Luisa Rocha · Esper A. Cavalheiro *Editors*

Pharmacoresistance in Epilepsy

From Genes and Molecules to Promising Therapies

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Chapter 1
Pharmacoresistance and Epilepsy

Francisco Rubio-Donnadieu

Abstract Although more than ten new antiepileptic drugs have been developed in the past decade, epilepsy remains resistant to drug therapy in about one-third of patients. Approximately 20 % of patients with primary generalized epilepsy and up to 60 % of patients who have focal epilepsy develop drug resistance during the course of their condition, which for many is lifelong. Managing these patients is a challenge and requires a structured multidisciplinary approach. The present chapter is a general overview of epilepsy as stigma, health and economical problem, and initiatives to change and the conditions of people with epilepsy. Special emphasis is focused to highlight the consequences of pharmacoresistant epilepsy.

Keywords Epilepsy • Pharmacoresistance • Epidemiology • Stigma • Antiepileptic drugs • Burden • Blood brain barrier • International League against Epilepsy • World Health Organization

1.1 Epilepsy

To "take hold of abruptly or to seize" is the meaning of the word Epilepsy, derived from a preposition and an irregular Greek verb *(Epilambanein).* Throughout the last five decades, the definition of epilepsy has been subjected to extensive controversy and debate by different neurological schools. It was not until 1973, that the International League against Epilepsy (ILAE) and the World Health Organization (WHO) published an Epilepsy dictionary in which epilepsy is defined as a chronic affliction of diverse etiology, characterized by recurring seizures due to excessive

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neuronal discharge (epileptic seizures), associated to diverse clinical and paraclinical manifestations. However, one must consider that there are many variables that are not encompassed within this definition, such as genetic aspects, age of onset, and triggering factors, on the one hand. On the other hand, this definition leaves out associated manifestation that have transcended to society, and throughout history led to interpretation of epilepsy as a supernatural phenomenon, as these paroxystic episodes cause fear, surprise and, as a rule, uncertainty.

1.2 Epilepsy as Stigma

The scientific development of medicine has often clashed with religious beliefs, leading to various erroneous concepts that classify epilepsy as the "sacred disease," oblivious to the warning by Hippocrates who tried to convince society that epilepsy was nowise more divine nor more sacred than other diseases, but had a natural cause like other affections. Regardless of Hippocrates wise concepts, absurd beliefs and conceptions multiplied and spread; the epileptic patient has been considered to "be possessed," which, in turn, has resulted in his/her rejection or exclusion not only by society in general, but often by the own family. It is well known that up to this date, the patient with epilepsy is submitted to exorcisms to liberate him/her from "demonic possession" both in highly developed and underdeveloped countries. Few diseases have been associated to such an accumulation of erratic beliefs, based on superstition, prejudice or ignorance, as epilepsy. In fact, in several cases it proves more difficult to control the environment where the epilepsy patient lives, than to obtain good seizure control. The stigma persists and is sustained on mystical bases.

The clinical manifestation of the disease and the different types of epileptic seizures were described since Babylonian times in the earliest handbooks of medicine in a clay tablet called antashubba, which is Sumerian for "falling disease." Regardless of this medical knowledge dating back over 4,000 years, religious beliefs were widely spread through the Bible, the Talmud, and the Koran. Paroxystic episodes are described in the Old Testament and considered as episodes of deep sleep (tardemah) that "took hold of Abra-ham." It is noteworthy that the word "Tardemah" used in Genesis and translated to Greek is understood as ecstasy, episodes that were frequently experienced by the prophets Isaiah, Daniel, Ezekiel and Jeremiah. On the other hand, the Book of Revelations in the New Testament contains a detailed description of what is now called "Saint John's malady," a disease suffered by the apostle himself with clinical features, consistent in auditive manifestations and falls with possible seizures, considered by Dostoyevsky as similar to his own episodes, and that can very likely be considered epileptic seizures.

Furthermore, even in the nineteenth century there were several evidences of erroneous interpretations of the epileptic phenomenon, always related to some famous character in the field of arts or science who exhibited these phenomena. Such is the case of the convulsive seizures presented by Vincent Van Gogh, who according to various reports was assumed to suffer epilepsy. This interpretation has been placed in

doubt, considering the clinical history of Van Gogh and the surrounding circumstances of his convulsive episodes, that were most probably non-epileptic seizures associated to alcohol ingestion. The confusion resulting from the interpretation of Van Gogh's convulsive episodes has often led to the association of epileptic disorders with other mental disorders, such as the one suffered by Van Gogh, who, in hindsight, might have suffered from bipolar affective disorder leading to suicide. We can likewise highlight the consequences of the religious beliefs in Christ's miracle when he exorcised the lunatic child to liberate him from the "demonic possession causing epileptic seizures." Unfortunately, there are many examples in the literature that have done nothing more than spread an erroneous understanding of epilepsy, which is ultimately responsible for the persistent stigmatization of the patient with epilepsy.

From the historical point of view, it is a reality that "knowledge" is based on anecdotes, which are translated into facts considered to be incontrovertible, as they are derived from the evolution of culture and, particularly, religion, whether monotheist or polytheist. Thus, throughout the centuries this anecdotic-type beliefs persist and offer resistance to the great advances derived from science, particularly in the last 50 years and the recent knowledge that has resulted from application of the scientific method and led to new theories and continued research related to basic mechanisms; in this case, on the nature of the epileptic discharge. This is why, at the level of society in general, whose behavior is a reflection of cultural beliefs transmitted from generation to generation, it is difficult to obtain a consensus with recent scientific development and advances and modify the concepts that, throughout the centuries, have identified epilepsy as a supernatural phenomenon. Persistence of these erroneous concepts renders epilepsy an even bigger health problem worldwide, and it is why in 1997, the WHO, in conjunction with the ILAE, launched the Global Campaign "Epilepsy out of the shadows."

1.3 Epilepsy and Pharmacoresistance

Epilepsy is a multifactorial disorder from the molecular, genetical, and environmental points of view, which has been the cause of multiple controversies and drawbacks in creating a universal consensus and uniformed criteria that allow determining the magnitude and transcendence of the epileptic phenomenon. Since 1973, according to the WHO and ILAE, epilepsy has been defined as a chronic and recurrent affection of paroxystic seizures (epileptic seizures) resulting from abnormal electrical discharges that have varied clinical manifestations of multifactorial origin and are associated to paraclinical abnormalities (electroencephalographic abnormalities) and present spontaneously. This definition has had the great advantage of being accepted by the different associations and organizations related to the neurosciences, allowing, in the last three decades, a more or less uniformed criterion on what is considered an epileptic phenomenon. This uniformed definition has also contributed largely to the completion of comparative epidemiological studies worldwide, which allow organization of effective and sustainable campaigns against epilepsy to benefit people who suffer epilepsy.

Epilepsy is characterized by abnormal synchronization of neural activity. Pharmacotherapy is the treatment of choice for control of epileptic seizures and the selection of antiepileptic drugs (AEDs) depends on several factors such as the type of epilepsy and drug tolerability (Browne and Holmes 2001).

Though the majority of patients respond to treatment with AEDs adequately, about one third of patients present pharmacologically resistant epilepsy, which is generally defined as the failure of seizures to come under complete control or acceptable control in response to AED therapy (Berg 2009). Clinical characteristics associated to resistance include early onset of epileptic seizures (before 1 year of age), elevated seizure frequency before onset of treatment, history of febrile convulsive seizures, brain lesions, malformations of cortical development and dysembryoplastic neuroepithelial tumors (Rogawski and Johnson 2008; Semah et al. 1998; Regesta and Tanganelli 1999; Sisodiya et al. 2002).

An innate high excitatory neurotransmission could be a neurobiological factor that may underlie augmented susceptibility to develop pharmacoresistance (Arroyo et al. 2002; Rogawski and Johnson 2008; Luna-Munguia et al. 2011). At the cellular level, intractability of epilepsy is associated to factors such as abnormal reorganization of neuronal circuitry, alteration in several neurotransmitter receptors, canalopathies, reactive autoimmunity as well as the abnormal inadequate penetration of AEDs into the epileptic focus due to changes in the blood brain barrier (BBB) (Fig. 1.1) (Kwan and Brodie 2002; Vreugdenhil and Wadman 1994, 1999; Remy et al. 2003; Ellerkmann et al. 2003). Other factors, such as the cell junctions in the vascular endothelium and astrocytes, which undergo important changes as a consequence of repetitive epileptic seizures (Kasantikul et al. 1983; Lamas et al. 2002), also may play a role in pharmacoresistance.

1.4 Epilepsy as Health Problem

Epilepsy is a chronic, recurrent, frequently progressive neurological disorder that affects 1–2 % of the population worldwide. Epilepsy is considered an important public health problem with significant social and economic impact (Engel and Taylor 1997). It is estimated that about 37 million individuals in the world have primary epilepsy, a number that increases to approximately 50 million when epilepsy secondary to other diseases or injuries is considered (World Health Organization 2001). Interestingly, it is calculated that at least 100 million people will have epilepsy at some time in their lives (Reynolds 2002).

In developed countries, the incidence of epilepsy is remarkably consistent across geographical areas, ranging from 24 to 53 per 100,000 person-years (Kurland 1959; Keränen et al. 1989; Olafsson et al. 1996), whereas the prevalence ranges from 3.5 to 10.7 (de Graaf 1974; Haerer et al. 1986). In contrast, epidemiological studies indicate higher prevalence and incidence rates of epilepsy in the general population of developing countries. For example, in Latin America, the median lifetime prevalence in all countries is 17.8 (range 6–43.2) per 1,000 people, and the incidence is

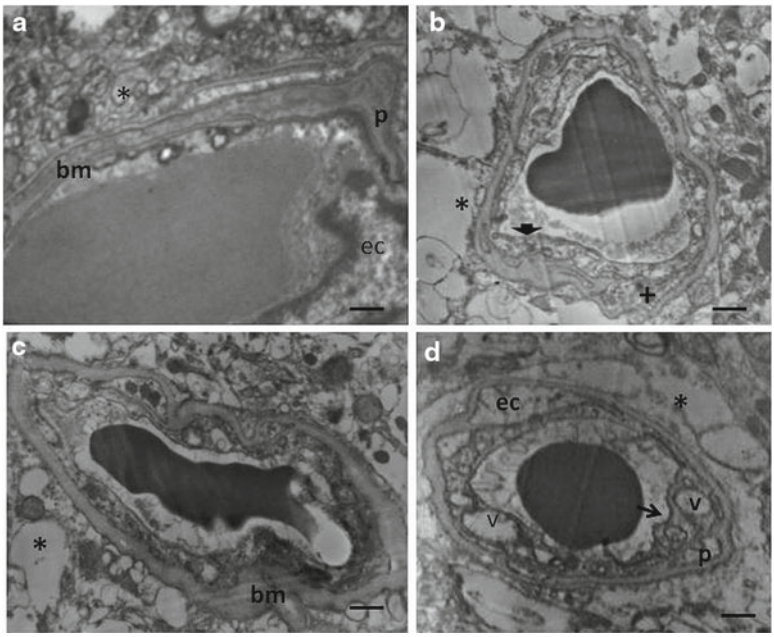

Fig. 1.1 Electron photomicrographs of vessels from epileptic focus of pediatric patients with epilepsy. (**a**) Normal microvessel with normal thickening of the basal membrane. Notice that endothelial cells, pericytes, and the astrocytes feet are preserved. (**b**, **c**) microvessels located in white matter from epileptic focus and (**d**) microvessel in epileptic neocortex. Notice in (**b–d**) degeneration of pericytes, swollen, vacuolation and multivesical bodies (+) inside endothelial cells as well as longitudinal folds and invaginations (*arrows*) on their surface. Abbreviations: *bm*, basal membrane; *ec*, endothelial cells; *p*, pericytes; *v*, vacuolation. Scale bars: (**a**) 0.5 μm, (**b**) 2 μm (**c**, **d**) 1 μm (from Dr. Sandra Orozco-Suárez with permission)

77.7–190 per 100,000 people per year (Burneo et al. 2005). Developing countries concentrate more than 80 % of persons with epilepsy, a situation associated with a lack of appropriate treatment (Carpio and Hauser 2009).

The WHO (2001) highlights that from the 40 million people suffering epilepsy worldwide, only 6 million receive adequate medical treatment. The 34 million people in developing and underdeveloped countries consume 18 % of the antiepileptic medications, whereas 6 million epilepsy patients in the so-called first world consume 82 % of the antiepileptic medications.

1.5 Burden of Pharmacoresistant Epilepsy

The burden of epilepsy is high and, for the year 2000, accounts for approximately 0.5 % of the whole burden of diseases in the world (Leonardi and Ustun 2002; World Health Organization 2001). Patients with epilepsy have significantly higher rates of health-related contacts and medication use as well as a higher

socioeconomic cost, lower employment rates and income. Socioeconomic impact of epilepsy has been evaluated in different countries. In the U.K. 400,000 people have active epilepsy and represent a cost of £600 million annually in direct care and £2 billion annually in overall cost to the nation (Bowis 2002). A Danish study indicates that the direct net annual health care and indirect costs are €14,575 for patients in contrast with €1,163 for people without epilepsy, giving a consequent excess cost of €13,412 (Jennum et al. 2011). In the U.S., the direct medical costs for patients with no seizures during the previous year was $US 251, for patients with less-than one-seizure-per-month $US 1,333, and $US 2,439 for patients presenting more than one seizure per month (Annegers et al. 1999; Platt and Sperling 2002). In Mexico, a study published in 2006 revealed that the mean annual healthcare cost per patient with epilepsy was $US 2,646 (García-Contreras et al. 2006). It is important to note that, according to multinational studies, costs of healthcare for patients with pharmacoresistant epilepsy are higher than those for non-refractory epilepsy patients (Begley and Beghi 2002).

In addition to the economical burden, epilepsy may have a substantial social impact because people with this disorder and their families all over the world experience prejudice and discrimination, isolation and exclusion. People with epilepsy are victim of society's stigma and live their life on the margins (Lee 2002). This situation is worsened for patients who experience pharmacoresistant epilepsy (Regesta and Tanganelli 1999).

In carefully selected cases of pharmacoresistant epilepsy, surgical removal of the epileptogenic zone is superior to continued medical treatment in completely controlling seizures and improving health-related quality of life (Wiebe et al. 2001). After epilepsy surgery, total costs for seizure-free patients decline 32 % at a 2-year surgical follow-up due to decreased use of AEDs and inpatient care needs. In the 18–24 months following evaluation, epilepsy-related costs are $US 2,094 in patients with persisting seizures vs. $US 582 in seizure-free patients (Langfitt et al. 2007).

In spite of the high economical burden that pharmacoresistant epilepsy represents, it is important to consider that not all patients with this disorder are candidates for resective epilepsy surgery. Then, there is a great need to develop other therapeutic strategies to control seizure activity for those patients who do not respond to AEDs.

1.6 Epilepsy Care

Given that epilepsy is a public health problem with important social transcendence, affecting the patient's opportunities in the personal, education and employment spheres, extending to the whole family, due to persistent social exclusion, it is the obligation to divulge -across all levels of our communities- that epilepsy is a treatable neurological disorder, which is frequently curable.

In the last few years, several important ILAE initiatives have been taken to change and improve the conditions of people with epilepsy. The main goal of ILAE

is to organize successful programs for improving expertise in epileptology in all countries. Under the auspices of the Global Campaign "Epilepsy out of the shadows," knowledge about differences in the pattern of provision of epilepsy care encountered by the ILAE chapters is helpful in the continuing efforts to develop high-quality management of epilepsy all over the world.

For example, the public health sector in Mexico supports and drives assistant programs such as the Epilepsy Priority Program (PPE for its Spanish initials) concerned with prevention, diagnosis, treatment and rehabilitation of patients with epileptic seizures through specialized groups, distributed across the different states and coordinated by neurologist and neuropediatricians, certified by the Mexican Neurology Council. The main objective of the PPE is establishing an efficient reference and contra reference system for patients with epilepsy that works across the three levels of medical health care attention, upon which the National Health System is based. Besides assistant activities, the PPE holds the responsibility to train general and family physicians as well as internists and pediatrician in the diagnosis and treatment of epilepsy, and divulge recent advances in medical and surgical treatment options, and, in many cases, cure of the disease.

Concerted efforts at a global level are needed to improve epilepsy care, and regional surveys concerning the provision of epilepsy care at different levels may be informative and helpful instruments. This is vital because patients identified at early stages may have a proper epilepsy care, avoiding the development or long-term consequences of pharmacoresistant epilepsy.

1.7 Conclusion

Several studies support that in some cases, epilepsy is pharmacoresistant from the onset, even if it appears initially to be benign. At present, there are no biomarkers that allow us to predict confidently whether a newly diagnosed patient will become pharmacoresistant. The knowledge of the mechanisms involved refractoriness, and new strategies in identifying individual genetic variations, might improve our ability to identify patients at risk. In addition, early identification and prompt (and adequate) therapeutic intervention might improve the overall outcome of the disease and maximize quality of life. The future developing of new classes of AEDs with antiepileptogenic properties or focused to block drug transporter effluxes, as well as neuromodulation strategies will change this expectation.

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Chapter 2
Genes Involved in Pharmacoresistant Epilepsy

Iris E. Martínez-Juárez, Laura Elena Hernández-Vanegas, Nayelli Rodríguez y Rodríguez, Jorge Alfredo León-Aldana, and Antonio V. Delgado-Escueta

Abstract This chapter is devoted to resistance to antiepileptic drugs (AEDs) and its genetic mechanisms. There are three general hypothesis proposed for pharmacoresistant epilepsy: (1) Target hypothesis, (2) Drug transporter hypothesis, and the (3) Intrinsic Severity Hypothesis (Gorter and Potschka, Jasper's basic mechanisms of the epilepsies, 4th ed. National Center for Biotechnology Information (USA), Bethesda, MD, 2012).

In diagnosing poor response to treatment, it is also important to separate drug resistance from incorrect diagnosis of epilepsy syndrome for example: (a) Epilepsy caused by mutations in Glucose transporter gene 1 (GLUT1) being treated with valproate (VPA) worsens the seizures in this disease whereas replacement of glucose with ketogenic diet alleviates seizures and the glucose deficit in the central

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nervous system. (Klepper, Epilepsia 49(Suppl 8):46–49, 2008; Klepper et al., Neuropediatrics 40(5):207–210, 2009) (b) Genetic or idiopathic epilepsies such as Childhood Absence Epilepsy (CAE), Juvenile Myoclonic Epilepsy (JME) and Dravet's Syndrome can be aggravated when treated with Na^+ channel blockers (Genton, Brain Dev 22(2):75–80, 2000; Guerrini et al., Epilepsia 39(5):508–512, 1998; Thomas et al., Brain 129(Pt 5):1281–1292, 2006; Martínez-Juárez et al., Brain 129(Pt 5):1269–1280, 2006) and (c) Mitochondrial disorders can also be aggravated by VPA (Finsterer and Zarrouk Mahjoub, Expert Opin Drug Metab Toxicol 8(1):71–79, 2012).

Herein, we describe the three general hypothesis; we also summarize the "difficult to treat" genetic epilepsies.

Keywords Genes • Drug resistant • Epilepsy • Pharmacoresistant • Target hypothesis • Drug transporter

2.1 Target Hypothesis

A drug must have one or more actions on target sites of the brain to exert its therapeutic action. The resistance to drugs is caused by a structural or functional change at the site of action of drugs causing change in the pharmacodynamics of the drug (Sanchez-Alvarez et al. 2007).

The molecular targets refer to the sites that act as ligands of AEDs by which they exert their mechanism of action. These groups of molecules can be divided into two: voltage-gated channels and neurotransmitter receptors associated with neuronal excitation. These alterations at the site of action may be genetically determined or developed as a result of epigenetic and exogenous environmental factors.

2.1.1 Alterations of Sodium (Na^+) Channels

Sodium channels are the main target of most AEDs, which act by blocking their resting phase (tonic block), preventing channel opening and Na^+ conductance selectively. It has been suggested that mutations in Na^+ channels may affect the clinical response to AEDs (Ragsdale and Avoli 1998).

The Na^+ channels are formed by a pore-forming α subunit and two auxiliary associated subunits β. The modification of any of its subunits may play an important role in drug resistance (Sanchez-Alvarez et al. 2007).

The target theory was based on studies in voltage-regulated Na^+ channel in hippocampal neurons with the use of carbamazepine (CBZ). Most antiepileptic drugs act blocking Na^+ channels in their resting phase (tonic block), which prevents channel opening and ion conductance. It is considered that this alteration may be genetic

or acquired. An alteration in the Na^+ channel can modify the sensitivity to one particular drug, but does not necessarily modify all responses to all drugs that share the same mechanism of action in the Na^+ channel.

In genetic drug resistance, it is known that some mutations in genes encoding VGSC subunits may cause refractory or drug-resistant epilepsy (Claes et al. 2001). The *SCN1A* gene is located on chromosome 2q24.3; it has been linked to several diseases, including severe myoclonic epilepsy of infancy or Dravet Syndrome and Generalized Epilepsy with Febrile Seizure Plus (GEFS+). *SCNA1* gene encodes for the α subunit of the VGSC; however, an exact physiological basis of drug resistance related to structural alterations of the subunit of VGSC in Dravet Syndrome has not been demonstrated.

In the *SCN1A* gene, exon 5 encodes for one of the four voltage sensitive channel, the I-S4 domain. This has two versions, one neonatal (N) and another adult (A), which differ in three amino acids. Normally both exons are coexpressed in the adult brain. In studies by Tate et al. (Tate et al. 2005), a G to A polymorphism was identified in the *SCN1A* gene that affects the alternative splicing of exon 5. This polymorphism has been observed in the intron adjacent to exon 5. Apparently this region determines which sequence, either neonatal or adult, is incorporated into each channel. Ancestral allele G allows both exons to be expressed, whereas the mutant allele alters the expression of neonatal exon by interrupting the consensus sequence, reducing the expression of this exon relative to exon 5A. By studying the minimum dose required of two AEDs, namely, CBZ and phenytoin (PHT) prescribed in 706 patients, Tate et al. (2006) found that AA homozygotes had an average dose of CBZ and PHT higher than that of heterozygotes, and the latter also had higher dose than GG homozygotes. A second study by the same authors failed to report this association; therefore, more studies are needed to confirm this (Tate et al. 2006).

An association between polymorphisms of the *SCN2A* gene channel, which codes for the α2 subunit of neuronal Na^+ channel, and resistance to drugs acting on Na^+ channels has also been found (Kwan et al. 2008).

The β subunits function is to modulate the membrane expression of the Na^+ channel. A mutation in the gene encoding for the β1 subunit has been linked with GEFS+. In epilepsy animal models a decreased expression of β1 and β2 subunit has been found. However, this lack of effect of CBZ on Na^+ channels in kindled rats is transient, and the inhibitory effect of CBZ on Na^+ channels is recovered. This effect has not been described in vivo (Gastaldi et al. 1998; Ellerkmann et al. 2003).

In acquired drug resistance, exogenous factors such as the presence of repeated seizures can promote transcriptional or post-transcriptional changes capable of inducing structural changes in VGSC, changes that are enough to induce refractory or drug-resistant epilepsy (Beck 2007). Remy et al. (2003) observed in brain tissue, from patients undergoing surgery for temporal lobe epilepsy with hippocampal sclerosis, a tonic loss of VGSC blockade, in contrast to the CBZ sensitive patient tissue samples.

2.1.2 Alterations of Voltage-Dependent Calcium (Ca^{+}) Channels

The Ca^{+} channels are voltage transmembrane ion channels with an excitatory function. There are at least six types of Ca^{+} channels (T, L, N, P/Q, R) classified in two categories on the basis of the voltage necessary for activation: low threshold and high threshold. The T-type channel is the only low-threshold Ca^{2+} channel current described (Shin et al. 2008).

Each VGCC is formed by an α1 subunit which serves as main pore and sensor in potential change, which is encoded by ten distinct genes, and several accessory subunits identified as β, γ, and α2δ subunits. The VGCC has a highly functional heterogeneity as a result of its wide distribution.

The T-type calcium channels are involved in generating thalamocortical discharges, involved in the pathophysiology of absence seizures. The α1G subunit of T-type calcium channels is related to the generation of spike and wave discharges, while the α1 subunit does not have this physiological property. Therefore, it is possible that an imbalance in the proportion of α1 and α1G subunits in the T Ca^{+} channel reduces the response to anti-absence AEDs such as ethosuximide (ESM), lamotrigine (LTG), VPA, and zonisamide (ZNS). However, there is not experimental evidence yet to confirm this hypothesis (Chioza et al. 2001).

2.1.3 Alterations of Gamma Aminobutyric Acid Channels

Gamma Aminobutyric Acid (GABA) is the major inhibitory neurotransmitter in the adult brain. There are two GABA receptors: $GABA_A$ and $GABA_B$. The $GABA_A$ receptor has specific binding sites for benzodiazepines and barbiturates. $GABA_A$ channels mediate most inhibitory neurotransmission in the brain. Most $GABA_A$ channels are assembled by seven different subfamilies, which are defined by similar sequences: α, β, γ, δ, π, θ, and ρ. Most of the $GABA_A$ channels are formed by α, β and γ subunits. The 60 % of $GABA_A$ subunits are assembled by $\alpha_1\beta_2\gamma_2$ subunits. The $GABA_A$ receptor subtypes are distinguished by their affinity for GABA, channel kinetics and the rate of desensitization, distribution and pharmacology. Changes in the composition of the channel may have implications on its role and sensitivity to AEDs, especially of benzodiazepines (Schmidt and Lösher 2005).

In some animal models of chronic epilepsy there has been a progressive decrease in GABA receptor response to benzodiazepines. In these models hippocampal neuronal loss has been observed and has been associated with recurrent seizures with the subsequent development of acquired resistance, secondary to altered $GABA_A$ receptor. Combined molecular and functional studies indicate that the transcriptional change occurs in the α subunit of the $GABA_A$ receptor, consistent with a decrease in the α_1 subunit expression and an increase in the α_4 (Brooks-Kayal et al. 1998).

2.2 Multidrug Transporter Hypothesis (See Fig. 2.1)

The multidrug transporter hypothesis is based on the modification of the drug pharmacokinetics causing an inadequate concentration of antiepileptic drugs in brain tissue. This phenomenon occurs by an increase in cell membrane proteins which expel the endogenous toxins and xenobiotics, thus preventing penetration the blood–brain barrier and a decreased concentration of the medications at the epileptogenic focus or zones.

Drug resistance that occurs as a result of an increase in membrane proteins has become evident in several diseases such as cancer and epilepsy. Now it is considered a major cause of treatment failure. Resistance may be evident from the start of therapy or after an adequate initial response. There may even be cross-resistance to several drugs as a result of overexpression of membrane transport proteins. This phenomenon is called multidrug resistance (MDR).

These proteins are expressed at the luminal surface of cells that form the blood–brain barrier, glial and endothelial cells, and neurons, thus acting as a "second barrier". This would explain why, despite the use of AEDs at maximum doses, these are not effective in patients with refractory or drug-resistant epilepsy (Dombrowski et al. 2001).

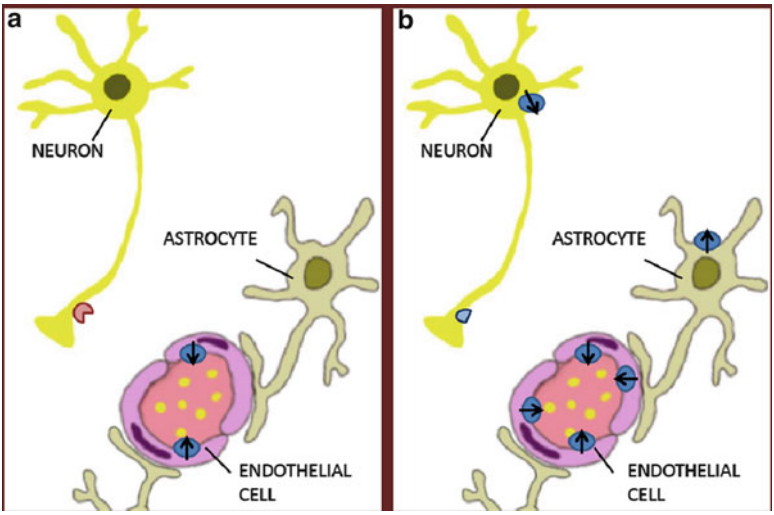

Fig. 2.1 (**a**) Schematic representation of blood brain barrier and normal expression of multidrug transporters. Expression of normal ion channel. (**b**) Epileptogenic-brain tissue with multidrug transporter overexpressed in capillary endothelial cells, astrocytes and neurons. Expression of mutated ion channel

2.2.1 ABC Superfamily (ATP-Binding Cassette Transporters)

The ABC proteins are associated with multiple drug resistance. These are members of an energy-dependent protein superfamily. Several members of the superfamily have been identified and classified into seven subfamilies (ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, and ABCG). Among these, the subfamilies ABCB, ABCC, and ABCG are involved in MDR.

The ABCB1 (*MDR1*) and its protein, P-glycoprotein (P-gp) were the first ones to be described and are the most studied among those associated with resistance to multiple drugs. The *MDR1* gene is located on chromosome 7q21.1. The P-gp is a membrane protein with 1,280 amino acids and sized/weighing 170 kDa. It is found in different tissues with excretory or secretory function, such as liver, pancreas, kidney, intestine, and others. Substances that interact with P-gp are very diverse and have great ability to cross plasma barriers for their characteristics: high molecular weight, positive charge, and high lipophilicity (Kwan and Brodie 2005).

Tishler et al. (1995) suggested that the poor response to AED treatment in patients with refractory epilepsy was secondary to a reduction in the penetration of the drugs to the central nervous system. They observed an increased expression of MDR1 mRNA in 11 of 19 brain tissue of patients undergoing surgery with an increase in P-gp immunostaining. The concentration of PHT in cells was reduced to ¼ in patients expressing MDR1 in contrast to those that did not express it.

The variable expression of P-gp suggests a genetic influence, environmental factors or both. *MDR1* gene is highly polymorphic. More than 50 single nucleotide polymorphism (SNP) along with insertion/deletion polymorphisms have been reported in the *MDR1* (ABCB1) gene that encodes P-gp. Environmental factors could cause the expression of *MDR1* in tissues where it was not previously found; this could explain the fact that symptomatic epilepsies are more resistant to AEDs than idiopathic or genetic epilepsies (Kwan and Brodie 2000, 2005).

An increased expression of multidrug transporters has also been associated with constitutive, genetic or hereditary mechanism. Increased expression of *MDR1* was demonstrated in endothelial cells of the blood–brain barrier up to 130 % in patients with epilepsy, *MRP5* was increased up to 180 % and *MDR2* up to 225 % in comparison to patients without epilepsy (Dombrowski et al. 2001). Siddiqui et al. (2003) reported an association between drug resistance in epilepsy and a polymorphism in the ABCB1 gene. They reported that patients with the CC genotype expressed more P-gp, which was associated with increased drug resistance versus patients with the TT genotype. However, this association has been poorly reproduced and other studies have even documented an inverse association (Tan et al. 2004; Sills et al. 2005).

The association of drug-resistant or refractory epilepsy and some specific etiologies, including mesial temporal sclerosis, cortical dysplasias, and glial tumors has been known now for some time (Semah and Ryvlin 2005). Overexpression of multidrug resistance protein is regionally selective areas, affecting mainly epileptic brain areas. Overexpression of *MRP1* and *MDR1* was demonstrated in perivascular astrocytes of patients with temporal lobe epilepsy due to hippocampal sclerosis.

An aberrant expression in neurons and glial cells was observed as well in patients with dysembryoplastic neuroepithelial tumors and malformation of cortical development (Sisodiya et al. 2002; Sanchez-Alvarez et al. 2007).

It has been proposed that sustained augmentation of glutamate secondary to seizures is the mechanism of acquired increased P-gp expression in cells of the blood–brain barrier. Glutamate acts through NMDA-R, which produces the signal for arachidonic acid, which is then oxidized by the cyclooxygenase 2 (COX-2) producing prostanoids, including prostaglandin E2 (PGE2). PGE2 acts on the Prostaglandin E receptor 1 (EP1-R), which by means of a second messenger system increases transcription of P-gp (Potschka 2012).

Among the drugs transported by P-gp are CBZ, felbamate (FBM), gabapentin (GBP), LTG, phenobarbital (PB), PHT, and topiramate (TPM). Levetiracetam (LVT) and benzodiazepines are not substrates of P-gp in the blood–brain barrier. Kwan et al. (2010) reported a negative relationship between seizure control after epilepsy surgery and P-gp expression on the resected tissue of patients. Various antiepileptic drugs, their mechanisms of action and their corresponding transporters are shown on Table 2.1.

2.3 Intrinsic Severity Hypothesis

A prognostic factor associated with drug-resistant epilepsy is the frequency of seizures at the beginning of the disease, in some cases associated with the number of seizures before the start of treatment (Kwan and Brodie 2000). Even some cases of drug-resistant epilepsies had prolonged episodes of remission in its initial phases. The intrinsic severity hypothesis implies that the frequency of seizures is associated with refractoriness: if seizures are easy to trigger, then seizures will be more difficult to suppress, and the usual dose of the drug will not be enough. There is as yet no evidence that genetic factors directly contribute to the severity of epilepsy in idiopathic (genetic generalized epilepsies, Rogawski and Johnson 2008).

2.4 Genetic Epilepsies “Difficult to Treat”

Pathogenic alterations or mutation in genes and structural abnormalities in chromosomes (deletions, insertions) are responsible of a variety of epilepsies. In some, the possibility of drug-resistant epilepsy is high.

Several mechanisms may be interrelated between genetic disorders and the presence of epilepsy. It is important to distinguish between the susceptibility to generate epilepsy caused by a functional abnormality of a gene, and epilepsy that results from structural or functional abnormalities in a chromosome. Some genetic disorders relate more to certain types of epilepsy but overall any seizure type may be present.

Table 2.2 summarizes some genetic pharmacoresistant epilepsies.

Table 2.1 Antiepileptic drugs, their mechanisms of action and their corresponding transporters

	Mechanism of action							Transporter			
Antiepileptic drug	Blockade of Na^+ channel	Blockade of T-type Ca^+ channel	Blockade of other Ca^+ channels	Blockade of K^+ channel	GABA agonist	Glutamine antagonist	SV2A Action	P-glycoprotein	MRP	MDR	Target
Carbamazepine	+	−	+	−	+	+	−	+	+	+	+
Clobazam/Clonazepam	+	−	+	−	+	−	−	?	?	−	+
Ethosuximide	−	+	−	−	−	−	−	−	?	?	?
Felbatame	+	−	+	−	+	+	−	+	−	+	?
Gabapentin	+	−	+	+	+	+	−	+	?	+	?
Lamotrigine	+	−	+	+	−	+	−	+	−	+	−
Levetiracetam	−	−	+	+	+	−	+	−	−	−	?
Oxcarbazepine	+	−	+	+	−	+	−	+	?	?	?
Phenobarbital/Primidone	+	+	+	−	+	+	−	+	−	−	+
Phenytoin	+	−	+	−	+	−	−	+	+	+	+
Pregabalin	−	−	+	−	−	+	−	?	?	?	?
Topiramate	+	−	+	+	+	+	−	+	?	+	?
Valproate	+	+	+	−	+	+	−	?	+	+	−

+ = effect
− = no effect
? = unknown