Epilepsy & Behavior 26 (2013) 253–260

Contents lists available at SciVerse ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Review

Searching for new targets for treatment of pediatric epilepsy

Yoav Noam [a,1], Yogendra H. Raol [b,1], Gregory L. Holmes [c,*]

[a] Department of Anatomy & Neurobiology, University of California-Irvine, Irvine, CA, USA
[b] Division of Neurology, Department of Pediatrics, School of Medicine, University of Colorado Denver, Aurora, CO, USA
[c] Department of Neurology, Geisel School of Medicine at Dartmouth, Hanover, NH, USA

ARTICLE INFO

Article history:
Accepted 6 September 2012
Available online 6 December 2012

Keywords:
HCN
Potassium channels
Ion channels
Seizure models
Neonatal
Flupirtine
Hypoxia
Trafficking
HCN
Epilepsy

ABSTRACT

The highest incidence of seizures in humans occurs during the first year of life. The high susceptibility to seizures in neonates and infants is paralleled by animal studies showing a high propensity to seizures during early life. The immature brain is highly susceptible to seizures because of an imbalance of excitation and inhibition. While the primary outcome determinant of early-life seizures is etiology, there is evidence that seizures which are frequent or prolonged can result in long-term adverse consequences, and there is a consensus that recurrent early-life seizures should be treated. Unfortunately, seizures in many neonates and children remain refractory to therapy. There is therefore a pressing need for new seizure drugs as well as antiepileptic targets in children. In this review, we focus on mechanisms of early-life seizures, such as hypoxia–ischemia, and novel molecular targets, including the hyperpolarization-activated cyclic nucleotide-gated channels.

This article is part of a Special Issue entitled "The Future of Translational Epilepsy Research".

Introduction

Gregory L. Holmes, MD

The highest incidence of seizures in humans occurs during the first year of life. The high susceptibility to seizures in neonates and infants is paralleled by animal studies showing a high propensity to seizures during early life. The immature brain is highly susceptible to seizures because of an imbalance of excitation to inhibition. Factors such as depolarizing GABA due to high intracellular concentrations of chloride, delayed $GABA_B$ development, high input resistances of neurons, and an over-exuberance of excitatory synapses result in a highly excitable network prone to seizures. While the primary outcome determinant of early-life seizures is etiology, there is evidence that seizures which are frequent or prolonged can result in long-term adverse consequences, and there is a consensus that recurrent early-life seizures should be treated. Unfortunately, despite the introduction of many new antiepileptic drugs, early-life seizures in many children remain refractory to therapy. There is a pressing need for new seizure drugs as well as antiepileptic targets in children. This paper highlights the work of two young investigators who are studying mechanisms of seizure, including early-life seizures, and who have innovative ideas regarding novel molecular targets.

Children during the first months of life are at particularly high risk for seizures, with the largest number of new-onset seizure disorders occurring during this time [1,2]. There is considerable evidence that the immature brain is more susceptible to seizures than the mature brain. For example, children under the age of six years are at risk for febrile seizures, whereas older children and adults rarely have seizures caused by fever alone. This increased propensity for seizures in humans has also been demonstrated in a wide variety of experimental models, including kainic acid [3,4], electrical stimulation [5], hypoxia [6], penicillin [7], picrotoxin [8], $GABA_B$ receptor antagonists [9], hyperthermia [10,11], and increased extracellular potassium [12,13].

Scientists have identified a number of reasons why the immature brain is highly susceptible to seizures [13,14]. During the early postnatal period, γ-aminobutyric acid (GABA), which in the adult brain is the primary inhibitory neurotransmitter, exerts a paradoxical excitatory action [12,13]. γ-Aminobutyric acid is initially excitatory because of a larger intracellular concentration of Cl^- in immature neurons than that in mature ones, resulting in E_{GABA} that is depolarized [15–17]. The E_{GABA} shift from depolarizing to hyperpolarizing occurs over an extended period depending on the age and developmental stage of the structure [18]. The shift is mediated by

* Corresponding author at: Department of Neurology, Geisel School of Medicine at Dartmouth, One Medical Center Drive, Lebanon, NH 03756, USA. Fax: +1 603 650 7617.
E-mail address: Gregory.L.Holmes@Dartmouth.edu (G.L. Holmes).
[1] Both authors contributed equally to this work.

1525-5050/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yebeh.2012.09.022

an active Na^+-K^+-$2Cl^-$ co-transporter (NKCC1) that facilitates the accumulation of chloride in neurons and a delayed expression of a K^+-Cl^- co-transporter (KCC2) that extrudes Cl^- to establish adult concentrations of intracellular chloride [19]. The depolarization by GABA of immature neurons is sufficient to generate Na^+ action potentials and to remove the voltage-dependent Mg^{2+} blockade of NMDA channels and activate voltage-dependent Ca^{2+} channels, leading to a large influx of Ca^{2+} that in turn triggers long-term changes of synaptic efficacy. The synergistic action of GABA with NMDA and calcium channels is unique to the developing brain and has many consequences on the impact of GABAergic synapses on the network. In addition, agents that interfere with the transport of Cl^- into the cell exert an antiepileptogenic action [19]. With maturation, there is increasing function of KCC2 and decreasing function of NKCC1, resulting in lower levels of intracellular Cl^-. The lack of an efficient time-locked $GABA_A$ inhibition and the delayed maturation of postsynaptic $GABA_B$-mediated currents place the immature brain in a vulnerable position. One of the few modes of inhibition in the young brain is the K^+ channel. Outflow of K^+ ions serves to hyperpolarize the membrane and limits action potentials.

In addition to lack of GABA inhibition, during the first few weeks of life, there is enhanced excitation due to an overabundance of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors [21,22]. High input resistance of immature neurons also facilitate the generation of action potentials and synchronized activities [9,18,20].

Unfortunately, the treatment of seizures in young children is suboptimal. In the case of neonatal seizures, little has changed over the past six decades with most physicians using phenobarbital or phenytoin as first-line therapies [21,22] despite well-conducted studies showing that both drugs are incompletely effective [23]. In addition to poor efficacy, animal data indicate that both drugs can lead to apoptosis when administered to developing animals [24]. In addition, phenobarbital following prolonged seizures in immature rats impairs spatial learning when the animals are evaluated as adults [25].

In the case of infants and toddlers, effective therapies also remain limited. Twelve second-generation antiepileptic drugs have been approved in the US for use in epilepsy over the past 15 years: felbamate, gabapentin, lamotrigine, topiramate, tiagabine, levetiracetam, oxcarbazepine, zonisamide, pregabalin, clobazam, vigabatrin, and ezogabine. Their use in children is fairly widespread, despite most of these agents not having US FDA indications for use. Clobazam is approved for children over two years and rufinamide for children over age four years. Only vigabatrin is approved for younger children and here the indication is restricted to infantile spasms, yet medically intractable epilepsy is common in children and the long-term consequences of recurrent seizures in the developing brain are tragic [26,27].

Young children have been terribly under-studied in regard to antiepileptic drug efficacy, tolerability, and safety. Clearly, there is a great need for new molecular targets for treatment of early-life seizures. In this paper, two young investigators, Yoav Noam and Yogendra Raol, provide compelling information to indicate that two novel molecular targets may be important targets of antiepileptic drugs.

Dr. Noam discusses hyperpolarization-activated, cyclic nucleotide-gated channels (HCN), a class of molecules that are open at subthreshold potentials and, therefore, are ideally suited for the fine-tuning of intrinsic excitability. As he notes, dysregulation of HCN channels in epilepsy occurs at multiple levels and at different timescales [28]. Transcriptional regulation of HCN channel protein has been observed in early-life seizure models resulting in altered I_h amplitude and gating properties and modified neuronal excitability [29,30]. Understanding the molecular and cellular mechanisms that underlie HCN channel dysregulation in epilepsy could contribute significantly to our understanding of early-life seizures and may provide an important target for the rational design of therapeutic strategies [31]; thus, targeting these channels may open an entirely new avenue of treatment.

Dr. Raol discusses potassium channels as a therapeutic target in neonatal seizures. There is increasing evidence that K^+ channels are important in the generation of early-life seizures. Newborns with benign familial neonatal convulsions (BFNC) typically begin having seizures between days 2 and 8 of life and remit by 16 months [32,33], with very little residual cognitive impairment. This syndrome is due to mutations in *KCNQ2* and *KCNQ3* which encode the voltage-gated K^+ channels, Kv7.2 and Kv7.3. Both *KCNQ2* and *KCNQ3* are expressed in the brain where the gene products form heteromultimeric channels that mediate the M-current, a slowly activating, non-inactivating potassium current that serves to inhibit neuronal firing. Mouse models for human *KCNQ2* and *KCNQ3* mutations demonstrate early-life seizures and reduced amplitude and increased deactivation kinetics of the M-current. Recently, Weckhuysen et al. [34] showed that a K^+ channel mutation may not be so quite benign. The authors studied a cohort of 80 patients with unexplained neonatal or early infantile epileptic encephalopathies for mutations in *KCNQ2* and *KCNQ3*. *KCNQ2* mutations occurred in 10% of patients. All of the patients had onset of seizures within the first three months of life followed by slowing of psychomotor development. In view of the important role of inhibition of K^+ channels in the developing brain, targeting this channel in early-life seizures may be an attractive therapeutic option.

Potassium channel modulators for treatment of neonatal seizures

Yogendra H. Raol, PhD

The neonatal brain is different from the mature brain anatomically and neurochemically, which can affect how the immature brain responds to both injury and treatment as compared to mature brain. Therefore, to find the most efficacious treatment for early childhood diseases, it is imperative to target age-specific mechanisms and test new therapies in neonatal disease models. Hypoxia–ischemia is a common cause of neonatal seizures and brain injury. Survivors of hypoxia–ischemia often experience neurological problems such as epilepsy and intellectual disability in later life. Studies suggest that seizures contribute to brain injury and affect long-term neurological outcomes. First-line drugs such as phenobarbital, which acts by augmenting $GABA_A$ receptor activity, are not fully effective in treating neonatal seizures and are associated with side effects.

Potassium channels play a uniquely important role in controlling brain excitability in early life. Our recent study showed that unlike diazepam and phenobarbital, flupirtine, a potassium channel opener, effectively treated chemoconvulsant-induced neonatal seizures. Currently, my laboratory is examining the efficacy of flupirtine to treat hypoxia–ischemia-induced neonatal seizures in an animal model. Our future studies will determine if treatment of early-life seizures can alter long-term neurological outcomes.

Although seizures can occur at any age, the risk is high in the neonatal period. In the United States, the occurrence of neonatal seizures is estimated at 1.8 to 3.5 per 1000 live births [35,36]. This increased risk can be attributed to a relative increase in the excitability of developing brain due to age-specific mechanisms such as overexpression of glutamate receptors and delayed development of GABA inhibition [37,38]. There is a broad range of insults that can cause seizures in neonates, but neonatal seizures are most commonly associated with perinatal hypoxic–ischemic encephalopathy [39]. Studies of human neonates and animal models suggest that seizures themselves may independently contribute to brain injury and poor neurological outcome [40–42]. Neonatal seizures can cause excessive fluctuations in brain oxygenation and blood perfusion, which may amplify