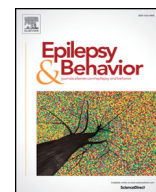




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Review

Drug-resistant epilepsy: From multiple hypotheses to an integral explanation using preclinical resources

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ABSTRACT

Drug-resistant epilepsy affects approximately one-third of the patients with epilepsy. The pharmacoresistant condition in epilepsy is mainly explained by six hypotheses. In addition, several experimental models have been used to understand the mechanisms involved in pharmacoresistant epilepsy and to identify novel therapies to control this condition. However, the global prevalence of this disease persists without changes. Several factors can explain this situation. First of all, the pharmacoresistant epilepsy is explained by different and independent hypotheses. Each hypothesis indicates specific mechanisms to explain the drug-resistant condition in epilepsy. However, there are different findings suggesting common mechanisms between the different hypotheses. Other important situation is that the experimental models designed for the screening of drugs with potential anticonvulsant effect do not consider factors such as age, gender, type of epilepsy, and comorbid disorders. The present review focuses on indicating the limitations for each hypothesis and the relationships among them. The relevance to consider central and peripheral phenomena associated with the drug-resistant condition in different types of epilepsy is also indicated. The necessity to establish a global hypothesis that integrates all the phenomena associated with the pharmacoresistant epilepsy is proposed.

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1. Drug-resistant epilepsy

Epilepsy is a neurological disease characterized by the presence of spontaneous and recurrent seizures [1], which affects approximately 50 million people worldwide [2,3]. Approximately 30% of these patients show drug-resistant epilepsy [4,5], which is defined as the persistence of epileptic seizures despite an adequate and well-tolerated anticonvulsant pharmacological treatment [6]. Regardless of the advances in the field of epilepsy and the acquisition of new antiepileptic drugs, the proportion of patients with drug-resistant epilepsy remains unchanged [4]. The present review focuses on analyzing the limitations of different hypotheses that explain drug-resistant epilepsy, as well as the models that try to reproduce the pathophysiology of the disorder.

2. Experimental models of drug-resistant epilepsy

Several animal models are used to reproduce pharmacological resistance in epilepsy, which allows to investigate their mechanisms and test drugs to modify this condition [7–9]. Three experimental approaches of

pharmacoresistant epilepsy are commonly used: animal models with recurrent and spontaneous epileptic seizures, animal models in which chemical or electrical stimulations induce epileptic seizures, and *in vitro* models of brain slices with epileptiform activity (Table 1).

Only some of these models are considered adequate in pharmacological screening according to their high efficiency in time, low cost, and the translational application of the obtained results [10,11]. These models are the following: 1) mice with epilepsy secondary to status epilepticus induced by kainate [12]; 2) mice with psychomotor seizures induced by electrical stimulation (6 Hz at 44 mA) [13]; 3) seizures induced with pentylenetetrazole in rats with epilepsy [14]; 4) rats with epileptic seizures secondary to electrical kindling and resistant to lamotrigine [15]; and 5) *in vitro* hippocampal slices with spontaneous epileptiform activity of rats previously submitted to kainate-induced status epilepticus [16]. Additionally, models in which convulsive drug-resistant seizures are induced as the consequence of the repetitive administration of chemoconvulsants are useful in pharmacological screening because of their short-term development: a) rodents with drug-resistant seizures secondary to the repeated administration of 3-mercaptopropionic acid or pentylenetetrazole [17–20] and b) zebrafish with convulsive seizures induced by ethylketopentenoate [21]. In these animal models, the lack of spontaneous recurrent seizures, a characteristic of

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Table 1
Experimental models of drug-resistant seizure activity.

Model	Species	Condition	Strategy of induction seizure/epilepsy	Characteristics and limitations	References
Spontaneous seizure subsequent to status epilepticus	Rat and mouse	<i>In vivo</i>	Status epilepticus by electrical stimulation with pilocarpine, kainate, or electrical stimulation	Animals present spontaneous seizures. However, the model represents high cost of resources and time.	[12,151,152]
Posttraumatic seizures	Rat	<i>In vivo</i>	Traumatic brain injury by fluid percussion	Animals present spontaneous seizures. However, there is no selection of responder and nonresponder animals. High mortality and high cost of resources and time	[153]
Induction of convulsive seizures in animals with epilepsy	Rat	<i>In vivo</i>	Acute seizures induced by pentylenetetrazole in rats with spontaneous seizures secondary to pilocarpine-induced status epilepticus	Animals present spontaneous seizures. Pharmacological resistance was found in all animals stimulated with pilocarpine, a condition that was independent to the existence of recurrent spontaneous seizures.	[14,154,155]
Kindled seizures resistant to phenytoin	Rat	<i>In vivo</i>	Amygdala-kindling electrical stimulation	Low number of animals (12–20%) with drug-resistant seizures. They do not present spontaneous seizures.	[156,157]
Chemical kindling	Rat	<i>In vivo</i>	Drug-resistant seizures subsequent to repetitive seizures induced by picrotoxicine or pentylenetetrazole	Animals do not present spontaneous recurrent seizures.	[103,158,159]
Repetitive convulsive seizures	Rat and mouse	<i>In vivo</i>	Repetitive generalized seizures induced by 3-mercaptopropionic acid	Pharmacoresistant seizures are obtained faster. Animals do not present spontaneous recurrent seizures.	[17,19,20]
Psychomotor seizures	Mouse	<i>In vivo</i>	Corneal electrical stimulation at 44 mA and 6 Hz	Pharmacoresistant seizures are induced faster. Animals do not present spontaneous recurrent seizures.	[13]
Lamotrigine-resistant corneal-kindled seizures	Rat and mouse	<i>In vivo</i>	Repetitive corneal electrical stimulation and chronic lamotrigine administration	Animals do not present spontaneous recurrent seizures. The resistance seems to be selective to the sodium channel inhibitors.	[160]
Lamotrigine-resistant kindled seizures	Rat	<i>In vivo</i>	Kindling electrical stimulation and chronic lamotrigine administration	Animals do not present spontaneous recurrent seizures. The resistance seems to be selective to the sodium channel inhibitors.	[15,161]
Seizures induced by ethyl ketopentenoate	Zebrafish	<i>In vivo</i>	Chemical stimulation with ethyl ketopentenoate	The model represents low cost of resources. Animals do not present spontaneous recurrent seizures.	[21]
Epileptiform activity in animals with cortical dysplasia	Rat	<i>In vitro</i> and <i>in vivo</i>	<i>In vivo</i> and <i>in vitro</i> exposure of animals with cortical dysplasia (induced with methylazoxymethanol acetate) to 4-aminopyridine	Animals do not present spontaneous recurrent seizures <i>in vivo</i> . The model represents high cost of resources and time.	[162]
Seizure-like events in brain slices	Rat	<i>In vitro</i>	Induction of seizure-like events by elevation of K^+ , lowering of Ca^{2+} , or lowering of Mg^{2+} in brain slices	No correlation with behavioral changes.	[163]

subjects with epilepsy, represents a critical limitation to explain the pharmacoresistant condition in patients with epilepsy.

At present, the Therapeutic Screening Program in Epilepsy—standardized by the National Institutes of Health—is the only program authorized for the screening of drugs with potential anticonvulsant effect [22,23]. This program includes the use of different experimental models to address the main problems of treatment in epilepsy: epileptogenesis, disease modifications, drug resistance, and comorbidities. This program includes two phases: the initial or identification phase, which focuses on the characterization of the anticonvulsant effect of the drug evaluated, as well as on identifying the effective dose 50 (ED₅₀) and the toxic dose 50 (TD₅₀) of the compound. Correspondingly, the secondary or differentiation phase involves the use of more complex models that reproduce the etiopathogenesis of the disorder. Pharmacological resistant models are included in both phases. Mice with psychomotor seizures induced by electrical stimulation (6 Hz at 44 mA) and hippocampal slices with spontaneous epileptiform discharges obtained from rats previously subjected to kainate-induced status epilepticus are the recommended models for the first phase. Mice with epilepsy secondary to kainate-induced status epilepticus as well as kindled rats with seizures resistant to lamotrigine are recommended for the second phase.

Even with the inclusion of several experimental models of drug-resistant seizures, no indication of the use of drug-resistant epilepsy models reproducing this disorder in young or aged subjects is specified in this program. In addition, the models indicated in the program reproduce focal seizures or focal epilepsy, leaving out generalized seizures including absence seizures. Therefore, it is necessary to rethink and design a program that integrates these features.

3. Hypotheses for drug resistance in epilepsy

Experimental findings from animal models and brain tissue from patients with drug-resistant epilepsy have helped to propose different hypotheses for the mechanisms underlying this disease (see Fig. 1) [24]. However, currently, no advances in the effective therapeutic control of drug-resistant epilepsy have been achieved [4,25]. This lack of progress could be explained because of the significant limitations in several hypotheses that attempt to explain the mechanisms of drug resistance in epilepsy. The hypotheses explaining the pharmacoresistant condition in different types of epilepsy and their limitations are discussed in the following sections.

3.1. The neural network hypothesis

This hypothesis is based on the fact that recurrent epileptic seizures generate neuronal death. It is known that patients with drug-resistant epilepsy present cellular alterations, such as neuronal death and the formation of aberrant and excitatory circuits (see Fig. 1) [26–28]. The surviving neurons aberrantly remodel the neural network, which generates hyperexcitable circuits challenging to control with the endogenous inhibitory mechanisms and antiepileptic drugs [29]. Also, aberrant connectivity affects multiple functions such as language execution [30]. In humans, these changes are evaluated by imaging studies, such as magnetic resonance imaging and positron emission tomography, which allow identifying both poor anatomical and functional connectivity during epileptic activity [31–33], as well as during brain activity at rest [34,35].

Unfortunately, a limitation of this hypothesis is its reproducibility. No studies have replicated the findings obtained in patients with

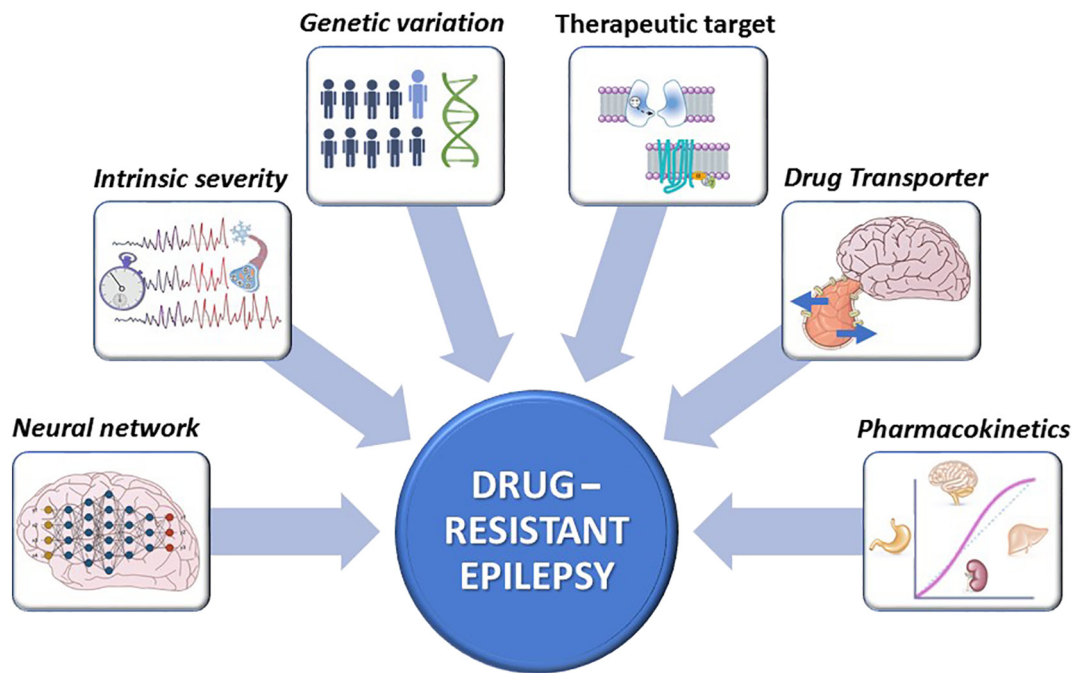


Fig. 1. Diagram showing the classical hypotheses of drug resistance in epilepsy. At present, there are 6 different hypotheses trying to explain the mechanisms producing drug resistance in epilepsy. Each hypothesis attempts to explain the pharmacoresistance in epilepsy by specific mechanisms. There are no relationships among the different hypotheses.

drug-resistant epilepsy in drug-resistant epilepsy models. Therefore, it has not been possible to determine if these observations are characteristic findings of either the drug resistance condition or epilepsy as a brain disorder. Furthermore, no cellular, anatomical, or cerebral connectivity alterations associated with epilepsy [36–38] have been characterized in drug-resistant epilepsy models.

3.2. The intrinsic severity hypothesis

Several clinical conditions are considered as predictive factors of drug-resistant epilepsy: early age at onset of the disease, the presence of interictal epileptiform activity, the history of status epilepticus, comorbidity of neuropsychiatric or neurological disorders, and retardation in the neurodevelopment process [39–44]. The hypothesis of intrinsic severity indicates that the high frequency of epileptic seizures is a relevant biomarker of the severity of the disorder and drug resistance (see Fig. 1) [45,46].

This hypothesis, which is based on studies in patients and experimental models, indicates that the increased intrinsic severity in drug-resistant epilepsy is associated with a high release of glutamate, the excitatory neurotransmitter par excellence [47,48]. High extracellular levels of glutamate have been detected in the epileptic focus of patients with drug-resistant temporal lobe epilepsy during the ictal and interictal activity [49]. This phenomenon is also observed in fully kindled rats that do not respond to antiepileptic treatment [50]. However, it is essential to mention that the kindling model is not considered as a typical model of epilepsy since the animals do not present spontaneous and recurrent epileptic seizures. Therefore, further experiments using animal models of drug-resistant epilepsy are necessary to support this hypothesis.

It is important to emphasize that the hypothesis of intrinsic severity can be associated with other hypotheses of drug-resistant epilepsy (see Fig. 2). For example, the high release of glutamate induces excitotoxicity and favors the formation of aberrant neuronal circuits [51], a condition associated with the neural network hypothesis (see Section 3.1). Similarly, glutamate favors the overexpression of P-glycoprotein, the most important ATP-binding cassette (ABC)-transporter producing

multidrug resistance [52,53], a phenomenon that in turn supports the hypothesis of the pharmacological transporter (see Section 3.5).

3.3. The genetic variation hypothesis

This hypothesis is based on pharmacogenomic studies in which gene variants represent risk factors of drug resistance in epilepsy (see Fig. 1). In this regard, the presence of changes in the functionality of specific proteins as a result of polymorphisms or gene variations can modify the pharmacokinetics and pharmacodynamics of antiepileptic drugs and affect their efficacy [11]. Gene variants related with drug-resistant epilepsy have been associated with the protein expression of gamma-aminobutyric acid A (GABA_A) complex subunits [54], ion channels [55–58], enzymes of the cytochrome P450 family [55,59], and drug transporters [60–63].

Surprisingly, this hypothesis does not include epigenetic changes, which can also modify protein functionality and facilitate drug resistance. Epigenetic changes alter gene expression through chromatin modifications without altering the Deoxyribonucleic acid (DNA) sequence [64]. These chromatin modifications may result in the activation or silencing of gene expression [65]. Epigenetic changes may persist even if the initial stimulus has disappeared [66,67]. The following mechanisms can mediate epigenetic changes:

1. DNA methylation. The promoter region of a gene contains cytosine–guanine-rich zones (cytosine–phosphate–guanine islands). Methylation consists of the covalent attachment of a methyl group to a cytosine residue in DNA, resulting in 5-methylcytosine [68].
2. Posttranslational modification of histones. This epigenetic mechanism consists of the posttranslational modifications of the amino (N)-terminal ends of histones [69].
3. Noncoding Ribonucleic Acids (RNAs). These RNAs (small interfering RNAs (siRNAs) and micro RNAs (miRNAs)) are functional molecules that do not encode for a protein but are capable of regulating gene transcription [70].

Recurrent epileptic seizures can produce epigenetic modifications [67,71]. Deoxyribonucleic acid (DNA) methylation patterns [72,73],

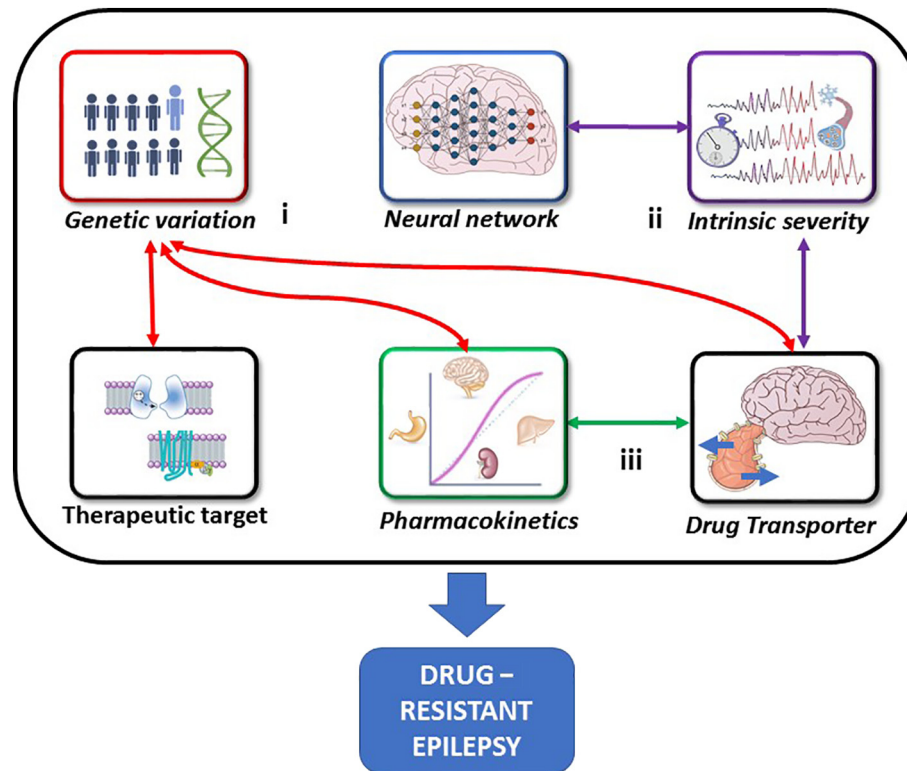


Fig. 2. Diagram showing connections between the different accepted hypotheses of drug resistance in epilepsy. (i) Genetic variations are also associated with changes in the expression of therapeutic targets, in genes that code for proteins involved in the pharmacokinetics of antiepileptic drugs, and in the expression of drug transporters. (ii) The intrinsic severity hypothesis is associated with the augmented release of glutamate. This effect induces neuronal damage and the reorganization of brain networks. High glutamate also leads to the overexpression of drug transporters. (iii) Cytochromes, important proteins for the metabolism of antiepileptic drugs, are coexpressed with the drug transporters in the blood–brain barrier. According to this information, it is important to establish an “Integrative Hypothesis of the Drug Resistance in Epilepsy”.

deacetylation of histones [74], and changes in the expression of miRNAs have been detected in patients with drug-resistant epilepsy [75–79]. It can be suggested that epigenetic changes induced by epilepsy modify the response to antiepileptic drugs. However, no experimental evidence currently exists to support this idea.

Moreover, antiepileptic drugs may induce epigenetic changes [80], a condition that may favor drug resistance. Unfortunately, evidence in animal models of pharmacoresistant epilepsy is still scarce and limited to alterations in the expression pattern of miRNAs [81]. This situation highlights the importance of implementing innovative strategies in animal models to elucidate the participation of epigenetic changes in drug-resistant epilepsy.

Furthermore, miRNAs are susceptible to genetic variants that might alter their function and possibly contribute to drug resistance in epilepsy [82,83]. Accordingly, it is clear that the hypothesis of genetic variants should consider the epigenetic changes, and it should be redefined as the Genome hypothesis to include any genome modifications associated with drug-resistant epilepsy.

3.4. The therapeutic target hypothesis

This hypothesis postulates that drug-resistant epilepsy is due to molecular changes in therapeutic targets, a situation that produces a lack of efficacy of antiepileptic drugs (see Fig. 1) [84]. These changes include alterations in transcriptional regulation mechanisms, second messengers, and posttranslational systems (phosphorylation, glycosylation, ubiquitination, S-nitrosylation, and protein carbonylation) [85]. This hypothesis is supported by the reduction of the efficacy of antiepileptic drugs such as phenytoin in patients with drug-resistant epilepsy, an alteration associated with changes in the voltage-dependent sodium channels [84]. Alterations in the distribution and expression of GABA_A receptors subunits have also been detected in the brain tissue of

patients with drug-resistant epilepsy [86–88]. Similar changes have been observed in animals with epilepsy secondary to the administration of kainic acid [89,90] and pilocarpine [84,91]. However, such findings have not been investigated in animal models of drug-resistant epilepsy.

3.5. The drug transporter hypothesis

Transporters are proteins expressed in protection (blood–brain barrier or hematotesticular barrier), elimination (renal glomeruli or hepatic canaliculi), or absorption (lung or intestine) barriers and are responsible for eliminating harmful substances exogenous or endogenous from the body as well [92–94]. The hypothesis of the drug transporter indicates that drug resistance in epilepsy results from the elevated expression of drug transporters in the blood–brain barrier (see Fig. 1). This situation produces low concentrations of antiepileptic drugs at the brain parenchyma and, consequently, a decrease in their effectiveness [95–98].

In drug-resistant epilepsy, the role of specific transporters is emphasized: P-glycoprotein [96,99], the multidrug resistance-associated protein (MRP) [96], and the breast cancer resistance protein (BCRP) [100]. The blood–brain barrier overexpression of these transporters in patients and drug-resistant epilepsy experimental models supports this hypothesis [96,97,100–105]. Also, this hypothesis is supported by the fact that the administration of drug-transporter antagonists reverts the low parenchymal concentration of antiepileptic drugs [98,103,104,106–111]. These findings support the need to use blockers to inhibit transporters as a therapeutic strategy to control pharmacoresistant seizures [112]. Indeed, clinical studies indicate that seizures are diminished in patients with pharmacoresistant epilepsy who use inhibitors of the P-glycoprotein (verapamil) as adjunctive therapy [113–116]. However, this strategy has significant limitations because the administration of P-glycoprotein blockers (or other transporters blockers)

induces substantial side effects thus, preventing their clinical application [117].

Moreover, this hypothesis explains only partially the pharmacological resistance in epilepsy because not every anticonvulsant drug is a substrate for drug transporters [107,118–121].

In addition to the blood–brain barrier, the drug transporters are overexpressed in neurons, astrocytes, and cardiomyocytes as a consequence of severe convulsive stress (repeated seizures or status epilepticus) [17,18,122]. The overexpression of P-glycoprotein at the neuronal level is associated with an increase in the membrane depolarization potential and increased neuronal excitability, effects that are reverted with exposure to a P-glycoprotein inhibitor [18]. In cardiomyocytes, the overexpression of P-glycoprotein is related to alterations in cell excitability that are reflected in QT segment prolongation, cardiac failure, and sudden unexpected death in epilepsy (SUDEP) [122]. Based on this information, it is necessary to include the pathophysiological consequences of transporter overexpression, both at the level of the blood–brain barrier and in other brain cells and peripheral organs in the present hypothesis.

3.6. The pharmacokinetics hypothesis

This hypothesis indicates that the pharmacological resistance in epilepsy is a consequence of alterations in the metabolism and elimination of antiepileptic drugs (see Fig. 1) [24]. Therefore, subjects with drug-resistant epilepsy show plasma levels below the reference values despite their adequate administration [95,123,124].

The main proteins involved in the metabolism and elimination of antiepileptic drugs are cytochromes and drug transporters. Polymorphisms in the CYP3A isozyme are related to drug-resistant epilepsy because they are associated with decreased plasma levels of several antiepileptic drugs and a poor therapeutic response [57,59,125–127]. The CYP3A is also expressed in the endothelium of the blood–brain barrier, and its atypical function can be involved in drug-resistant epilepsy [128–131].

This hypothesis partially explains the phenomenon of resistance in epilepsy because not all antiepileptic drugs share the same pharmacokinetic mechanisms [132,133]. Furthermore, the present hypothesis is associated with the hypothesis of drug transporters. Studies suggest a synergistic interaction between P450 enzymes and multidrug transporters in the blood–brain barrier. Therefore, dysfunction of such interaction might play a role in drug-resistant epilepsy [134].

4. Future directions in the study of drug-resistant epilepsy

Although a wide variety of experimental models of drug-resistant epilepsy currently exists [8], some populations are not represented, for which the prediction of their results is partial. The female population is not considered in the models of drug-resistant epilepsy. The influence of sex hormones on epileptic activity is a complex phenomenon [135]. Recently, efforts have been made to encourage the inclusion of female individuals in clinical and preclinical biomedical research [136–138]. Some of the first models of drug-resistant epilepsy were initially characterized in both male and female rats [139,140]. Therefore, it is now essential to extend this measure to all existing models.

Young and aged subjects are vulnerable populations to epileptic activity because of their physiological characteristics [141]. In this regard, aged patients with drug-resistant epilepsy show a poor neuropsychological performance [142] and a high rate of side effects as a result of the chronic administration of antiepileptic drugs, particularly under polypharmacy [143,144].

Moreover, comorbidities are not considered in drug-resistant epilepsy animal models. Patients with drug-resistant epilepsy frequently display neuropsychiatric disorders such as anxiety and depression [145]. The impact of these comorbidities can be even more severe than that produced by epileptic seizures. Thus, the importance of

treatments focused on solving these comorbidities associated with pharmacoresistant epilepsy [146,147].

Finally, chronic treatment with antiepileptic drugs is not considered in drug-resistant epilepsy studies. The chronic administration of antiepileptic drugs can induce tolerance to their effects [148], alterations on the GABAergic inhibitory system [149], epigenetic changes [80], and alterations in the expression of proteins [150], which can facilitate the condition of pharmacological resistance. Overall, further investigation on the consequences of prolonged treatment with antiepileptic drugs equivalent to the time of exposure and their mechanisms in patients is essential.

5. Conclusions

Drug resistance in epilepsy is a current scientific dilemma that demands attention. The lack of accessibility to human tissue limits the progression in the study of the mechanisms associated with resistance, for which experimental models are of high relevance. At present, experimental models of drug resistance in epilepsy include a broad spectrum ranging from *in vitro* models to animals with spontaneous drug-resistant seizures. These models have helped to design hypotheses allowing a better understanding of pharmacological resistance. However, none of these hypotheses can thoroughly explain the drug resistance phenomenon. The connections among the different hypotheses support the complexity of considering them in isolation. Therefore, it is essential to observe the relation between hypotheses and how they complement each other. Consequently, the pharmacological resistance in epilepsy would be explained comprehensively, with a dynamic relationship between the different mechanisms enunciated in each hypothesis (see Fig. 2). According to this information, it is essential the establishment of a global hypothesis that integrates all the phenomena associated with the drug-resistant condition in epilepsy.

Finally, although a wide variety of experimental models of drug-resistant epilepsy is available, some populations are not represented by these models. Therefore, it is essential to incorporate these populations, which unique characteristics require an individual approach.

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