Clarification of refractory epilepsy mechanisms is of great significance for its precise diagnosis and adequate therapeutic approach. This review presents current hypotheses of refractory epilepsy formation: 1. Hypothesis of genetic factors; 2. Hypothesis of multi-drug transporters; 3. Target hypothesis; 4. Hypothesis of mechanisms related to antiepileptic drugs – development of tolerance and ineffective mechanisms of action; 5. Hypothesis of epilepsy related factors – seizure etiology, epilepsy progression, structural brain changes or neural net alterations. The latter hypothesis is closely related to the hypothesis of intrinsic severity as a determinant of antiepileptic drug resistance. A combination of mechanisms is also a possible explanation of refractoriness. Each of these hypotheses characterizes refractory epilepsy to a different extent without attaining an explicit and complex explanation of epileptogenesis.

**Keywords:** refractory; epilepsy; genetic; tolerance; multi-drug transporter

**Introduction**

Refractory epilepsy (RE) is a complex phenomenon. It is found in 20-35% of patients with epilepsy. Clarification of refractory epilepsy mechanisms is of great significance for its precise diagnosis and adequate therapeutic approach. There are several current hypotheses of refractory epilepsy formation: 1. Hypothesis of genetic factors; 2. Hypothesis of multi-drug transporters; 3. Target hypothesis; 4. Hypothesis of mechanisms related to antiepileptic drugs – development of tolerance and ineffective mechanisms of action; 5. Hypothesis of epilepsy related factors – seizure etiology, epilepsy progression, structural brain changes or neural net alterations. The latter hypothesis is closely related to the hypothesis of intrinsic severity as a determinant of antiepileptic drug resistance. One mechanism does not exclude the others, therefore co-existence of various mechanisms may explain intractability in some cases.

**Genetic factors**

The identification of genetic mutations is essential for specifying epilepsy etiology and diagnosing epilepsy as refractory. Genetic variations could explain partially etiology, mechanisms, and prognosis of the epileptic syndrome. Monogenic Mendelian epilepsies are rare. Clinical syndromes like Juvenile myoclonus epilepsy often have multiple possible genetic causes, and conversely, different mutations in one gene can be associated with various epileptic syndromes. Usually these factors contribute to lower seizure thresholds. Various mutations, the most frequent being missense mutations of SCN1A gene which encodes the α-subunit of sodium channels, underlie a broad spectrum of epilepsies – from severe myoclonus epilepsy in infancy to the more benign generalized epilepsies with febrile seizures plus. The prodynorphin gene encodes dynorphin which is expressed during focal hippocampal seizures and could assist seizure termination and...
prevention of a secondary generalization and status epilepticus. Allele polymorphism has been described (H or L – high or low dynorphin levels). In cases with family risk of temporal epilepsy the L-allele is overexpressed and partial seizures with secondary generalization and status epilepticus are more frequent. Another gene (PRNP) encodes the cellular prion protein. The allele variation Asn 171SER has been discovered in 23% of patients 18 months after anterior temporal lobectomy. It might be associated with intractability - 92% of patients having the normal allele and 68% of those having the allele variation become seizure free [7]. Idiopathic generalized epilepsies have been considered as a result of the combined effect of environmental factors and polygenic or oligogenic susceptibility [8]. The study results of Lakhan et al. demonstrate significant involvement of CYP2C9 genetic variants in the modulation of epilepsy pharmacotherapy confirming the important role of CYP2C9 mutants in prevention from developing drug resistance [9].

Multi-drug transporter hypothesis

In a way the effect of genetic factors is associated with the hypothesis of overexpression of MDR1 and ABCB1 genes which encode the so called multi-drug transporters – P-glycoprotein (PGP) and MRP (multi-drug resistance associated protein) [10, 11]. A frequent polymorphism of exon 25 of MDR1 gene correlating with these proteins overexpression has been described in patients with RE [12]. Normally multi-drug transporters exist in the apical parts of endothelial cells in the brain tissue and act as efflux pumps of lipophilic molecules, being a natural protection against xenobiotics. It has been found that their concentration is elevated in epileptogenic foci from patients with RE which is suggested to be associated with a limited access of antiepileptic drugs (AEDs) to the brain tissue. Expression was studied immunohistochemically in lesional tissue from surgical resections and compared with expression in histologically normal adjacent tissue [4]. Cation-chloride co-transporters (CCTs), particularly NKCC1, might contribute to the epileptogenesis in cases with hippocampal sclerosis and focal cortical dysplasia. Sen et al. have found increased immunoreactivity of NKCC1 in these cases (which is typical of the neonatal period), but not in adjacent histologically normal cortex. During ontogenesis NKCC1 are usually replaced by NKCC2 and GABA is transformed from an excitatory to an inhibitory neurotransmitter. Persisting of NKCC1 might contribute to epileptogenesis through intracellular accumulation of chlorine ions, GABA_B-receptors activation, depolarization, and excitation [13].

There is no precise answer to the question whether the overexpression of multi-drug resistance proteins is a consequence of epilepsy, uncontrolled seizures, chronic treatment with AEDs, a combination of these factors, or a result of resistance associated with gene polymorphism [1].

An activation of a secondary epileptogenic focus and drug resistance formation are also possible after a resection of the primary epileptogenic focus followed by antiepileptic treatment termination. It has been suggested that seizure frequency is more likely to correlate with multi-drug transporters overexpression. On the other hand however, overexpression can be also found in cases with structural alterations (malformations), which are neither acquired, nor induced. Volk et al. have proved histologically and immunohistochemically increased expression of PGP antibodies C219 after an induced status epilepticus in rats which are non-responders to treatment in the ipsilateral to the stimulation endothelial cells and the contralateral limbic regions. The lack of a statistically significant difference between rats’ responders and non-responders with regards to seizure frequency and brain tissue PGP expression necessitates a prospective study with the purpose of investigating AED levels in these regions in both groups. Rizzi et al. have proven a significant (30%) decrease in the ratio of Phenytoin (PHT) level in the brain/plasma, while Potschka and Löschter – a decreased PHT level in the hippocampus of rats following amygdala stimulation [14].

Functional polymorphism is also important. Multi-drug transporters overexpression has been described in other tissues as well – small intestines for example, and results in a decreased resorption of AEDs and their subtherapeutic blood levels. With regard to this, multi-drug transporter inhibitors have been proposed for an adjunctive treatment in cases with RE. Most of them are not usually recommended because of their inhibitory effect on cytochrome p450 and induced ataxia. Evidence about their positive effect on RE improvement would confirm this hypothesis. PET is a method which could visualize how PGP influence the brain intake of AEDs [11]. Calcium antagonists might be also effective, especially nifedipin, which is not associated with neurotoxicity [15-17]. Sen et al. have described a single case with refractory partial seizures and an effective verapamil application as a PGP inhibitor [13].

The first limitation of the multi-drug transporter hypothesis is that many reports are purely correlative and any compelling proof particularly in humans is still lacking. The data about PGP genetic polymorphism in RE are also contradictory [18]. PGP overexpression in the epileptogenic focus in patients with RE has not been
detected by PET in the study of Langer et al. \[18\]. Besides, not all AEDs are multi-drug transporters substrates (levetiracetam for example), but patients non-responders to other AEDs are refractory to levetiracetam as well. The molecular principle of overexpression has not been clarified – whether it is result of a second-line protection of the blood-brain barrier caused by seizure impairment, or a result of a chronic barrier dysregulation. Seizure induced overexpression is a possible explanation because high seizure frequency before treatment has a negative impact on AEDs response \[1, 4, 16\].

**Target hypothesis**

According to this hypothesis AED molecular targets could be modified functionally or genetically and become less sensitive to them. These targets include various receptors and ion (sodium, potassium, calcium) channels. Based on experimental models of Wadman and Vreugdenhil with rats having temporal epilepsy, Beck et al., Remy et al., it has been suggested that RE is caused by sensitivity loss to carbamazepine and phenytoin of sodium channels \[16\]. The study results concerning the action of valproate on sodium channels in the dentate gyrus are contradictory. Some investigators have found that valproate does not modify them, while others have described delayed recovery from inactivation. Recent studies have not confirmed different valproate effect in patients with and without mesial temporal sclerosis or in rats after kindling and controls \[16\]. Heinemann et al. have studied in vitro resected brain tissue from patients with RE and drug responders after induced convulsions refractory to high doses of carbamazepine. A possible cause of refractoriness is the finding of alterations of α- and β-subunits of sodium channels or posttranslational modifications – ROS production, glycosylation or phosphorylation. According to Schmidt and Löscher there is no answer to the question why the loss of carbamazepine effect on sodium channels of rats after kindling is temporary (from 1 day after the last generalized seizures to 5 weeks later). Besides, the reported in vitro decreased effect on sodium channels in CA1 neurons is not definitely associated with a decreased or lacking response to carbamazepine in vivo (the response of the rats of Wadman and Beck has not been investigated before the electrophysiological in vitro intervention) \[3, 16\]. The finding that despite the clinically confirmed refractoriness to carbamazepine, phenytoin, and lamotrigine (having a similar mechanism of action), lamotrigine remains effective, while the other two AEDs lose partially or completely their modulating effect on sodium channels, has not been explained yet. The study results of Vreugdenhil et al. about valproate effects on sodium channels in patients with RE do not support the statement that modifications of these channels could explain pharmacoresistance \[16\].

A participation of GABA in spontaneous epileptic discharges generation in human hippocampal tissue during early ontogenesis has been described. This function could be potentiated by GABA-mimetic drugs with the participation of carbonic anhydrase. That is why carbonic anhydrase inhibitors are effective in some patients. There are hypotheses about alterations in GABA-receptors, GABA-ergic synaptic transmission and potential reorganization of the GABA-ergic neural net. In rats with epilepsy expression of α1-subunit is decreased, while that of δ- and α4-subunits is increased. According to some studies these alterations are temporary for the α1-subunit and more continuous for the β1-subunit. In fact there is no specific model of expression which correlates with seizure genesis. In cases with temporal lobe epilepsy the sensitivity to GABA\(_{\text{A}}\)-receptors of AEDs in dentate gyrus is modified significantly - it is increased to zinc (concentrated in mossy fibers), and is decreased to the benzodiazepine receptor agonist Zolpidem. GABA-receptors blockade of zinc could be associated with lower efficacy of GABA-mimetic drugs and other agents having the same target \[12\]. Levetiracetam counteracts the effects of zinc in animals with recurrent seizures after a pilocarpine-induced status epilepticus, but it’s effective in no more than 40% of cases \[16\]. Based on experimental models Macdonald et al. have proven that continuous status epilepticus is associated with impairment of functional qualities of GABA\(_{\text{A}}\)-receptors in the granular layer and reduced chances of seizure termination by GABA-mimetic drugs \[3\].

One limitation of this hypothesis is that it does not offer an explanation of the fact that no modifications in many types of drug targets have been found in patients’ non-responders to lots of AED. It has been suggested to search for alternative targets like adenosine, cholinergic, glycine receptors, receptors of peptides with proved anticonvulsive potential, instead of recommending combinations with agents having different targets \[12\].

**Hypothesis of mechanisms related to AED – development of tolerance and ineffective mechanisms of action**

The target hypothesis has a lot of common with the hypothesis of development of tolerance and ineffective mechanisms of action. Tolerance is an adaptive response of the body of reduced response to a drug after repeated administration. It develops to some drug effects much more rapidly than to others. The extent of tolerance depends on the drug and individual (probably genetic) factors. Two major types of tolerance are known: 1.
Pharmacokinetic (metabolic) tolerance which is due to induction of AED-metabolizing enzymes and has been shown for most first-generation AEDs, it is easy to overcome by increasing dosage; 2. Pharmacodynamic (functional) tolerance is due to "adaptation" of AED targets (e.g. loss of receptor sensitivity) and has been shown experimentally for all AEDs that lose activity during prolonged treatment. Functional tolerance may lead to complete loss of AEDs activity and cross-tolerance to other AEDs.

Hypothesis of epilepsy related factors

RE is frequently associated with various neuropathological changes \cite{20}. In cases with mesial temporal sclerosis and cortical dysplasia epilepsy is usually refractory. Some neurobiological characteristics typical of the hippocampal net have been discovered. Aggregates of protein hexamers (connexins) forming gap junctions are found in the hippocampal net. This is the way of connexon formation that makes a semi-channel in the cellular membrane which is important for intercellular communications (electric currents and molecules) associated with hyperexcitability and seizures. The role of the numerous glial cells connected by gap junctions and modulating directly neuronal excitation and epileptogenesis in the hippocampal tissue and neocortex is significant \cite{21}. In cases with hippocampal sclerosis changes in receptors of neurotransmitters due to segment neuronal loss in CA1 and CA4 hippocampal regions have been found. Investigators have described a state of hyperexcitability which is a result of replacement of the normal information transport by means of glutamatergic mechanisms with transport based on interactions between interneurons through facilitating seizure generation disinhibition increased by GABA-mimetic drugs \cite{12, 22}. Alterations of the structure, distribution, and functions of GABA-ergic and glutamatergic receptors have been also reported. Leung has described sodium channels opening and more intensive sodium currents \cite{23}. Besides, the afterdischarge seizure threshold of amygdala and hippocampus is very low, which is an explanation of their involvement in temporal lobe epilepsy \cite{5}. Another proposed mechanism of epileptogenesis in mesial temporal sclerosis is the loss of perivascular Kir4 potassium channels which is associated with a partial destroying of dystrophin-associated protein complex and changes in potassium homeostasis \cite{24}. Wang et al. have found an increased expression of one type of neurofilament (nestin) in the temporal lobe of patients with RE. Nestin is considered a marker of cellular migration and proliferation and participates in cellular remodeling. A correlation between this finding and intractability has been suggested \cite{25}. Xiao et al. have described a significantly higher expression of synaptotagmin I in the cytoplasm and the cytomembrane of neurons in patients with RE. Synaptotagmin I is a key synaptic protein involved in both endocytosis and exocytosis. The same authors suggest that synaptotagmin I might be involved in human refractory epilepsy \cite{26}.

In some patients it has been observed an autoimmune response to GluR3-subunit of glutamate receptors (Rasmussen encephalitis) or to glutamate decarboxylase which catalyzes the decarboxylation of glutamate to GABA. This finding is also considered to correlate with RE formation. Michalak et al. have suggested that IgG leakage may contribute to neuronal dysfunction, accumulation, and epileptogenesis in drug-refractory epilepsies with blood-brain barrier disruption \cite{27}.

Cavernous angiomas may also cause RE through chronic lipid peroxidation increase, impairment of glutamate transport, decreased synthesis of nitrogen oxide in the cortex, and increasing in hippocampal epileptogenicity \cite{23}. Rogawski et al. have proposed a novel approach that considers epilepsy pharmacoresistance in terms of intrinsic disease severity which determines the response to AEDs. This hypothesis is supported by the following statements: 1. Despite the release of many new AEDs, rates of remission of seizures in newly diagnosed epilepsy have changed little in 20 years; 2. Despite the heterogeneity of epilepsy etiology, a consistent finding across the studies is the single most important factor associated with prognosis is the frequency of seizures in the early phase of epilepsy, with an association between increased number of seizures in this period and poorer outcome. The observation that the occurrence of frequent seizures is associated with poorer outcome suggests that common neurobiological factors may underlie both epilepsy severity and drug refractoriness. The influence of genetic factors on the severity of seizures is unknown. In spite of these findings, with the exception of an attempt of a single study to measure the heritability of epilepsy outcome, clinical and experimental studies in epilepsy have generally ignored the concept of disease severity \cite{28}.

No matter what the underlying mechanism, RE is a progressive and self-maintaining disease based on an epileptic vicious cycle. The onset in any stage cycle leads to the other stages and it is hard to determine the causes and consequences: Abnormality (anatomical, physiological) ⊧ Interictal epileptic activity ⊧ Ictal epileptic activity ⊧ Abnormality enlargement → Cognitive dysfunction, cellular death → Abnormality \cite{20, 29}.

There are still some proofs in support of the statement that "seizures beget seizures": 1. The existence of rapid, seizure-induced changes of synaptic functions and the
transformation of inhibitory GABA-ergic potentials to excitatory ones; 2. The observed changes in potassium homeostatic mechanisms and alterations of interactions between the processes of excitation and inhibition; 3. Frequency and severity of induced seizures increase after a short-time stimulation of amygdala in rats. This finding has not been confirmed clinically in all cases, because no more than 50% of patients with one seizure have a second one [29]. In the course of studying the kindling phenomenon in experimental animals it has been found that seizures may transform seizure-resistant mammals to seizure-susceptible ones [23].

Experimental models from temporal lobe medial regions have shown that seizures cause: 1. Apoptotic neuronal death (by means of increase in glutamate, NMDA receptors activity, and intracellular penetration of calcium ions); 2. Continuous changes of excitatory and inhibitory receptors (e.g. increased expression of Glu R2 subunit of AMPA-receptors in CA1 and CA3 regions and of glutamate receptors mGluR2, mGluR5 and the receptor subunits of kainate KA1 (Grik4) and KA2 (Grik5)), ion channels (increased expression of a1 subunit of class C L-type calcium channels); 3. Changes of inflammation factors (COX-2, TGF-β, NF-κB); 4. A cascade expression of early genes like c-fos, neurotrophic factors, and late genes, calpain, coding peptides, receptors, and cellular skeleton proteins (dystrophin and alpha syntrophyn) [30]. Fanq et al. have 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 does not only avoid the inhibitory effect of endogenous antiepileptic system, but also prevents the traditional AEDs from entering their targets [31].

The neurotrophic brain factor produced from terminal buttons of mossy fibers after depolarization is also important. Xu et al. have shown that its amplification facilitates epileptogenesis in kindling and have suggested that it could change directly neuronal excitability [22]. Neuronal plasticity of this kind may be associated with various combinations of disinhibition and hyperexcitability. By means of microscopic examination Chengyun et al. have described neuronal degenerative damage, reactive proliferation of astrocytes, as well as overexpression of glial fibrillary acidic protein and MDR1 in surgically removed brain tissues of 26 patients with RE no matter what etiology and clinical manifestations [32]. Najjar et al. have hypothesized that microglial activation and proliferation initiate a cycle of inflammation-induced seizures and seizure-induced inflammation [33]. Some of these alterations are involved in secondary epileptogenesis when the primary epileptogenic lesion induces epilepticiform activity in a primarily normal cellular population. Thus a mirror-focus is created during kindling by means of consecutive stages of excitotoxicity, neuronal death, abnormal synaptogenesis, recurrent circles, and glial reaction. This focus becomes an independent source of epileptic activity and causes progression of disease intractability [29].

**Conclusion**

Each of these hypotheses characterizes refractory epilepsy to a different extent without attaining an explicit and complex explanation of epileptogenesis. Further detailed investigations of cellular and molecular mechanisms of RE are highly needed for the purpose of timely and adequate treatment of these patients.

**References**


Current hypotheses of mechanisms of refractory epilepsy


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