex. In fact, no ictal discharges could be observed during 4AP application in non-FCD slices of frontal and occipital cortices (Avoli et al., 1994). We have demonstrated previously that the ictal activity in FCD slices is abolished by NMDA receptor antagonists (Avoli et al., 1999), while the interictal events recorded in both types of tissue are mostly contributed by GABA receptor-mediated conductances (Avoli et al., 1994, 1999). Therefore, the present findings support the view that epileptogenicity is a functional feature of FCD tissue; such a conclusion is in line with data obtained from animal models of cortical maldevelopment, even though associated with histopathological patterns that are dissimilar to Taylor's type FCD (Baraban and Schwartzkroin, 1996; Jacobs et al., 1996; Luhmann and Raabe, 1996; Castro et al., 2001; Roper et al., 1997; Luhmann et al., 1998; DeFazio and Hablitz, 2000; Benardete and Kriegstein, 2002; Gabel and Lo Turco, 2002; Aronica and Gorter, 2003; Hagemann et al., 2003). It should be emphasized that the ictal activity induced by 4AP in FCD slices resembled the electrographic activity seen during pre-convulsion ECoG in these patients (Palmini et al., 1991, 1995; Gambardella et al., 1996), a pattern that is believed to be a specific and sensitive indicator of FCD lesions.

We have also identified a close temporal relationship between ictal discharge onset and the occurrence of GABA receptor-mediated interictal events in FCD slices. Accordingly, interictal discharges recorded in the presence of 4AP were preceded shortly by negative-going events similar to those seen in isolation during the interictal period. However, the field potentials leading to ictal discharge onset were always of larger amplitude and were often followed by a secondary, slow negative field event from which ictal oscillations emerged. Studies performed in rodent brain slices have shown that 4AP application induces patterns of epileptiform activity that depend upon the type of cortical network. For instance, adult isolated hippocampal slices can only generate interictal-like discharges that include short-lasting, CA3-driven events and slow GABA receptor-mediated potentials (Perreault and Avoli, 1992), while ictal discharges occur in adult entorhinal networks (Avoli et al., 1996a; Buchheim et al., 2000). Moreover, the occurrence of ictal synchronization in isolated hippocampal slices treated with 4AP depends on the maturity of the tissue (Avoli et al., 1993, 1996b), a characteristic that is mirrored by the higher propensity of young animals to generate seizures in vivo (Purpura, 1969; Cavalheiro et al., 1987; Moshe 1993). Hence, one may speculate that the dysplastic cortex reverts to, or rather retains ontogenetically immature properties and is therefore susceptible to seizure generation in a way similar to the young rodent hippocampus. It should be emphasized that in these animal studies as well, ictal activity onset is characterized by the occurrence of GABA receptor-mediated events similar to those recorded in FCD tissue (Avoli et al., 1993, 1996a, b). Hence, the ability of human FCD slices to generate ictal discharges, as compared with cortical MTLE tissue, may reflect the abnormal neuronal connectivity seen in this type of tissue (Spreafico et al., 1998; Köhling et al., 1999).

Pharmacological manipulations of the GABA_A receptor modulate ictal discharges in FCD slices

The involvement of GABA receptor-mediated synchronization in initiating ictal activity in FCD tissue is supported by pharmacological manipulations that were aimed at decreasing or enhancing the function of GABA (mainly type A) receptors. Accordingly, we have found that activation of μ-opioid receptors (a pharmacological procedure that blocks the release of GABA from interneuron terminals) (Capogna et al., 1993; Madison and Nicoll, 1988) or blockade of GABA_A receptors abolished the ictal discharges induced by 4AP along with the GABA receptor-mediated interictal events; under both conditions, FCD slices only generated recurrent epileptiform activity at ~0.3 Hz. A similar pattern of robust interictal discharge has been recorded in rodent brain slices during application of 4AP and GABA_A receptor antagonists (Avoli et al., 1996a, b; Lopantsev and Avoli, 1998; Bruckner et al., 1999; Barbarosie et al., 2002). It has been proposed that interictal bursts may have anti-seizure properties (Barbarosie and Avoli, 1997; Barbarosie et al., 2002). Hence, we cannot exclude that the loss of 4AP-induced ictal activity seen in FCD slices during application of either BMI or DAGO was also contributed by such a mechanism.

Conversely, potentiating GABA_A receptor function with minimal concentrations of phenobarbital (Nicoll et al., 1975; Barker and McBurney, 1979) caused ictal discharge prolongation along with potentiation of slow interictal events. Although both types of epileptiform activity were abolished by further increasing the concentration of this barbiturate, the facilitating action identified with low doses supports the view that a GABA_A receptor-mediated mechanism can lead to ictogenesis in FCD neuronal networks. A recent comparative study performed in isolated hippocampal slices obtained from rats treated with methyloxyazoxymethanol in utero (which represents an experimental model of cortical dysplasia) and from control animals has shown that phenobarbital does not
influence 4AP-induced interictal activity in dysplastic tissue (Smyth et al., 2002).

The paradoxical role played by GABA receptor-mediated mechanisms in initiating ictal activity was originally reported in the CA3 subfield of young (<30-day-old) rat slices treated with 4AP (Avoli et al., 1993, 1996b) and later confirmed in adult rodent entorhinal cortex (Avoli et al., 1996a; Lopantsev and Avoli, 1998; Barbarosie et al., 2002). It is worth emphasizing that intracellular recordings obtained from entorhinal cortex neurons during application of 4AP indicate that GABA_A receptor-mediated conductances may also play a role in the maintenance of ictal synchronization (Lopantsev and Avoli, 1998). GABA_A receptor-mediated mechanisms also contribute to epileptiform afterdischarges induced in the hippocampus by tetanic stimulation (Higashima et al., 1996; Velazquez and Carlen, 1999). Similar conclusions have been drawn by Köhling et al. (2000) while analyzing the mechanisms responsible for ictogenesis in hippocampal slices superfused with Mg^{2+}-free medium.

The pro-convulsant effects exerted by GABA receptor-mediated mechanisms may rest on the entrainment of interneuronal network via GABAergic interactions (presumably, GABA_A receptor-mediated depolarizing events) (Köhling et al., 2000) along with increased axonal backfiring (which has been proposed to be under the control of presynaptic GABA receptors) (Stasheff et al., 1993). However, the evidence obtained in rodent slices during 4AP application (Avoli et al., 1996a, b) or high-frequency stimulation (Smirnov et al., 1999) suggests that GABA receptor-dependent elevations in [K^+]_o may play an even more relevant role (see below).

Elevations in [K^+]_o and ictal discharge initiation

Our data indicate that GABA receptor-dependent [K^+]_o, homeostasis may contribute to the occurrence of ictal discharges in the human FCD tissue. First, we have found that the elevations in [K^+]_o recorded in these slices during the slow 4AP-induced interictal events attained maximal values that were often larger than those accompanying a similar type of synchronous activity in MTLE slices. Secondly, the GABA receptor-mediated potentials that shortly preceded the ictal discharge onset were characterized by rises in [K^+]_o that were larger than those seen in association with similar field potentials occurring during the interictal period. We previously have reported an association of similar large elevations in [K^+]_o and ictal discharge onset in the deep layers of the entorhinal cortex (Avoli et al., 1996a; Barbarosie et al., 2002) and in the CA3 stratum radiatum of isolated hippocampal slices obtained from young rats (Avoli et al., 1996b). Data obtained in the latter study have revealed that the occurrence of ictal activity in the CA3 area is characterized by developmental changes in GABA receptor-dependent [K^+]_o, homeostasis. Indeed, elevating [K^+]_o can disclose seizure activity both in vivo (Zuckermann and Glaser, 1968) and in vitro (Traynelis and Digledine, 1988; Traub and Dingledine, 1990).

Several studies have shown that slow GABA receptor-mediated events induced by 4AP in hippocampal (Michelson and Wong, 1991; Perreault and Avoli, 1992; Staley et al., 1995; Lamsa and Kaila, 1997), entorhinal (Avoli et al., 1996a) and neocortical networks (Avoli et al., 1994; Benardo, 1997) continue to occur during blockade of ionotropic glutamatergic transmission. Moreover, under these experimental conditions, glutamatergic-independent synchronous events are still accompanied by transient increases in [K^+]_o (Avoli et al., 1996a, b; Lamsa and Kaila, 1997). These [K^+]_o elevations, which are abolished by GABA_A receptor antagonists, reflect depolarization of neurons and glia caused by the activation of GABA_A receptors that may lead to outward counter/co-transport of K^+ due to the action of the K^+/Cl^- exchanger KCC2, possibly in combination with a depolarizing action of GABA caused by Cl^- equilibrium and Cl^-/HCO_3^- anion shift (Kaila et al., 1993, 1997; Kaila, 1994; Staley et al., 1995; Avoli et al., 1996a, b; Rivera et al., 1999).

We have confirmed these findings here by recording field potential and [K^+]_o in FCD and MTLE slices during application of 4AP and glutamatergic receptor antagonists. However, some important differences emerged when comparing the depth distribution and the size of the [K^+]_o increases recorded in these two types of human cortical tissue. First, we could confirm that the largest increases in [K^+]_o in slices obtained from MTLE patients were localized between 600 and 1400 μm from the pia (see Louvel et al., 2001), while in FCD slices the [K^+]_o increases attained maximal values at depths that varied greatly from one experiment to another. The lack of a typical depth profile points to a severe disturbance of the neuronal network in dysplastic tissue. In agreement with this, the initiation foci for Mg^{2+}-free-induced discharges appear to be heterogeneous and multilayered in FCD slices (Köhling et al., 1999). Secondly, the elevations in [K^+]_o recorded in MTLE slices superfused with 4AP and glutamatergic receptor antagonists reached maximal values that never exceeded 4 mM; in contrast, values as large as 5.5 mM could be identified in localized areas of FCD tissue. It should be emphasized that the largest increases in [K^+]_o, during blockade of excitatory transmission spatially coincided with the cortical location from which ictal discharges appear to initiate under control conditions (i.e. during application of medium containing 4AP only). Hence, these data, by demonstrating a co-localization of the largest increases in [K^+]_o with the site of ictal discharge onset, underscore the importance of GABA receptor-dependent mechanisms in FCD ictogenesis. Interestingly, immunocytochemical data obtained by analysing Taylor’s type FCD tissue (Sprefacico et al., 1998; Garbelli et al., 1999; Tassi et al., 2001, 2002) indicate that even though GABAergic cells are decreased in number, they provide an ‘overexpression’ of GAD-positive terminals that surround large, dysmorphic glutamatergic neurons. Aasly et al. (1999), by using proton
magnetic resonance spectroscopy, have found an increase in GABA content in biopsies obtained from patients with cortical dysplasia.

**GABA<sub>B</sub> receptor function in FCD and MTLE slices**

We have found in FCD slices that application of baclofen at concentrations as low as 2 μM can abolish 4AP-induced ictal discharges, but not the interictal events (which are largely contributed by a GABA receptor-mediated mechanism); indeed, this latter activity disappeared only with baclofen concentrations >40 μM. Moreover, these baclofen effects were fully antagonized by CGP 35348, even though it has been reported that this antagonist may have weak effects on presynaptic GABA<sub>B</sub> receptors (Deisz, 1999). Hence, we are inclined to conclude that the changes induced by baclofen were caused mainly by the activation of GABA<sub>B</sub> receptors that are known to be located both post- and presynaptically on principal (glutamatergic) cells and interneurons (Newberry and Nicoll, 1984, 1985; Misgeld et al., 1989; Thompson and Gähwiler, 1992; Williams and Lacaille, 1992; Lambert and Wilson, 1993). Our results also suggest that the relative resistance of the slow interictal activity to baclofen may reflect a downregulation of GABA<sub>B</sub> receptors within the interneuron network in the FCD tissue. Early studies have demonstrated that baclofen may depress excitatory transmitter release to a greater extent than GABA release (Pierau and Zimmermann, 1973; Potashner, 1978).

To better identify the mechanisms underlying the resistance of the GABA receptor-dependent interictal events to baclofen, we also analysed the effects exerted by this GABA<sub>B</sub> receptor agonist on the synchronous activity recorded in FCD and MTLE slices during application of 4AP and glutamatergic receptor antagonists. The synchronous potentials recorded under these experimental conditions reflect the firing of interneurons leading to GABA release and to the consequent activation of postsynaptic GABA receptors. We have, indeed, found that the half-maximal doses of baclofen required to depress the glutamatergic-independent events (and thus to inhibit the release of GABA from interneuron terminals) in FCD and MTLE slices were significantly higher in the former (i.e. 40 and 20 μM). Therefore, these findings suggest that the function of GABA<sub>B</sub> receptors located on interneurons may be downregulated in FCD tissue. Interestingly, Uusisaari et al. (2002) have reported that epileptiform activity mediated by GABA<sub>A</sub> receptors and gap junctions occurs in rat hippocampal slices during prolonged application of GABA<sub>B</sub> receptor antagonists.

**Conclusions**

The results obtained in this study suggest that GABA receptor-mediated synchronization represents a mechanism related to the ability of GABA to cause sizeable increases in [K<sup>+</sup>]<sub>e</sub>. Moreover, the findings obtained with baclofen show a decreased ability of this GABA<sub>B</sub> receptor agonist to control the release of GABA from interneuron terminals. Overall, we propose that in FCD tissue, the clusters of increased interneuron terminals impinging on glutamatergic neurons along with a loss in release control by GABA<sub>B</sub> receptors leads to an abnormally large release of GABA, leading to pathologically increased synchronization, presumably due to CI<sup>−</sup> influx-dependent, KCC2-driven K<sup>+</sup> accumulation. In addition, since ictal discharges are abolished by NMDA receptor antagonists and since an abnormal distribution of NMDA receptors has been reported in human FCD tissue, ictal synchronization appears to depend on this glutamatergic mechanism.

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