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Research report

The NMDA receptor NR2B subunit contributes to epileptogenesis in human cortical dysplasia

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Abstract

Cortical dysplasia (CD) is often associated with pharmacoresistant epilepsy. Previous studies showed increased expression of the NMDA receptor subunit NR2B in dysplastic and epileptic human neocortex. We tested the hypothesis that differential increase of NR2B constitutes an epileptogenic mechanism in humans. Dysplastic neocortex and lateral temporal lobe regions resected for treatment of pharmacoresistant seizures were processed for electrophysiological, histological, and immunocytochemical studies. Assignment to the "dysplastic" (n = 8) and "non-dysplastic" (n = 8) groups was based on histology. Neurons in "dysplastic" samples differentially stained for NR2B. Western blot (n = 6) showed an immunoreactive band for NR2B in three out of four "dysplastic" samples. Epileptiform field potentials (EFP) were elicited in vitro by omission of magnesium from the bath. EFP in "dysplastic" slices were characterized by multiple afterdischarges, occurring at a significantly higher repetition rate than EFP in non-dysplastic slices. The NR2B-specific NMDA receptor inhibitor ifenprodil (10μM) suppressed EFP in dysplastic slices. In non-dysplastic slices, burst repetition rate did not change with ifenprodil application. In both dysplastic and non-dysplastic slices, EFP were suppressed by a non-specific NMDAR antagonist (APV) or AMPA receptor antagonist (CNQX). These results provide additional evidence that the differential expression of NR2B in dysplastic human neocortex may play a role in the expression of in-situ epileptogenesis in human CD. NR2B may constitute a target for new diagnostic and pharmacotherapeutic approaches.
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Topic: Epilepsy: basic mechanisms

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1. Introduction

Cortical dysplasia (CD) [42] is commonly associated with medically intractable epilepsy and is associated with a less favorable epilepsy surgery outcome than other pathologies [12,48]. CDs are developmental disorders caused by disruption of neuronal migration. They are characterized by changes in neocortical microarchitecture: disturbance of laminar organization, ectopic neurons, and cellular abnormalities such as cytomegalic or dysmorphic neurons [7]. The association of CD with epilepsy is well documented [13,30,35].

The processes involved in the generation of epileptiform activity in human CD remain unclear. Possible mechanisms include changes in network connectivity, excitatory and

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inhibitory neurotransmitter function, as well as disturbances of glial physiology and the regulation of extracellular space and ion concentrations.

One of the possible causes that may lead to abnormal neuronal synchronization is an imbalance between excitatory and inhibitory neurotransmitter receptor activation. Increased expression of excitatory amino acid (glutamate) receptors has been found in human epileptic cortex [51]. However, besides increased receptor quantity, changes in receptor subunit composition may also account for hyperexcitability, as these changes can dramatically alter physiologic properties of receptors [43].

NMDA receptors (NMDAR) are likely to play an important role because (1) the NMDAR channel is permeable to Ca^{2+} which acts as a second messenger in signaling cascades attributed to synaptic plasticity [19,26] and (2) NMDARs display slow kinetics with a long inactivation time constant.

Three families of NMDAR subunits have been identified, termed NR1, NR2, and NR3. The NR1 subunit is a single gene product with eight different splice variants [52]. The four NR2 subunits (termed NR2A-D) are each encoded by separate genes. Native NMDA receptors are heterotetramers [22,38] or -pentamers [37] consisting of multiple NR1 and at least one NR2 subunit. NR1–NR2A receptors dominate in the mature neocortex. In contrast, NR1–NR2B receptors are physiologically expressed during fetal development and feature higher peak ionic currents and a six times slower inactivation time constant [31,32,43], resulting in an increased Ca^{2+} influx.

Several reports have shown a differentially increased expression of NR2B (or NR2A/B) in tissue resected from patients with drug-resistant epilepsy [9,27,33,49,50]. Mathern et al. [27] found increased NR2B expression in the hippocampi of epileptic patients. Crino et al. [9] reported increased NR2B mRNA in dysplastic neocortical neurons in human CD. Our own group has demonstrated differential NR2B expression in dysplastic neocortex from patients with pharmacoresistant focal epilepsy [49] and showed that NR2B subunits are coexpressed with and colinked to NR1 subunits [50], indicating that they are likely to form functional receptors. Moreover, we correlated the density of immunocytochemical (ICC) staining for NR2B with in-situ epileptic activity, assessed by prolonged direct cortical recordings [33].

The goal of this study was to test the hypothesis that NR2B-NMDA receptors contribute to epileptogenicity in dysplastic lesions in vitro: (a) we compared in vitro epileptiform field potentials (EFP) activity in brain slices prepared from dysplastic human neocortex with EFP in non-dysplastic control slices, and (b) we investigated the effect of the NR2B-subunit-specific NMDA receptor antagonist ifenprodil [45,46] on EFP in slices from dysplastic and non-dysplastic human neocortical tissue.

2. Materials and methods

2.1. Human neocortical tissue

Human neocortical tissue was obtained from 20 patients who underwent surgical treatment for pharmacoresistant focal epilepsy. All patients had undergone prolonged surface with or without subdural electrode video EEG monitoring and MRI studies prior to surgery. The tissue samples consisted of portions of neocortex with underlying white matter approximately 2 cm^3 in size, which were excised as part of the planned surgical treatment. No tissue was resected for sole experimental purposes. One half of each sample was immediately cut into slices for electrophysiological experiments (see below), the other half was fixed in paraformaldehyde (PFA) for histological and immunocytochemical (ICC) studies and partly frozen on dry ice for Western immunoblot analysis.

Dysplastic tissue was chosen from the most epileptogenic area as determined by chronic subdural grid or intraoperative electrocorticographic recordings or, in cases with a clear morphological abnormality detected on imaging studies, from the region corresponding to the MRI abnormality. The presence of CD was confirmed by histology.

Non-dysplastic neocortical samples were obtained from the inferior or middle temporal gyrus as part of a standard temporal lobectomy in patients who presented either with hippocampal sclerosis as the sole imaging abnormality or with normal MRI; the absence of pathological changes in the neocortex was confirmed histologically (the histopathological diagnoses were: hippocampal sclerosis [n = 4], mild hippocampal gliosis [n = 2], and no abnormal findings [n = 2]).

The material was freshly obtained during operations performed at the Cleveland Clinic Foundation between March and November 2003 (n = 20; 8 male, 12 female). Histopathological analysis of the tissue revealed cortical dysplasia in 8 out of 12 cases in the “dysplastic” group. In four cases with preoperatively suspected cortical dysplasia, histopathological analysis revealed neocortical pathologies different form CD: two patients had ischemic lesions, one had a cavernous angioma, and one had perivenous inflammatory changes consistent with the diagnosis of Rasmussen’s encephalitis. These four cases were excluded post-hoc.

The use of human tissue was approved by the Cleveland Clinic Foundation Institutional Review Board.

2.2. Histological and ICC studies

Histological and immunocytochemical (ICC) stainings were performed from blocks of neocortical tissue obtained during surgery from sites directly adjacent to the ones used for electrophysiological experiments. Additionally, representative specimens of the resected tissue were independ-

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