

CORTICAL DYSPLASIA AND EPILEPTOGENESIS: MODELS AND MECHANISMS

Electrophysiological Characteristics of Reactive Astrocytes in Experimental Cortical Dysplasia

Bordey A, Lyons SA, Hablitz JJ, Sontheimer H

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Neocortical freeze lesions have been widely used to study neuronal mechanisms underlying hyperexcitability in dysplastic cortex. Comparatively little attention has been given to biophysical changes in the surrounding astrocytes that show profound morphological and biochemical alterations, often referred to as reactive gliosis. Astrocytes are thought to aid normal neuronal function by buffering extracellular K^+ . Compromised astrocytic K^+ buffering has been proposed to contribute to neuronal dysfunction. Astrocytic K^+ buffering is mediated, partially, by the activity of inwardly rectifying K^+ channels (K_{IR}) and may involve intracellular redistribution of K^+ through gap-junctions. We characterized K^+ channel expression and gap-junction coupling between astrocytes in freeze-lesion-induced dysplastic neocortex. Whole cell patch-clamp recordings were obtained from astrocytes in slices from postnatal day (P) 16–P24 rats that had received a freeze-lesion on P1. A marked increase in glial fibrillary acidic protein immunoreactivity was observed along the entire length of the freeze lesion. Clusters of proliferative (bromo-deoxyuridine nuclear staining, BrdU+) astrocytes were seen near the depth of the microsulcus. Astrocytes in cortical layer I surrounding the lesion were characterized by a significant reduction in K_{IR} . BrdU-positive astrocytes near the depth of the microsulcus showed essentially no expression of K_{IR} channels but markedly enhanced expression of delayed rectifier K^+ (K_{DR}) channels. These proliferative cells showed virtually no dye coupling, whereas astrocytes in the hyperexcitable zone adjacent to the microsulcus displayed prominent dye-coupling as well as large K_{IR} and outward K^+ currents. These findings suggest that reactive gliosis is accompanied by a loss of K_{IR} currents and reduced gap junction coupling, which in turn suggests a compromised K^+ buffering capacity.

Electrophysiological and Morphological Characterization of Neurons within Neocortical Ectopias

Gabel LA, LoTurco JJ

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Focal developmental abnormalities in neocortex, including ectopic collections of neurons in layer I (ectopias), have been associated with behavioral and neurological deficits. In this study, we used infrared differential interference contrast microscopy and whole cell patch-clamp to complete the first characterization of neurons within and surrounding neocortical ectopias. Current-clamp recordings revealed that neurons within ectopias display multiple types of action potential firing patterns, and biocytin labeling indicated that $\sim 20\%$ of the cells in neocortical ectopias can be classified as nonpyramidal cells and the rest as atypically oriented pyramidal cells. All cells had spontaneous excitatory (glutamatergic) and inhibitory (GABAergic) postsynaptic currents. Excitatory postsynaptic currents consisted of both N-methyl-D-aspartate (NMDA) receptor-mediated and AMPA/kainate (A/K) receptor-mediated currents. The NMDA receptor-mediated component had decay time constants of 15.35 ± 2.2 (SE) ms, while the A/K component had faster decay kinetics of 7.6 ± 1.7 ms at -20 mV. GABA_A receptor-mediated synaptic currents in ectopic cells reversed at potentials near the Cl^- equilibrium potential and had decay kinetics of 16.65 ± 1.3 ms at 0 mV. Furthermore we show that cells within ectopias receive direct excitatory and inhibitory input from adjacent neocortex and can display a form of epileptiform activity.

Abnormal Morphological and Functional Organization of the Hippocampus in a p35 Mutant Model of Cortical Dysplasia Associated with Spontaneous Seizures

Wenzel HJ, Robbins CA, Tsai LH, Schwartzkroin PA.

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Cortical dysplasia is a major cause of intractable epilepsy in children. However, the precise mechanisms linking cortical malformations to epileptogenesis remain elusive. The neuronal-specific activator of cyclin-dependent kinase 5, p35, has been recognized as a key factor in proper neuronal migration in the neocortex. Deletion of p35 leads to severe neocortical lamination defects associated with sporadic lethality and seizures. Here we demonstrate that p35-deficient mice also exhibit dysplasia/heterotopia of principal neurons in the hippocampal formation, as well as spontaneous behavioral and electrographic seizures. Morphological analyses using immunocytochemistry, electron microscopy, and intracellular labeling reveal a high degree of abnormality in dentate granule cells, including heterotopic localization of granule cells in the molecular layer and hilus, aberrant dendritic orientation, occurrence of basal dendrites, and abnormal axon origination sites. Dentate granule cells of p35-deficient mice also demonstrate aberrant mossy fiber sprouting. Field potential laminar analysis through the dentate molecular layer reflects the dispersion of granule cells and the structural reorganization of this region. Similar patterns of cortical disorganization have been linked to epileptogenesis in animal models of chronic seizures and in human temporal lobe epilepsy. The p35-deficient mouse may therefore offer an experimental system in which we can dissect out the key morphological features that are causally related to epileptogenesis.

Reduced Inhibition in an Animal Model of Cortical Dysplasia

Zhu WJ, Roper SN

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Cortical dysplasia has a strong association with epilepsy in humans, but the underlying mechanisms for this are poorly understood. In utero irradiation of rats produces diffuse cortical dysplasia and neuronal heterotopia in the neocortex and hippocampus. Using in vitro neocortical slices, whole-cell patch-clamp recordings were obtained from pyramidal neurons in dysplastic cortex and control neocortex. Spontaneous IPSCs were reduced in amplitude (35%) and frequency (70%) in pyramidal cells from dysplastic cortex. Miniature IPSCs were reduced in frequency (66%) in dysplastic cortex. Two additional measures of cortical inhibition, monosynaptic evoked IPSCs and paired pulse depression of evoked EPSCs, were also

impaired in dysplastic cortex. Spontaneous EPSCs were increased in amplitude (42%) and frequency (77%) in dysplastic cortex, but miniature EPSCs were not different between the two groups. These data demonstrate significant physiological impairment in inhibitory synaptic transmission in experimental cortical dysplasia. This supports previous immunohistochemical findings in this model and observations in humans of a reduction in the density of inhibitory interneurons in dysplastic cortex.

COMMENTARY

Cortical dysplasia is often associated with epilepsy. Because cortical dysplasia can be induced in experimental animals in a controlled fashion, it may provide a powerful model to study mechanisms of epileptogenesis (see review by Baraban). Several recent studies have described new ways of inducing experimental dysplastic lesions and are beginning to examine the pathophysiological mechanisms underlying seizure generation in these models.

Wenzel et al. showed that mice with deletion of the p35 gene have profound structural abnormalities in the hippocampus, with alterations in the location of neuronal cell bodies, axons, and dendrites. Of particular note, these mice exhibit granule cell dispersion and mossy fiber sprouting, two alterations that are known to occur in temporal lobe epilepsy. They also have electrographic and behavioral seizures, and thus, this animal model is attractive for studies aimed at understanding how cortical dysplasia leads to seizures. Although cortical dysplasia can occur without epilepsy and epilepsy can occur without cortical dysplasia, the p35 knockout mouse has both cortical dysplasia and epileptic seizures. It seems likely that some type of reorganization of neuronal circuits leads to or at least contributes to the seizures. The challenge for the future is to identify the cellular and network mechanisms linking cortical dysplasia and epileptogenesis in this model.

Bordey et al. induced a dysplastic lesion in cortex by freezing and investigated specific adaptations in glia. A long-standing and important issue in epilepsy research is the function of glia in seizures and epileptogenesis and in particular the role of astrocytes in regulating the concentration of extracellular potassium after injury-induced gliosis. Potassium channels and gap junctions in astrocytes are two elements that are likely important, but the manner in which they may change after an injury has not been adequately studied with modern techniques. This study focused on inwardly rectifying potassium currents and dye coupling between astrocytes as a measure of gap junctions. Potassium currents and gap junctional coupling were *decreased* in the reactive astrocytes near the injury, but the astrocytes in hyperexcitable cortex further from the injury ac-

tually had *increased* potassium currents and intercellular coupling. This report thus offered an explanation for differing views concerning the effect of cortical injury on astrocytic function.

The functional consequence of the gliosis that is often associated with epilepsy has been an important and relatively unapproachable problem for decades. For example, numerous studies have discussed the mesial temporal sclerosis generally found in temporal lobe epilepsy, but virtually nothing is known about the physiological ramifications of this histological phenomenon. Studies in this and similar models should help clarify the functional implications of alterations in glia.

Zhu and Roper induced cortical dysplasia by exposing rats in utero to gamma irradiation. Synaptic mechanisms were studied quantitatively with whole-cell recordings under direct visual observation in neocortical slices prepared from these animals. The alterations in the amplitude and frequency of spontaneous inhibitory postsynaptic currents, combined with alterations in evoked synaptic events, provided evidence that GABA-mediated inhibition is altered in this animal model of cortical dysplasia. Excitatory synaptic mechanisms were enhanced, but this could have been the secondary result of the reduced inhibition. These electrophysiological data support the view that dysplastic cortex has a reduced density of inhibitory interneurons. However, caution is warranted. It is necessary to evaluate the status of inhibitory mechanisms in other types of cortical malformations that have been associated with epilepsy to verify the generality of the conclusions of this report. This is challenging because the structural disorganization in focal malformations makes it difficult to compare circuit properties in the affected tissue with that in normal brain regions.

Gabel and LoTurco characterized the electrophysiological properties of neurons in ectopias in two strains of autoimmune mice and concluded that the neurons appeared to be electrophysiologically normal. The range of firing patterns and synaptic mechanisms in both ectopic and nearby normotopic cortex were similar to previous observations in neocortical neurons. The ectopias received synaptic input from adjacent normotopic cortex, but synaptic activation did not evoke epileptiform bursts. It was possible to induce epileptiform activity with treatments that block inhibition, but the ectopias did not display spontaneous epileptiform activity in normal medium. This article emphasized the concept that tissue with dramatic structural alterations can show electrophysiological responses that appear qualitatively, and even quantitatively, normal. An interesting anecdotal result in this study was the observation that one preparation with two adjacent ectopias did show epileptiform abnormalities. Based on this observation, the authors suggested the hypothesis that a larger area of abnormal cortex, compared with the relatively small ectopias examined in these experiments, is a critical substrate necessary for generating epileptiform activity. This hypothesis, combined with the other observations in this study, implies that epileptogenesis associated with cortical malformations results from large-scale network interactions. Future research using the different animal models that are now available for studying cortical malformations can test this hypothesis in well-controlled experiments. In addition, it may be possible to evaluate the idea in human tissue from surgical resections.

by F. Edward Dudek, Ph.D.