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Review

Gender issues in antiepileptogenic treatments

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ABSTRACT

Disease modification of epilepsy refers to the alleviation of epileptogenesis or comorbidities after genetic or acquired epileptogenic brain insults. There are currently 30 proof-of-concept experimental pharmacologic studies that have demonstrated some beneficial disease-modifying effects. None of these studies, however, has yet passed from the laboratory to the clinic. The International League Against Epilepsy and American Epilepsy Society working groups on antiepileptogenic (AEG) therapies recently released recommendations for conducting preclinical AEG studies, taking into account many of the critiques raised by previous study designs. One of the issues relates to the lack of analysis of AEG efficacy in both sexes. A review of the literature reveals that most of the preclinical studies have been performed using male rodents, whereas clinical study cohorts include both males and females. Therefore, it is important to determine whether sex differences should be taken into account to a greater extent than they have been historically at different phases of experimental studies. Here we address the following questions based on analysis of available experimental AEG studies: (a) whether sex differences should be considered when searching for novel AEG targets, (b) how sex differences can affect the preclinical AEG study designs and analysis of outcome measures, and (c) what factors should be considered when examining the effect of sex on outcome of clinical AEG trials or the clinical use of AEGs.

Contents

Introduction

Epileptogenesis refers to the development and extension of tissue capable of generating spontaneous seizures, resulting in (a) the development of an epileptic condition and/or (b) the progression of

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epilepsy after it is established. Antiepileptogenesis (AEG) is a process that counteracts the effects of epileptogenesis, including prevention, seizure modification, and cure. Complete prevention arrests the development of epilepsy. Partial prevention delays the development of epilepsy or reduces its severity, e.g., seizures occur but may be fewer in frequency, shorter, or milder (seizure modification). Antiepileptogenesis can also prevent or reduce the progression of epilepsy after it has been established. Cure refers to a complete and permanent reversal of epilepsy, such that no seizures occur after treatment withdrawal (Pitkänen and Engel, 2014).

Antiepileptic drugs (AEDs), such as phenytoin, phenobarbital, carbamazepine, or valproic acid were used in the first attempts to prevent epileptogenesis in humans (for review, see Temkin, 2001). More recent clinical trials in which the development of epilepsy was the primary or secondary outcome measure investigated molecules with neuroprotective properties, in addition to newer AEDs (Pitkänen and Immonen, 2014; Trinka and Brigo, 2014). The first proof-of-concept experimental AEG studies approximately 15 years ago were also performed using standard AEDs, and treatments targeting the molecular and cellular mechanisms related to circuitry reorganization were introduced more recently (see Pitkänen and Kubova, 2004; Pitkänen and Lukasiuk, 2011). Still, in 2014, there are no clinically available AEGs, and epileptogenesis is not considered a treatment indication. Preclinical proof-of-concept studies, however, have provided promising evidence that epileptogenesis triggered by genetic or acquired insults can be modified. The success has been shadowed by criticism related to study design, however, including the fact that none of the studies has analyzed the effect of sex on the outcome. Consequently, the working group established by the International League Against Epilepsy and the American Epilepsy Society proposed that potential sex effects can be assessed in statistically powered preclinical trials performed after proof-of-concept studies (Pitkänen et al., 2013). According to the guidelines "*demonstration of AEG efficacy in one gender is considered adequate before advancing to a clinical study. However, if the resources are available, AEG testing should be conducted in both males and females*".

As there are currently no AEG treatments available clinically, sex effects and antiepileptogenic treatment cannot be analyzed from the existing clinical data. The present review, therefore, is based on 47 experimental treatments in mostly adult animals, most of which have shown some favorable disease-modifying effect in experimental proof-of-concept studies (Table 1). Here we address sex-related issues that could arise if these treatments proceed to preclinical studies and eventually to the clinic. This is of particular interest as clinical AEG trials will likely be conducted in patient cohorts including both males and females. We believe that our discussion provides an acceptable framework to address the questions of (a) whether a sex issue should be considered when searching for targets for antiepileptogenesis, (b) how sex issues affect pre-clinical AEG study designs and analysis of outcome measures, and (c) what are the factors that should be considered with regard to the effect of sex on outcome in clinical AEG trials or the use of AEGs clinically when AEGs finally become available.

Target expression

Does sex affect target expression during epileptogenesis?

Is it possible that AEG treatment could work better in one sex because target expression during epileptogenesis or interactions of treatment with molecular networks in the epileptogenic focus differ between sexes?

Sexual dimorphism in gene expression in the brain is well-known and could therefore be a factor in target expression during epileptogenesis (Rinn and Snyder, 2005). Only a few studies have, however, considered sex differences in target expression during genetic or acquired epileptogenesis. The little information available comes from a study in which the effect of sex on levels of GABAA receptor subunit mRNAs was investigated in a rat model of absence epilepsy triggered by the treatment of Long-Evans Hooded rats with the cholesterol synthesis inhibitor, AY9944, on postnatal days (PN) 1, 5, and 9, resulting in a life-long increase in the occurrence of electroencephalographic spike-wave discharges (SWDs) (Li et al., 2007). Analysis of tissue from the thalamus or somatosensory cortex at PN35 and PN60 revealed that *Gabra1* and *Gabrg2* mRNA levels in the somatosensory thalamus were higher in males than in females. Males also had higher *Gabra1* and *Gabrg2* mRNA levels in the somatosensory cortex at PN35 and higher *Gabra1* mRNA levels also at PN60 compared with females. Interestingly, female rats had a more severe disease phenotype than males, as females had up to three-fold more SWDs per hour and twice the duration of SWDs than male rats (Persad et al., 2002). As summarized in Table 1, the severity of absence epilepsy in animal models can be modified by initiating treatment with AEDs or other compounds before the onset of epilepsy. All but one of these studies, however, were conducted using male animals. As absence epilepsy is known to be more prevalent in females than males (Janz, 1997), the AEG data obtained raises questions about whether sex affects target expression during absence epileptogenesis, and thereby treatment outcome. Another dimension that complicates the target search of AEG treatments relates to the age-dependency of sex-related target expression as pointed out in other chapters of this volume.

One study reported sex differences in gene expression in models of acquired epileptogenesis (Sharma et al., 2009). The microarray analysis performed by Sharma et al. (2009) revealed a sex difference at the transcriptomic level in a fly model of epileptogenesis induced by repeated administration of pentylenetetrazol ("kindling"). In male *Drosophila*, JAK/Stat, Wnt, mitogen-activated protein kinase, transforming growth factor-β, cell communication, and dorsoventral axis formation pathways were downregulated, whereas in females, only genes regulating dorsoventral axis formation were downregulated. Pyruvate metabolism and ribosomal pathways were downregulated in females only. Interestingly, the decrease in locomotor speed used as a functional outcome measure did not differ between males and females.

Neuroinflammatory diseases are more prevalent in females than males, and there is some indication that sex also affects the type and severity of a central nervous system inflammatory response after epileptogenic brain insult, such as stroke or traumatic brain injury (TBI) (Bruce-Keller et al., 2007; Manwani et al., 2013). The sex-dependent differences apparently relate to circulating levels of steroid hormones that can regulate both peripheral and central nervous system inflammation (Roof and Hall, 2000; Pettus et al., 2005; Bruce-Keller et al., 2007; Chen et al., 2013; Manwani et al., 2013). There have been at least nine studies of AEG treatments that target the immune/inflammatory response, five of which were conducted in male rats and four in female rats (Table 1). None of the studies, however, compared the AEG effect between males and females.

Are there links between signaling cascades that are affected by AEG treatments and sex-related hormonal pathways?

Traditional antiepileptic treatments (reviewed by Löscher et al., 2013) target ligand-gated or voltage-gated ion channels and thus regulate the activity of GABA receptor signaling, calcium signaling, or glutamate signaling. Based on our analysis of interactions between AEDs and sex-hormone signaling using String analysis (Jensen et al., 2009), only valproic acid has direct connections with sex hormone receptors through histone deacetylation (data not shown).

As summarized in Fig. 1, our String analysis revealed several interactions between targets of candidate AEGs (Table 1). Ingenuity Pathway Analysis (IPA, Ingenuity® Systems, www.ingenuity.com) indicated that the interaction site most often localized to the cell cytoplasm (20 targets) > cell membrane (19 targets) > nucleus