Medical Hypotheses 76 (2011) 871–876

Contents lists available at ScienceDirect

Medical Hypotheses

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

A new hypothesis of drug refractory epilepsy: Neural network hypothesis

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ARTICLE INFO

Article history:
Received 3 November 2010
Accepted 20 February 2011

ABSTRACT

Drug refractory is an important clinical problem in epilepsy, affecting a substantial number of patients globally. Mechanisms underlying drug refractory need to be understood to develop rational therapies. Current two prevailing theories on drug refractory epilepsy (DRE) include the target hypothesis and the transporter hypothesis. However, those hypotheses could not be adequate to explain the mechanisms of all the DRE. Thus, we propose another possible mechanism of DRE, which is neural network hypothesis. It is hypothesized that seizure-induced alterations of brain plasticity including axonal sprouting, synaptic reorganization, neurogenesis and gliosis could contribute to the formation of abnormal neural network, which has not only avoided the inhibitory effect of endogenous antiepileptic system but also prevented the traditional antiepileptic drugs from entering their targets, eventually leading to DRE. We will illustrate this hypothesis at molecular and structural level based on our recent studies and other related researches.

Introduction

Epilepsy is the most prevalent chronic neurological disorder characterized by an enduring predisposition to generate epileptic seizures [1], affecting up to 50 million people worldwide [2]. Although many patients with epilepsy have their seizures controlled effectively by antiepileptic drugs (AEDs), around 30% of patients continue to have seizures, despite trying a range of appropriate AEDs (mono- or poly-therapy) [3,4]. Drug refractory epilepsy (DRE) is established when there is inadequate seizure control despite at least two potentially effective AEDs taken in tolerable doses for 1–2 years [5]. The consequences of DRE can be quite severe, including substantial deleterious effects on individual health, life quality, and a heavy burden on society [6,7]. Although clinicians have been aware of the problem of pharmacoresistance in epilepsy for years, the mechanisms underlying its development are still not understood.

Several putative mechanisms underlying DRE have emerged in recent years. Two main hypotheses have been proposed to account for DRE, the target hypothesis and the transporter hypothesis [8]. Based on the target hypothesis, inherited or acquired alterations in the molecular targets of AEDs lead to reduced pharmacodynamic effects of the drugs, whereas the transporter hypothesis claims that there is inadequate access of AEDs to epileptic tissue because they are removed by multidrug transporters that are pathologically over-expressed [9,10]. However, valuable as each of these hypotheses is, none could be adequate to explain the molecular and cellular mechanisms of pharmacoresistance in epilepsy for the following reasons: (1) The phenomenon of multidrug resistance does not exist in all the cases with DRE; (2) Multidrug resistance can not be adequate to explain some pathological changes in epilepsy brain tissue, such as neuronal loss, sprouting, gliosis, and the recurrent seizures induced by those pathological changes; (3) The drug designed for multidrug resistance gene (probenecid, flunarizine, etc.) have made only part of the treatment effect in DRE; (4) The target hypothesis lack of electrophysiological and morphological evidence. All above indicate there may be other unknown mechanisms involved in mechanisms of DRE.

In recently years, the theory of abnormal neural network in epilepsy has attracted more attention [11,12]. Neural network hypothesis suggests that, under the influence of gene and microenvironment, pathological disorders with recurring episodes of excessive neural activity can induce neuronal degeneration and necrosis, gliosis, axonal sprouting, synaptic reorganization and remodeling of neural network. Under the guidance of the error message, the residual neurons in injured brain induced by seizures will extend toward non-physiological direction, and form abnormal connection with the inferior synapse. Thus, the novel neural network under pathological conditions has come into being and the origin or pathway of epileptiform discharges has been changed. The formation of abnormal neural network has not only avoided the inhibitory effect of endogenous antiepileptic system but also prevented the traditional antiepileptic drugs from entering their targets, eventually leading to the DRE.

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doi:10.1016/j.mehy.2011.02.039

Although the neural network hypothesis has been concerned much more, its participation factors, crucial points, key genes, direct and indirect pathways, and also its role in DRE are still unclear. The molecular biology evidence in supporting this hypothesis is, however, still lacking. Here, we will illustrate the hypothesis at molecular and structural level based on our recent studies and other related researches.

Growth cone is an important structure potentially involved in DRE

DNA microarrays, which provide global insight into transcriptional events occurring in a studied phenomenon, are now widely used tools for large-scale studies of gene expression in the brain of neuronal disorders [13–15]. There are also some available studies describing large-scale analysis of gene expression in conditions relevant to epilepsy [16–18]. One function of DNA microarrays analysis is the generation of new hypotheses to guide future research, and one such hypothesis can be proposed based on these data.

Using a complementary DNA microarray representing 4096 human genes, we have analyzed differential gene expression in the anterior temporal neocortex of DRE patients relative to controls [16]. Novel genes involved in cytoskeleton, synaptic plasticity, and structural/cellular reorganization have been identified in the brains of DRE patients. Our gene chip data are generally in agreement with the published results on epilepsy [19,20]. We had verified those novel genes by real-time fluorescence-quantitative polymerase chain reaction as well as immunohistochemistry, immunofluorescence and western blot analysis. After analysis of the results, we have found an interesting phenomenon that most of those abnormal expressed genes are associated with growth cone (Table 1).

Table 1
Expressions of growth cone related genes in the temporal lobe brain tissue of drug refractory epilepsy.

Genbank_ID	UniGene	Definition	cy5/cy3* Ratio
NM_002291	Hs.489646	Homo sapiens laminin, beta 1 (LAMB1), mRNA	4.650
NM_014325	Hs.330384	Homo sapiens coronin, actin binding protein, 1C (CORO1C), mRNA	3.212
NM_004998	Hs.370392	Homo sapiens myosin IE (MYO1E), mRNA	2.500
AB003592	Hs.387300	Homo sapiens mRNA for neural adhesion molecule NB-3, complete cds.	2.011
NM_004540	Hs.177691	Homo sapiens neural cell adhesion molecule 2 (NCAM2), mRNA	2.111
NM_016261	Hs.463638	Homo sapiens tubulin, delta 1 (TUBD1), mRNA	0.453
NM_001070	Hs.279669	Homo sapiens tubulin, gamma 1 (TUBG1), mRNA	7.455
X17033	Hs.482077	Human mRNA for integrin alpha-2 subunit	4.285
BC031051	Hs.317632	Homo sapiens cadherin 18, type 2, mRNA (cDNA clone MGC:33098 IMAGE:5278717), complete cd	2.866
NM_014748	Hs.278569	Homo sapiens sorting nexin 17 (SNX17), mRNA	2.979
NM_005909	Hs.335079	Homo sapiens microtubule-associated protein 1B (MAP1B), transcript variant 1, mRNA	0.194
NM_031846	Hs.368281	Homo sapiens microtubule-associated protein 2 (MAP2), transcript variant 3, mRNA	2.466
NM_002373	Hs.194301	Homo sapiens microtubule-associated protein 1A (MAP1A), mRNA	6.380
NM_001839	Hs.483454	Homo sapiens calponin 3, acidic (CNN3), mRNA	2.195
NM_183387	Hs.325846	Echinoderm microtubule-associated protein like 5 (EML5)	3.427

Cy5/Cy3 refer to the ratio of the fluorescent signals of patient/control groups, and were analyzed by GenePixPro3.0 software. Gene expression in epileptic tissue was considered to be over-expressed if the intensity value was double that of the control group (Cy5/Cy3* > 2). Conversely, gene expression was considered down-expressed when Cy5/Cy3* < 0.5. [16,83].

Growth cone at the tip of each axon is a highly motile structure that can help each neuron to extend an axon and find its ultimate destination amongst a complex environment during nervous system development [21,22]. Growth cone completes its movement function relying on filopodia, lamellipodia, and the internal cytoskeleton [23]. There are two cytoskeletal filaments present in growth cone: microtubules and actin filaments. Cytoskeletal proteins including microtubule-associated proteins (MAPs), tubulin, myosin etc. have been reported to play an important role in growth cone steering [24]. Coincidentally, those cytoskeletal genes were also abnormally expressed in the brains of DRE patients in our research (Table 1) [16]. It is complicated for the intracellular and extracellular signaling pathways that regulate the cytoskeletal reorganization during growth cone pathfinding. Cytoskeletal effectors in Rho-family GTPase signaling such as Cdc42, RhoA, N-WASP and actin-related protein 2/3 are known to regulate all aspects of the actin cycle which affect growth cone steering [21,25], and they have also been reported to be abnormally expressed in DRE brains [26,27].

Intriguingly, growth cone plays its role primarily during nervous system development [28,29], but why it remains active under pathologic condition of DRE? Elliot et al. [20] have used DNA microarray analysis to characterize gene expression in the dentate gyrus of the hippocampus and identify genes exhibiting similar patterns of regulation during epileptogenesis and development. In their study, 37 genes had an altered level of expression during both epileptogenesis and development. Most of the genes shared between the two groups are implicated in cell morphology and axon outgrowth. Therefore, the authors hypothesized that epileptogenesis shares the same features with normal development of nervous system. Based on this intriguing hypothesis, we speculate that the cell-biological events taking place in growth cone during development could also occur during epileptogenesis or development of DRE.

"Integrin – growth cone system" for neural network hypothesis

The formation of neural circuits requires proper connectivity that is established between neurons during development. Failure to achieve correct connectivity would result in dysfunction of nervous system, which might be associated with disorders. Growth cone is a crucial structure in central nervous system that is relevant to circuit formation and plasticity, including cell positioning and migration, synapse formation and plasticity, dendrite development, axon outgrowth and regeneration [30]. Local protein synthesis in growth cone has been described in the adult, and more interestingly, associated with neurological disorders and neural repair [31–33]. Therefore, our studies investigated the neural network hypothesis primarily focused on the growth cone.

An axon grows mainly at its tip, and the growth cone at its foremost senses and integrates signaling from multiple guidance cues [34], guiding the growth of neural fibers toward specific directions to gradually form neural networks. The growth cone is guided by extracellular guidance cues. Some axon guidance cues that play important role during embryonic development have also been reported to be possibly involved in epilepsy, such as netrin-1 [35], ephrin-A3 [36], the class 3 semaphorins [37–39]. In our previous study of epileptic patients and experimental animals, we observed axon guidance cues slit2 and netrin-G2 were abnormally expressed in the epileptic brains [40,41]. We found some cell adhesion