Drug Resistance in Epilepsy: Clinical Impact, Potential Mechanisms, and New Innovative Treatment Options

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Drug-Resistant Epilepsy

I. Introduction

Epilepsy is one of the most common and most disabling chronic neurologic disorders (Devinsky et al., 2018). People with epilepsy have recurrent unprovoked (spontaneous) seizures, which can be focal or generalized in nature. Seizures cannot be fully controlled in about a third of people with epilepsy, even though multiple antiseizure drugs (ASDs) may have been employed singly or in various combinations; this phenomenon is drug resistance. In theory, at least four clinical patterns of drug resistance can be observed: 1) de novo (or ab initio) ASD resistance, whereby the patient never enters a useful period of seizure freedom from the onset of the epilepsy; 2) delayed resistance, which is when the patient initially becomes seizure-free but seizures recur and become uncontrollable; 3) a waxing-and-waning (or fluctuating) pattern, which occurs when the epilepsy alternates between being controlled and uncontrolled; or 4) the epilepsy is initially drug-resistant but with time responds to treatment (Schmidt and Löscher, 2005). Long-term outcome studies in newly treated patients with epilepsy suggest that, after failure of two well-tolerated ASD schedules appropriately chosen for the seizure type(s), the chance of success with further drug manipulation becomes progressively less likely (Chen et al., 2018). Thus, drug-resistant

Abstract—Epilepsy is a chronic neurologic disorder that affects over 70 million people worldwide. Despite the availability of over 20 antiseizure drugs (ASDs) for symptomatic treatment of epileptic seizures, about one-third of patients with epilepsy have seizures refractory to pharmacotherapy. Patients with such drug-resistant epilepsy (DRE) have increased risks of premature death, injuries, psychosocial dysfunction, and a reduced quality of life, so development of more effective therapies is an urgent clinical need. However, the various types of epilepsy and seizures and the complex temporal patterns of refractoriness complicate the issue. Furthermore, the underlying mechanisms of DRE are not fully understood, though recent work has begun to shape our understanding more clearly. Experimental models of DRE offer opportunities to discover, characterize, and challenge putative mechanisms of drug resistance. Furthermore, such preclinical models are important in developing therapies that may overcome drug resistance. Here, we will review the current understanding of the molecular, genetic, and structural mechanisms of ASD resistance and discuss how to overcome this problem. Encouragingly, better elucidation of the pathophysiological mechanisms underpinning epilepsies and drug resistance by concerted preclinical and clinical efforts have recently enabled a revised approach to the development of more promising therapies, including numerous potential etiology-specific drugs (“precision medicine”) for severe pediatric (monogenic) epilepsies and novel multitargeted ASDs for acquired partial epilepsies, suggesting that the long hoped-for breakthrough in therapy for as-yet ASD-resistant patients is a feasible goal.

Significance Statement—Drug resistance provides a major challenge in epilepsy management. Here, we will review the current understanding of the molecular, genetic, and structural mechanisms of drug resistance in epilepsy and discuss how the problem might be overcome.
drug resistance as to be operational. An ad hoc Task Force of the In-}

clear understanding, definitions of drug resistance tend to shape our understanding more clearly. In the absence of largely unknown, though recent work has begun to the mechanisms underlying drug resistance remain challenges in epilepsy. Despite many years of research, the mechanisms underlying drug resistance remain largely unknown, though recent work has begun to shape our understanding more clearly. In the absence of clear understanding, definitions of drug resistance tend to be operational. An ad hoc Task Force of the International League Against Epilepsy (ILAE) defined drug resistance as “failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom” and considered this a testable, working hypothesis to be refined with time (Kwan et al., 2010). We can expect that this definition will change, especially in light of the current tension between the clinically observed phenomenon of multidrug resistance (MDR), an understanding of common mechanisms in epileptogenesis and the generation of seizures shared across the epilepsies, large-scale genetic studies that also indicate shared susceptibility across the epilepsies, and the growing data on the separate biologies of the many conditions that together constitute the epilepsies. In due course, we may come to discover that there are many mechanisms or contributors to drug resistance. For now, it is reassuring that categorization according to the definition has proven to be dependable in practice (Mula et al., 2019; Zaccara et al., 2019).

Drug resistance is common. A review of 35 studies showed that the pooled prevalence proportion was 0.30 and pooled incidence proportion was 0.15 (although few studies employ the ILAE definition of drug resistance) (Kalilani et al., 2018). Clinical factors associated with drug resistance were noted to be age at onset, symptomatic epilepsy, abnormal neuroimaging, abnormal electroencephalography, history of mental retardation, neuropsychiatric disorders, prolonged febrile seizure, and status epilepticus (SE) (Kalilani et al., 2018). A single-center 30-year longitudinal cohort study found a similar proportion of people not seizure-free at terminal outcome, identifying as risk factors for this outcome the number of seizures occurring in the year before treatment began, previous recreational drug use, and a family history of epilepsy in first-degree relatives (Chen et al., 2018). Other risk factors have been proposed and models predicting drug resistance generated, but, as with many studies in this area, there is typically a lack of replication and robust evidence for many such suggestions. These factors probably point to underlying causes yet to be established and which might mediate both drug resistance and the epilepsy with its concurrent features, or there may be other causal models. Studies of drug resistance may be complicated by unexplained temporal dynamics; the same person may have prolonged periods of seizure freedom, with intervals during which seizures cannot be controlled (Berg et al., 2003), making classification challenging. It may be better to speak of a spectrum of drug resistance.

The known clinical risk factors for drug resistance in epilepsy also indicate that drug resistance is often associated with comorbidities that increase the overall disease burden for affected individuals; seizure control can ameliorate comorbidities and vice versa (Kezeer et al., 2016). Moreover, drug resistance means that seizures are not controlled and continue to occur; ongoing seizures, especially tonic-clonic seizures, are the best recognized risk factor for sudden unexplained death in epilepsy (Ryvlin et al., 2019) and also increase the risk of direct negative consequences from seizures, including injuries (Mahler et al., 2018) and drowning (Watila et al., 2018).

The personal burden of drug resistance in epilepsy is reflected in its economic impact for health care systems and beyond. There is associated loss of productivity and employment, for example. The socioeconomic burden is sizeable; older studies show that in the United States, the estimated total cost is about $4 billion per annum (Murray et al., 1996), whereas in Europe, drug resistance accounts for a substantial proportion of an estimated total cost of epilepsy, €15.5 billion per annum (Pugliatti et al., 2007).

Overall, therefore, drug resistance is a key challenge in the epilepsies. This review focuses on existing data, the prevailing concepts, and future prospects for tackling drug resistance.

II. Pharmacology of Antiseizure Drugs

ASDs, previously also termed antiepileptic or anticonvulsant drugs, are the main form of symptomatic treatment of people with epilepsy. About 30 ASDs are currently used, of which most were approved over the last 30 years (Fig. 1). Most ASDs were discovered by initial demonstration of their antiseizure activity in simple, classic rodent seizure models, such as the maximal electroshock (MES) and pentylenetetrazol (PTZ) tests, which are highly predictive of clinical efficacy in epilepsy but not in drug-resistant epilepsy (DRE) (Lösch et al., 2013a). ASDs are administered chronically with the intent of preventing the occurrence of epileptic seizures in a person with already diagnosed epilepsy. The symptomatic relief from seizures by ASDs occurs through interactions with a variety of cellular targets (Rogawski et al., 2016; Sills and Rogawski, 2020). The actions on these targets can be categorized...
into four broad groups: 1) modulation of voltage-gated ion channels, including sodium, calcium, and potassium channels; 2) enhancement of GABA-mediated inhibition through effects on GABAA receptors, the GABA transporter 1 (GAT1), the GABA-synthesizing enzyme glutamic acid decarboxylase, or the GABA-metabolizing enzyme GABA transaminase; 3) direct modulation of synaptic release through effects on components of the release machinery, including synaptic vesicle protein (SV) 2A and the α2δ subunit of voltage-gated calcium channels; and 4) inhibition of synaptic excitation mediated by ionotropic glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate receptors (Table 1). The result of the interactions at these diverse targets is to modify the intrinsic excitability properties of neurons or to alter fast inhibitory or excitatory neurotransmission (Rogawski et al., 2016). These actions reduce the probability of seizure occurrence by modifying the bursting properties of neurons and reducing synchronization in localized neuronal ensembles. In addition, ASDs inhibit the spread of abnormal firing to adjacent and distant brain sites.

As shown in Table 1, several ASDs act by more than one mechanism. Most ASDs were discovered by screening, structural alterations of known ASDs, or serendipity, whereas only relatively few, more recent, ASDs were the result of rational, target-based drug discovery (Lösch et al., 2013a). For most ASDs, mechanisms of action were only identified after their discovery or clinical approval. Target-based strategies have been based on previously presumed mechanisms of seizure generation, that is, impaired GABAergic inhibition and increased glutamatergic excitation, resulting in ASDs that either potentiate GABA transmission (such as vigabatrin and tiagabine) or inhibit glutamate receptors (such as perampanel). However, the old reductionistic

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**Fig. 1.** Introduction of antiseizure drugs (ASDs) to the market from 1853 to 2019. Licensing varied from country to country. We give here the year of first licensing or the first mention of clinical use in a country of Europe, the United States, or Japan. We have not included all derivatives of listed ASDs nor ASDs used solely for treatment of status epilepticus. The first generation of ASDs, entering the market from 1857 to 1958, includes potassium bromide, phenobarbital, and a variety of drugs that were mainly derived by modification of the barbiturate structure, including phenytoin, primidone, trimethadione, and ethosuximide. The second-generation ASDs, including carbamazepine, valproate, and the benzodiazepines, which were introduced between 1960 and 1975, differed chemically from the barbiturates. The era of the third-generation ASDs started in the 1980s with “rational” (target-based) developments such as progabide, vigabatrin, and tiagabine, i.e., drugs that were designed to selectively target a mechanism that was thought to be critical for the occurrence of epileptic seizures. The figure also illustrates the impact of preclinical seizure models on ASD development. The use of seizure models for drug screening started with the experiments performed by Merritt and Putnam in the 1930s, who used an electroshock seizure model in cats, leading to the discovery of phenytoin. Subsequently, the electroshock model was adapted to rodents and, together with chemical seizure models, used for drug screening in diverse laboratories, leading to discovery of various additional ASDs. In 1975, the NIH/NINDS ASP was established in the United States as part of a larger Antiepileptic Drug Development program to promote industry interest in ASD development. Since its start, the seizure tests have been performed at a contract facility based at the University of Utah, using three rodent models, i.e., the maximal electroshock seizure (MES) test, the pentylenetetrazole (PTZ) seizure test, and the rotarod test for assessing neurotoxicity. Later, other seizure models were added. The seizure tests were performed on a blinded and confidential basis and at no cost to the ASP participants, thus providing opportunities for researchers from academia and industry in the United States and abroad to submit compounds for screening in a battery of well established rodent seizure models. Approximately 32,000 compounds from more than 600 participants from 38 countries have been screened by this program, and the ASP has contributed to bringing nine currently available ASDs to market since 1990 (Kehne et al., 2017). More recently (2016), the ASP has been renamed Epilepsy Therapy Screening Program (ETSP) with the refocused mission to identify novel agents that will help address the considerable remaining unmet medical needs in epilepsy, particularly ASD-resistant seizures (Kehne et al., 2017). Figure modified from Lösch and Schmidt (2011). For further details, see text and Lösch et al. (2013a).
view that seizures or epilepsy are due to an imbalance between GABAergic inhibition and glutamatergic excitation ignores the complexity of the alterations within these neurotransmitter systems in the brain of a person suffering from epileptic seizures (Lösch et al., 2013a). In view of the various limitations and challenges of previous ASD development, it is mandatory to revisit conventional ASD discovery and development, which will be discussed in section V. A. Development of New Antiseizure Drugs by Using New Drug Screening Paradigms and V. B. Precision Medicine. The more complex experimental models of drug-resistant seizures discussed in the following sections are important in this respect. Furthermore, models of severe pediatric epilepsies that allow the development of etiology-specific drugs have recently become important in ASD development (Baraban and Lösch, 2014; Galanopoulou and Moshé, 2015; Grone and Baraban, 2015; Demarest and Brooks-Kayal, 2018; Griffin et al., 2018). Two recently approved etiology-specific treatments are listed in Table 1.

### III. In Vivo and In Vitro Models of Drug Resistance

#### A. General Aspects

In vivo and in vitro models have significantly contributed to our current understanding of the mechanisms of DRE. As further discussed below, analysis of epilepsy-associated molecular, cellular, and network alterations, and of their association with drug-responsiveness, has resulted in the formulation of different hypotheses. These hypotheses received important support by findings from comparative studies exploring differences between ASD responder and nonresponders in animal models of DRE.

Models of DRE (Table 2) are not only considered key tools for the identification of the pathophysiological mechanisms of therapeutic failure but also for the selection of novel drug candidates targeting difficult-to-treat epilepsy with refractoriness to available ASDs. The importance of these models is underlined by the fact that they became a mainstay in the recently reorganized screening program of the NINDS/National Institutes of Health (NIH), which has been renamed the Epilepsy Therapy Screening Program (ETSP) (Kehne et al., 2017, Lösch, 2017a). One main focus of the ETSP is the pharmacoresistant epilepsy workflow (Fig. 2), which integrates several models with a poor responsiveness to selected or several available ASDs. Such in vivo models belong to the first category of models introduced in the following section.

More elaborate models build on the selection of subgroups of responders and nonresponders from a group of animals with interindividual variation in ASD responsiveness. These models form the second category of in vivo models, which is further introduced below.

One main limitation of the majority of studies, except for some with responder and nonresponder selection, is that they do not consider the ILAE definition of pharmacoresistant epilepsy. Moreover, many studies fail to report and consider effective dose limitations because of adverse effects as, for instance, calculated by the protective index (i.e., the median minimal “neurotoxic” dose, TD50, divided by median effective dose, ED50), which is relevant for conclusions about responsiveness of a paradigm.

#### B. Rodent and Zebrafish Models with Poor Responsiveness to Antiseizure Drugs

A wide variety of preclinical models of seizures or epilepsy is in common use for evaluating novel compounds for antiseizure effects (Lösch, 2016). Some of these models, e.g., the MES and PTZ rodent models, have been discussed in section II (Pharmacology of Antiseizure Drugs); such models are particularly important in identifying novel compounds with antiseizure effects that are not effective in clinical trials.

#### Table 1: Molecular targets of clinically used ASDs

| Molecular target | ASDs that act on target |
|------------------|-------------------------|
| **Voltage-gated ion channels** | Phenobarbital, primidone, stiripentol, benzodiazepines (including diazepam, lorazepam, midazolam and clonazepam), possibly topiramate, felbamate, retigabine (ezogabine) |
| **GABA-mediated inhibition** | GABA receptors |
| **Presynaptic release machinery** | SV2A |
| **Ionotropic glutamate receptors** | AMPA receptor |
| **Carbonic anhydratase inhibition** | Disease-specific mTORC1 signaling |
| **Lyosomal enzyme replacement** | Mixed/unknown |
| **Other** | |

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AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate. A phosphoenolpyruvate carboxykinase is a prodrug for phenytoin. Oxicarbazepine serves largely as a prodrug for licarbazepine, mainly S-licarbazepine. *Elicarbazepine acetate is a prodrug for S-licarbazepine. In patients with epilepsy because of tuberous sclerosis complex (TSC). In patients with epilepsy because of neuronal ceroid lipofuscinosis type 2.
efficacy but are not likely to discover new compounds with higher efficacy against DRE. In the following, we will focus on a subset of preclinical seizure or epilepsy models that exhibit poor responsiveness to standard ASDs.

The mouse 6-Hz model was first described by Brown et al. (1953). In this acute seizure model, a low frequency (6 Hz) and long duration (3 seconds) corneal stimulation triggers a minimal clonic seizure with forelimb clonus and twitching of vibrissae and subsequent stereotyped behavioral patterns (Brown et al., 1953; Barton et al., 2001). Early pharmacological studies demonstrated a response to phenobarbital, phenytoin, and carbamazepine but not to other ASDs. Because of the lack of phenytoin sensitivity, the paradigm was discarded shortly after its first description by Brown et al. (1953).

The fact that a relevant percentage of patients did not respond to standard ASDs including phenytoin and carbamazepine resulted in increasing efforts to develop screening programs aiming to select ASDs with superior efficacy. In this context, the 6-Hz model has been reactivated in 2001 (Barton et al., 2001). Barton et al. (2001) demonstrated that the effects of ASDs depend on the stimulation strength. At a high stimulation strength of 44 mA, only levetiracetam and valproate exerted relevant anticonvulsant effects, whereas a series of other ASDs failed to protect from seizure induction (Barton et al., 2001). Interestingly, an unexpected variation in potency of drugs became evident when different mouse strains were compared in the 6-Hz model. Despite the lack of relevant pharmacokinetic differences, the responsiveness of NMRI (Naval Medical Research Institute) mice to levetiracetam and phenytoin exceeded that of C57Bl/6J mice (Leclercq and Kaminski, 2015). These findings suggest that strain comparisons may provide information about the molecular mechanisms of differences in pharmacodynamics and drug resistance. Based on these data and its ease of use, it was decided to implement the mouse 6-Hz model (in CF-1 [Carworth Farms] mice) as an early screening tool and “high-hurdle” seizure assay in the identification phase of the pharmacoresistant epilepsy workflow of the ETSP (Kehne et al., 2017; Löschner, 2017a) (Fig. 2).

Recently, it has been suggested that a rat 6-Hz paradigm might serve as an alternate model (Metcalf et al., 2017). The availability of a comparable model in a different species can be of particular relevance considering species differences in pharmacodynamics and pharmacokinetics. Moreover, the rat 6-Hz screening model can provide important guidance for dose selection and study design for subsequent drug candidate

### TABLE 2
In vivo models of drug-resistant seizures

| Seizure or epilepsy model | Species | Mode of seizure or epilepsy induction | Chronic model | Development of spontaneous seizures | Selection of responders and nonresponders reported | Throughput |
|--------------------------|---------|--------------------------------------|---------------|-------------------------------------|---------------------------------------------------|------------|
| 6-Hz seizure model       | Mouse   | Transcorneal electrical stimulation  | No            | No                                  | n.a.                                              | High       |
| 6-Hz seizure model       | Rat     | Transcorneal electrical stimulation  | No            | No                                  | n.a.                                              | High       |
| Allylglycine-induced      | Mouse   | Intraperitoneal administration of    | No            | No                                  | n.a.                                              | High       |
| 6-Hz seizure model       | Zebrafish larvae | Bath application of chemoconvulsant  | No            | n.a.                                | Very high                                         |           |
| Lamotrigine-resistant     | Mouse   | Transcorneal electrical stimulation  | Yes           | No                                  | No                                                | Intermediate|
| kindled animals           | Rat     | Repeated electrical stimulation of the amygdala | Yes           | No                                  | No                                                | Intermediate|
| Lamotrigine-resistant     | Mouse   | Repeated electrical stimulation of the amygdala | Yes           | No                                  | No                                                | Intermediate|
| kindled animals           |         |                                      |               |                                     |                                                   |            |
| Intrahippocampal         | Mouse   | Intracerebral injection of kainate   | Yes           | Yes                                 | Yes                                               | Intermediate|
| kainate model            |         |                                      |               |                                     |                                                   |            |
| Post-traumatic seizures  | Rat     | Fluid percussion injury             | Yes           | Yes                                 | No                                                | Low        |
| Cortical dysplasia model | Rat     | In utero exposure to methylazoxymethanol acetate plus kainate exposure | Yes           | No                                  | No                                                | Low        |
| Dravet models            | Mice    | Genetic modulation                  | Yes           | Yes                                 | No                                                | Low        |
| NMDA model of epileptic   | Rat     | Intraperitoneal administration of chemoconvulsant | Yes           | Yes                                 | No                                                | Low        |
| spasms                   | (immature) |                                        | No            | No                                  | High                                              |            |
| Multiple-hit model of     | Rat     | PN3 unilateral i.c.v. doxurubicin and intracortical lipopolysaccharide plus PN5 intraperitoneal p-chlorophenylalanine (→ increases spasm frequency) | Yes           | Yes                                 | No                                                | Low        |
| infantile spasms         |         |                                      |               |                                     |                                                   |            |
| Phenytin-selected         | Rat     | Repeated electrical stimulation of the amygdala | Yes           | No                                  | Yes                                               | Low        |
| kindled animals           |         |                                      |               |                                     |                                                   |            |
| Phenobarbital-selected    | Rat     | Prolonged electrical stimulation of amygdala induction of a status epilepticus | Yes           | Yes                                 | Yes                                               | Low        |
| animals with spontaneous seizures |         |                                      |               |                                     |                                                   |            |
| Canine patients with DRE | Dog     | Natural disease (structural or idiopathic according to IVETF guidelines) | Yes           | Yes                                 | Yes (based on clinical response)                 | Very low   |

i.c.v., intracerebroventricular; IVETF, International Veterinary Epilepsy Task Force; n.a., not applicable; PN, postnatal day.
assessment in chronic rat models. With head nodding, jaw clonus, and forelimb clonus, behavioral seizure patterns proved to be comparable to “psychomotor” seizures described in the mouse model (Metcalf et al., 2017). Metcalf et al. (2017) assessed the efficacy of 16 standard ASDs in this new rat seizure model. At the highest stimulus intensity, i.e., twice the convulsive current that elicits seizures in 97% of the rats, compounds that were effective with a protective index \( \geq 1 \) comprised ezogabine, phenobarbital, and sodium valproate (Metcalf et al., 2017).

As an alternate to electrical seizure induction, the administration of chemoconvulsants has always been a mainstay of seizure model generation. However, the fact that chemoconvulsants induce seizures by interfering with different neurotransmitter systems (e.g., GABA, glutamate, glycine) will necessarily lead to different efficacy of drugs against chemoconvulsant-induced seizures, depending on the mechanism of action of the drug and the chemoconvulsant to be tested. For instance, allylglycine modulates GABAergic neurotransmission by weak inhibition of glutamate decarboxylase, resulting in the induction of electrographic and behavioral seizure events (Leclercq et al., 2015). Leclercq et al. (2015) compared the pharmacoresponsiveness of allylglycine-induced seizures in mice and larval zebrafish. While diazepam and valproate protected against chemically induced seizures, levetiracetam, phenytoin, and topiramate exhibited only a limited protective effect (Leclercq et al., 2015). The fact that the cross-species validation indicated a comparable responsiveness profile of the allylglycine model in zebrafish led the authors to conclude that the zebrafish model might serve as a high-throughput model of treatment-resistant seizures (Leclercq et al., 2015).

In contrast to acute seizure models, chronic rodent models may better reflect the situation following epilepsy manifestation with a multitude of molecular, cellular, and network alterations. Kindling paradigms are based on repeated seizure induction resulting in a progressively increasing seizure severity and duration and a persistent lowering of seizure threshold. Repeated 50-Hz corneal stimulations in mice resulted in a fully kindled state with a good responsiveness to most of the ASDs tested in this paradigm (Matagne and Klitgaard, 1998; Rowley and White, 2010). As these data indicated that the model provides a sensitive screening tool, the paradigm has been integrated in the identification phase of the ETSP (Fig. 2).

In this context, it of interest that Leclercq et al. (2014) addressed the hypothesis that a corneal kindling paradigm with twice daily 6-Hz stimulations (3 seconds, 44 mA) in mice may provide a better tool concerning robustness and responsiveness. The authors reported a kindling progression with seizure activity evolving in severity and duration in response to repeated stimulations, finally resulting in a reproducible induction of generalized seizure activity (Leclercq et al., 2014). A direct comparison with a traditional 50-Hz corneal kindling paradigm revealed that the 6-Hz model proved to be advantageous regarding the persistence of the kindled state (Leclercq et al., 2014). Moreover, the ASDs...
clonazepam, valproate, carbamazepine, and levetiracetam all showed a relatively lower potency in the 6-Hz than the 50-Hz kindling paradigm (Leclercq et al., 2014). Thus, as concluded by the authors, the new 6-Hz corneal kindling model may serve as a new tool for selection of ASDs targeting DRE (Leclercq et al., 2014). Considering the relevance of chronic models for the selection process of new drug candidates, it has been decided to additionally implement further chronic models in the differentiation phase of the ETSP (Fig. 2).

The lamotrigine-resistant kindling model in rats was first described by Postma et al. (2000). In this paradigm, exposure to lamotrigine during kindling acquisition with repeated electrical stimulations of the amygdala results in a fully kindled state characterized by a poor drug responsiveness to lamotrigine and some other ASDs (Postma et al., 2000; Srivastava et al., 2013). Interestingly, ASD resistance in this model proved to extend from lamotrigine to carbamazepine, phenytoin, and topiramate (Postma et al., 2000; Srivastava et al., 2013). In contrast, valproate exerted potent anticonvulsant effects in lamotrigine-resistant kindled rats. More recently, Metcalf et al. (2019) described the dose response effects of numerous ASDs in this model. Five sodium channel blockers (ebsolcarbazepine, lacasamide, lamotrigine, phenytoin, and rufinamide) were either not efficacious or effective only at doses that were not well-tolerated in this model. Similarly, topiramate and levetiracetam were not effective at the doses tested, indicating that pharmacoresistance is not limited to sodium channel blockers. In contrast, compounds targeting either GABA receptors (clobazam, clonazepam, phenobarbital) or GABA-uptake proteins (tiagabine) produced dose-dependent efficacy against convulsive seizures. Similarly, ezogabine and valproate were also highly effective. Compounds acting to modulate Ca\(^{2+}\) channels (ethosuximide, gabapentin) showed differential activity. Taking into account that ASD exposure in combination with seizure elicitation results in the poor responsiveness of lamotrigine-resistant kindled rats, the failure of these animals to respond to selected ASDs may also reflect contingent or cross-tolerance.

More recently, efforts have been made to establish and characterize a comparable model in mice (Koneval et al., 2018). Therefore, the rat protocol was adapted to the 60-Hz corneal-kindling mouse model. Lamotrigine exposure during the kindling phase did not affect kindling progression (Koneval et al., 2018). However, fully kindled mice failed to respond to lamotrigine, carbamazepine, retigabine, and valproate (Koneval et al., 2018). Interestingly, drug resistance in these kindled mice proved to be associated with more pronounced kindling-associated behavioral alterations with increased hyperexcitability and anxiety-associated behavior (Koneval et al., 2018). The paradigm has been suggested as a moderate-throughput platform, which can be applied for early compound selection (Koneval et al., 2018).

Seizures in kindling paradigms are elicited by repeated electrical or chemical stimulation. This approach allows a precise determination of an impact of a test compound on seizure threshold and seizure characteristics at threshold stimulation. Spontaneous seizures only occur following several weeks to months of repeated stimulation, thus rendering it impractical to study drug effects on generation of spontaneous seizures in kindling paradigms. This is in contrast to post-SE models, in which electrical or chemical induction of SE results in the manifestation of epilepsy with spontaneous recurrent seizures. One of the available models is standing out with a high nonconvulsive seizure frequency, which renders the model suitable for rapid screening of ASD candidates. The model is induced by intrahippocampal kainate injection in mice, which results in a limbic SE followed by the development of spontaneous recurrent electrographic and electroclinical seizures. Though generalized convulsive seizures occur occasionally in this model, nonconvulsive electrographic seizures recorded in the Cornu Ammonis sector 1 (CA1) region typically occur multiple times per hour (Lévesque and Avoli, 2013; Löschler, 2016). Pharmacoresponsiveness of the paradigm has been intensively studied. Riban et al. (2002) reported a resistance of electrographic seizures to different ASDs, including phenytoin, carbamazepine, and valproate. Further support for a poor drug responsiveness of focal electrographic seizures in the intrahippocampal kainate model of mesial temporal lobe epilepsy (TLE) came from a recent comprehensive study, which confirmed resistance to phenytoin and carbamazepine (Klein et al., 2015). Although the group observed only moderate effects of valproate and phenytoin, they demonstrated a relevant response to phenobarbital and diazepam (Klein et al., 2015). In line with these studies, Duveau et al. (2016) reported that valproate, carbamazepine, and lamotrigine suppressed electrographic seizures only at high doses that were associated with adverse effects, such as drowsiness and reduced locomotion, whereas levetiracetam, pregabaline, tiagabine, vigabatrin, diazepam, and phenobarbital all suppressed the seizures at doses that were not associated with obvious adverse effects. As described in more detail below, the study by Klein et al. (2015) also revealed pronounced interindividual differences in the effects of ASDs in this paradigm.

Considering the efforts to develop precision medicine approaches tailored to the etiology and pathogenesis of the epilepsy (see section V. B. Precision Medicine), it is of interest to characterize epilepsy models with different constructive validity. There are numerous rodent epilepsy models reflecting different types of etiopathogenesis in human patients. However, the drug responsiveness and predictive validity of the majority of these paradigms has so far been only poorly characterized with a focus on single or selected ASDs.
In a rat fluid percussion model of head injury with post-traumatic development of spontaneous seizures, carisbamate exerted only limited and transient anticonvulsant effects (Eastman et al., 2011). The fact that this finding is rather in line with the compound’s limited efficacy in patients with drug-resistant TLE and in contrast with the broad spectrum anticonvulsant activity in conventional ASD screening tests resulted in the authors’ conclusion that the paradigm may serve as a valuable model of DRE for preclinical drug development (Eastman et al., 2011).

In utero exposure of rats to methylazoxymethanol acetate (MAM) causes cortical molecular and cellular alterations reflecting pathologic hallmarks of cortical dysplasia (Smyth et al., 2002). In hippocampal slices from MAM-exposed rats, 4-aminopyridine-induced epileptiform bursts proved to be resistant to high concentrations of valproate, ethosuximide, and lamotrigine (Smyth et al., 2002). Moreover, seizure activity triggered in MAM-exposed rats by kainate administration failed to respond to valproate (Smyth et al., 2002). In addition, such paradigms can guide the identification of biomarkers of drug resistance [reviewed by Köpp (2014), Köpp et al. (2017)]. Although the models may be applied for assessment of preselected drug candidates, they are not suitable for early drug screening purposes because of their elaborate nature with time-consuming procedures to select responders and nonresponder animals (Löscher, 2016).

The concept of selecting subgroups with divergent responsiveness to standard ASDs has been developed by Löscher et al. (1993), with early studies focused on the rat amygdala-kindling model. Allocation of animals to subgroups was based on repeated testing of maximum tolerated doses of the ASD phenytoin (Löscher, 2016). A retrospective analysis of a series of studies revealed that about 16% of kindled rats exhibit a reproducible response to phenytoin or its prodrug fosphenytoin and can thus be categorized as responders (Löscher, 2016). In contrast, about 61% and 23% show a variable response or no relevant response, respectively (Löscher, 2016). These animals are referred to as variable responders and nonresponders. A failure to respond to an ASD can be related to pharmacokinetic aspects such as rapid metabolization and excretion. Thus, it was of utmost relevance to assess the plasma concentrations at the time of drug testing in responder and nonresponder rats. The fact that plasma concentrations proved to be in a comparable range in all subgroups strongly argued against pharmacokinetic differences (Löscher et al., 1993).

In a series of follow-up studies, the value of the paradigm as a DRE model received further support. Comparative assessment of different standard ASDs in phenytoin responders and nonresponders demonstrated either a reduced efficacy or no effect for all examined ASDs except for levetiracetam (Löscher, 2016).

A successful selection procedure resulting in the identification of responders and nonresponders has also been reported with repeated valproate testing in fully amygdala-kindled rats (Töllner et al., 2011). These findings suggest that the general approach allows studying resistance mechanisms specific for a selected ASD. The promising findings in the kindling paradigm motivated efforts to test selection of ASD responders.
and nonresponders in an electrical post-SE model with development of spontaneous recurrent seizures (Brandt et al., 2004). Rats with spontaneous seizures were selected based on their responsiveness to prolonged phenobarbital exposure. Therefore, drug efficacy was evaluated based on a 2-week video/electroencephalogram seizure-monitoring phase in comparison with pre- and/or postdrug monitoring data (Brandt et al., 2004). Animals, which exhibited an at least 50% or 75% reduction in seizure frequency, were considered phenobarbital responders. Groups of responders and nonresponders did not differ with regard to phenobarbital plasma concentrations or adverse effects of phenobarbital (Brandt et al., 2004). These findings indicated that the interindividual variation in drug responsiveness are neither related to differences in drug distribution to the brain nor to differences in drug metabolism and excretion. The success of the selection procedure proved to be reproducible in internal and external follow-up studies (Löscher, 2016). Interestingly, exposure to other ASDs revealed that the majority of phenobarbital nonresponders also do not respond to phenytoin (Bethmann et al., 2007).

In subsequent experiments, it was shown that ASD responders and nonresponders can also be selected in the rat pilocarpine model of TLE (Bankstahl et al., 2012). In epileptic rats of this model, 50% did not adequately respond to prolonged treatment with phenobarbital, whereas this ASD significantly decreased seizure frequency and severity in another 50% of the animals. Responders and nonresponders did not differ in predrug seizure frequency, drug plasma levels, or hippocampal neurodegeneration, but behavioral differences were observed in anxiety models. As already mentioned above, interindividual variation in the effect of ASDs has also been described in the intrahippocampal kainate model in mice (Klein et al., 2015). ASD responders and nonresponders were identified with exposure of mice to diazepam, levetiracetam, phenobarbital, phenytoin, and valproate (Klein et al., 2015). When using >75% decrease in seizure frequency as criterion for response, the highest number of responders was observed with diazepam and phenobarbital, whereas only few responders were observed with valproate and phenytoin and no responder with carbamazepine. With levetiracetam, 30% and 60% of the epileptic mice responded to doses of 400 and 800 mg/kg, respectively. In the majority of nonresponders to the different ASDs, resistance proved to extend to one or more other ASDs (Klein et al., 2015).

D. Dogs with Spontaneous Seizures

As species differences in pharmacokinetics and pharmacodynamics need to be considered as confounding factors in preclinical drug testing, assessment in different species can be of particular relevance for preclinical efficacy testing. Moreover, the high level of standardization in animal facilities and in the design of preclinical studies in laboratory animals can contribute to a poor reproducibility and limited translation of preclinical findings to clinical application (Richter et al., 2009). Though multilaboratory designs and intentional heterogenization of experimental conditions can improve the accuracy of effect size estimates, robustness, and predictive validity (Richter et al., 2009; Voelkl et al., 2018), testing in veterinary patients has been discussed as an alternate option (Potschka et al., 2013; Löscher, 2016). Epilepsy is a frequent disorder in dogs, with an estimated prevalence of 0.6%–0.75% in the canine population (Berendt et al., 2015). Studies in canine patients with DRE have been discussed for identification of biomarkers and assessment of the efficacy of novel therapeutic strategies (Potschka et al., 2013; Löscher, 2016). Although ethical approval and enrollment of patients is often easier to achieve for veterinary studies compared with clinical trials in humans, the expenditure of time and complexity of a veterinary clinical trial with enrollment according to inclusion and exclusion criteria and with sequential clinical visits and examinations should not be underestimated. Thus, testing of drug candidates in canine DRE is not suitable for screening purposes and can only be considered for promising preselected compounds. Further major limitations need to be taken into account, including the uncertainties related to owner-based monitoring and reporting and the tendency for rapid compound excretion in dogs.

E. In Vitro Models

In line with the principles of the 3R concept (replace, reduce, refine in vivo models), efforts have been made to develop and validate in vitro models of DRE. Respective models can serve as valuable tools to study selected mechanisms of drug resistance. Moreover, the models can be applied for screening purposes during early drug development. However, considering the complexity of drug-resistance mechanisms, limitations of in vitro models need to be kept in mind. In particular, the fact that one can only study part of the epileptic network with preparation of selected brain regions and that the function of the blood-brain barrier (BBB) is compromised as a consequence of the preparation procedure should be taken into account when drawing conclusions. These limitations imply that in vitro models can serve as a screening tool but cannot fully replace in vivo studies.

Interestingly, the responsiveness of chemically induced epileptiform activity in brain slice preparations largely depends on the type of the convulsive trigger. Though exposure to low-Ca2+/high-K+ results in epileptiform discharges responding well to available ASDs, discharges and seizure-like events triggered by prolonged low-Mg2+ or 4-aminopyridine exposure failed to be controlled by ASDs, including phenytoin, carbamazepine, and valproate (Wahab et al., 2010; Kovács and
In addition, the fact that patient-derived iPSCs can be differentiated to generate multiple cell types opens up opportunities to explore the role of both neuronal and non-neuronal cells in DRE (Lybrand et al., 2019). Development of three-dimensional organoid structures from iPSCs provides a basis to study neuronal network function and its modulation (Lybrand et al., 2019).

IV. Current Hypotheses of Mechanisms of Drug Resistance

The mechanisms of drug resistance are likely to be variable and multifactorial according to the underlying cause of DRE and, in theory, to the drug’s site of action (Kwan et al., 2011). As it has occurred in oncology, studying the basis of DRE is important to predict poor response to ASD treatment and hopefully offer new treatment approaches (Margineanu and Klitgaard, 2009; Dalic and Cook, 2016; Tang et al., 2017). However, in view of the likely possibility that several of the mechanisms outlined in the following could act together and possibly even interact in an individual patient or a group of patients (Löschers and Schmidt, 2016), overcoming drug resistance remains a challenge.

Mechanistic hypotheses of drug resistance can be broadly categorized into three groups (Fig. 3), i.e., disease-related mechanisms, drug-related mechanisms, and genetic mechanisms, which may be interlinked. Among the various mechanisms that have been proposed (Fig. 3), the target hypothesis and transporter hypothesis are the most deeply explored theories of ASD resistance, but neither can fully explain the neurobiological basis of this phenomenon (Schmidt and Löschers, 2014). The responsiveness can be further reduced by combining low-Mg2+ with bicuculline exposure, resulting in immediate resistance (Wahab et al., 2010; Kovacs and Heinemann, 2014). Hippocampal and combined entorhinal cortex-hippocamal slice preparations from adult or juvenile rats have been used for these studies.

Comparable approaches using high-K+, low-Mg2+ with or without picrotoxin or bicucullin as a convulsive trigger have also been applied to brain slice preparations from chronic epileptic rodents or from epilepsy patients (Kovacs and Heinemann, 2014; Doeser et al., 2015). Tissue from these preparations is characterized by epilepsy-associated molecular and cellular alterations. Therefore, studies in tissue from epileptic animals and patients may provide more meaningful results. In this respect, a recent study by West et al. (2018) is of interest, in which the effect of 20 ASDs on recurrent epileptiform discharges (REDs) in entorhinal cortex slices derived from epileptic rats of the kainate model of TLE were examined. ASDs targeting sodium and potassium channels (carbamazepine, eslicarbazine, ezogabine, lamotrigine, lacosamide, phenytoin, and rufinamide) attenuated REDs at concentrations near their average therapeutic plasma concentrations. ASDs modulating GABAergic synaptic transmission (clonazepam, midazolam, phenobarbital, stiripentol, tiagabine, and vigabatrin) attenuated REDs only at higher concentrations and, in some cases, prolonged RED durations. ASDs (and other drugs) with other/mixed mechanisms of action (bumetamide, ethosuximide, felbamate, gabapentin, levetiracetam, topiramate, and valproate) and glutamate receptor antagonists weakly or incompletely inhibited RED frequency, increased RED duration, or had no significant effects. The authors suggested that the different sensitivities of REDs to these ASDs may reflect persistent molecular, cellular, and/or network-level changes resulting from disease (West et al., 2018). This in vitro model has been added to the ETSP flowchart for refractory epilepsy (Fig. 2).

Selected mechanisms of DRE can be studied in less complex in vitro or ex vivo setups such as dissociated hippocampal neurons or brain capillaries (e.g., Remy and Buck, 2006; Löschers et al., 2011; Avermay et al., 2013). Moreover, patient-derived induced pluripotent stem cells (iPSCs) provide an elegant basis to develop and analyze precision medicine approaches targeting specific genetic deficiencies. As reviewed recently by Lybrand et al. (2019), different imaging-based, electrophysiological, and molecular assays can be applied to patient-derived iPSCs exploring the impact of drug candidates on cell morphology and viability, neuronal hyperexcitability, and gene expression. Thus, options have become available to apply drug screening in a patient-specific manner. In genetic epilepsies, respective studies will provide valuable information about the responsiveness of cells from patients with different types of mutations.
As suggested by Sisodiya (2003), at least four criteria must be satisfied for a proposed drug-resistance mechanism of epilepsy to be accepted; the mechanism must 1) be detectable in epileptogenic brain tissue, 2) have appropriate functionality, 3) be active in drug resistance (and not be an epiphenomenon), and 4) drug resistance should be affected when the mechanism is overcome. These criteria are based on the famous Koch's postulates, which were originally proposed by Robert Koch in 1890 to establish a causal relationship between a bacterium and a disease.

TABLE 3

| Drug-resistance hypothesis in epilepsy | Detectable in brain (or peripheral) tissues of nonresponders | Appropriate functionality | Active in ASD resistance | Resistance reversed when mechanism is overcome |
|---------------------------------------|----------------------------------------------------------|--------------------------|--------------------------|-----------------------------------------------|
| Target hypothesis                      | + (rat) + (human)                                         | + (rat)                  | + (rat)                  | ? (rat) + (rat)                               |
| Transporter hypothesis                 | + (rat) + (human)                                         | + (rat)                  | + (rat)                  | + (rat)                                      |
| Pharmacokinetic hypothesis             | + (human) + (rat)                                         | ? (human) + (rat)        | ? (human) + (rat)        | ? (human) + (rat) + (human)                  |
| Neural network hypothesis              | + (rat) + (human)                                         | ? (rat) + (human)        | ? (rat) + (human)        | ? (human) + (rat) + (human)                  |
| Intrinsic severity hypothesis          | + (rat) + (human)                                         | + (rat) + (human)        | + (human) + (rat)        | + (rat) + (human) + (human)                  |
| Gene variant hypothesis                | + (rat) + (human)                                         | + (rat) + (human)        | + (human) + (rat)        | + (rat) + (human) + (human)                  |
| Epigenetic hypothesis                  | + (rat/mouse) + (human)                                  | + (rat/mouse) + (human)  | + (human) + (rat/mouse)  | + (human) + (rat/mouse) + (human)            |
| Neuroinflammation/blood-brain barrier  | + (rat/mouse) + (human)                                  | + (rat/mouse) + (human)  | + (human) + (rat/mouse)  | + (human) + (rat/mouse) + (human)            |

*Increased seizure frequency/density compared with ASD responders.

2005; Kwan et al., 2011; Potschka, 2013; Löscher and Schmidt, 2016; Tang et al., 2017). Although, several alternative hypotheses have been proposed (Fig. 3), preclinical and, more importantly, clinical evidence is quite limited (Table 3). One approach to identify potential mechanisms of drug resistance in epilepsy has...
been to develop rat models of TLE in which ASD responders and nonresponders can be selected, followed by mechanistic studies in the two subgroups (Löschler, 1997; Löschler, 2002; Potschka, 2013; Löschler, 2017c). Findings in such animal models and commonalities in patients with drug-resistant TLE are summarized in Fig. 4. In the following sections, the most prominent hypotheses of ASD-resistance mechanisms are discussed. It is interesting to note that some of these hypotheses, e.g., the transporter and gene variant hypotheses, are thought to also be relevant in other brain diseases, including brain cancer and depression (Löschler and Potschka, 2005; O’Brien et al., 2012; Brückl and Uhr, 2016).

A. Alteration of Drug Targets in the Brain

The “target hypothesis” postulates that acquired (epilepsy-induced) alterations to the structure and/or functionality of brain targets of ASDs lead to a reduction in their sensitivity to treatment (Remy and Beck, 2006). To exhibit antiseizure activity, a drug must act on one or more target molecules in the brain. As shown in Table 1, these targets include voltage-dependent ion channels, neurotransmitter receptors, and transporters or metabolic enzymes involved in the release, uptake, and metabolism of neurotransmitters (Rogawski et al., 2016). The target hypothesis is primarily based on studies with carbamazepine on voltage-gated sodium channels in hippocampal neurons. Remy et al. (2003a) showed that the use-dependent block of voltage-dependent Na⁺ channels of dentate granule cells by carbamazepine is completely lost in patients with carbamazepine-resistant TLE in comparison with patients clinically responsive to this ASD. In addition to the loss of use-dependent inhibition of Na⁺ channels by carbamazepine, the fast recovery from inactivation of the fast Na⁺ current was carbamazepine-insensitive in pharmacoresistant patients, whereas recovery was markedly slowed in cells from carbamazepine-responsive patients. Consistent with these data from patients with intractable TLE, Remy et al. (2003a) also showed that use-dependent block of Na⁺ channels by carbamazepine in dentate granule cells is absent in the pilocarpine rat model of TLE. Based on these data, the authors suggested that a loss of Na⁺ channel drug sensitivity could explain the development of DRE, which formed the core of the target hypothesis. In a subsequent study in the rat pilocarpine model of TLE, Remy et al. (2003b) demonstrated that the effects of phenytoin on fast recovery from inactivation of Na⁺ channels of hippocampal granule neurons were significantly reduced, though not as pronounced as observed with carbamazepine, substantiating the concept that reduced pharmacosensitivity of Na⁺ channels may contribute to the development of drug resistance. In contrast to carbamazepine and phenytoin, lamotrigine slowed the time course of recovery from fast inactivation both in epileptic and control rats without significant intergroup difference (Remy et al., 2003b). In the pilocarpine model, a loss of sensitivity of sodium channels to carbamazepine and phenytoin was also found in hippocampal CA1 neurons, although the loss of ASD sensitivity was less pronounced in CA1 neurons than in dentate granule neurons (Schaub et al., 2007). Thus, these results suggested that target mechanisms of drug resistance are cell type- and ASD-specific. More recently, Doeser et al. (2015) reported that eslicarbamepine may possess advantages over conventional Na⁺ channel modulators such as carbamazepine because it maintained activity in chronically epileptic tissue.

Which mechanisms can account for altered sensitivity of Na⁺ channels in CA1 or dentate granule cells in epileptic tissue? One possibility is that the subunit composition of these channels is altered, resulting in channels with lower ASD sensitivity (Remy and Beck, 2006). Several changes in Na⁺ subunit expression have been observed in both human and experimental epilepsy. For instance, in the pilocarpine model of TLE, the accessory β1 and β2 subunits were downregulated, which was suggested to play a role in the altered pharmacosensitivity of Na⁺ channels (Ellerkmann et al., 2003). A critical question in studying target alterations in epilepsy is the relation of changes at the cellular level in vitro to ASD sensitivity in vivo. Although such a correlation has been observed in patients with TLE (Remy et al., 2003a; Jandová et al., 2006), such a correlative analysis has not been performed for the pilocarpine model of TLE, which has been used in most studies. Jeub et al. (2002) used the amygdala kindling model of TLE to study whether ASD responders and nonresponders differ in pharmacological sensitivity of voltage-dependent sodium channels (Jeub et al., 2002). Responders and nonresponders were selected by repeated testing with phenytoin in vivo, followed by evaluation of phenytoin’s in vitro effects on voltage-gated Na⁺ channels of hippocampal CA1 neurons isolated from the kindled subgroups (Fig. 4). The in vivo resistance to phenytoin was not associated with altered tonic block of Na⁺ channels by phenytoin, but recovery from Na⁺ channel inactivation and use-dependent blocking effects were not analyzed in this study (Jeub et al., 2002). Although ASD responders and nonresponder can also be selected from the pilocarpine model (Bankstahl et al., 2012), these subgroups have not yet been analyzed with respect to alterations in ASD sensitivity of Na⁺ channels or other ASD targets.

Apart from voltage-dependent Na⁺ channels, other drug targets, such as GABA_A receptors, may be altered in patients and animal models with intractable epilepsy. Using the rat pilocarpine model of TLE, Brooks-Kayal et al. (1998) demonstrated that expression of GABA_A receptor subunit mRNAs is substantially altered in hippocampal dentate granule cells of pilocarpine-treated rats versus controls. These changes in GABA_A.
receptor subunit expression correlated with profound alterations in receptor function and pharmacology (Coulter, 2000, 2001). In epileptic rats, expression of the α1 and β1 subunits of the GABA_A receptor decreases and expression of α4 and δ subunits increases, leading to an assembly of GABA_A receptors that are strikingly zinc-sensitive. In addition to the enhanced zinc sensitivity, GABA_A receptors from the epileptic hippocampus lose their sensitivity to augmentation by the benzodiazepine site modulator zolpidem. However, none of these studies examined whether ASD-resistant epileptic rats differ from responsive rats in these changes in GABA_A receptor function.

Thus, subsequent studies examined whether ASD-resistant epileptic rats differ from ASD responders in expression and pharmacological sensitivity of GABA_A receptors, using a model in which epilepsy develops after sustained electrical stimulation of the basolateral amygdala, followed by responder/nonresponder selection with phenobarbital (Volk et al., 2006; Bethmann et al., 2008). As shown in Fig. 4, striking differences were found in phenobarbital-resistant epileptic rats when compared with responsive rats in autoradiographic imaging of diazepam-sensitive and diazepam-insensitive GABA_A receptor binding in the dentate gyrus, with greater diazepam-insensitive binding in nonresponders (Volk et al., 2006). To address the hypothesis that diazepam-insensitive receptors contain the α4 and δ subunits that mediate tonic inhibition in the dentate gyrus, the expression of various GABA_A receptor subunits was determined in ASD responders and nonresponders (Bethmann et al., 2008). In nonresponders, decreased expression of several subunits, including α1, β2/3, and γ2, was observed in CA1, CA2, CA3, and dentate gyrus, whereas much less widespread alterations were determined in responders. Furthermore, upregulation of the α4 subunit was observed in CA1 pyramidal cells of nonresponders. Phenobarbital’s antiseizure effect is thought to be primarily related to enhancement of GABA-mediated inhibitory synaptic transmission via modulation of GABA_A receptors (Löschler and Rogawski, 2012). Although the effects of barbiturates on the GABA_A receptor depend largely on the β subunit, their agonist activity is substantially influenced by the α-subunit subtype. The marked decreases in β and α subunits observed in phenobarbital nonresponders are likely to reduce the effect of phenobarbital on GABA_A receptors and thus could be involved in the lack of antiseizure efficacy of phenobarbital in these animals. Profound alterations in GABA_A receptor subtype expression have also been reported in adult patients with ASD-resistant TLE and pediatric epilepsy patients undergoing epilepsy surgery (Loup et al., 2000; Pirker et al., 2003; Porter et al., 2005).

Further evidence that changes in GABA_A receptors can lead to ASD resistance came from studies in the pilocarpine model, showing that internalization of GABA_A receptors, i.e., trafficking of these receptors from the synaptic membrane to submembranous compartments, causes a decrease in the number of functional postsynaptic GABA_A receptors that could explain the pharmacoresistance to ASDs that act via the GABA_A receptor (Goodkin et al., 2005; Naylor et al., 2005). Apart from alterations in GABA_A receptor subunit expression and receptor trafficking, a third potential mechanism to explain loss of pharmacological sensitivity of these receptors is a shift from adult hyperpolarizing (inhibitory) to neonatal depolarizing (excitatory) GABA_A receptors during epileptogenesis (Ben-Ari et al., 2012). Such a shift in GABAergic response polarity from hyperpolarizing to depolarizing was first described in human epileptic neurons recorded in the subiculum of hippocampal slices obtained from resections in adult patients suffering from mesial TLE (Cohen et al., 2002). This shift is thought to be a result of increased intraneuronal Cl^- levels caused by increased neuronal expression of NKCC1, an inwardly directed Na^+ K^+ 2Cl^- cotransporter that facilitates the accumulation of intracellular Cl^-, and downregulation of KCC2, an outwardly directed K^+ Cl^- cotransporter (Ben-Ari et al., 2012). Upregulation of NKCC1 and downregulation of KCC2 in the hippocampus have been described both in patients with TLE and the kindling and pilocarpine models of TLE (Löschler et al., 2013b). Furthermore, the GABA shift is thought to be involved in the resistance of neonatal seizures to GABA mimetic ASDs such as phenobarbital and benzodiazepines (Puskarjov et al., 2014). Inhibition of NKCC1 by bumetanide has been proposed as a strategy for overcoming ASD resistance in neonates (Kahle et al., 2008); however, a clinical trial with bumetanide as an add-on to phenobarbital for treatment of neonatal seizures did not support this proposal (Pressler et al., 2015).

As a proof-of-principle for the target hypothesis, it will be important to demonstrate that ASD-resistant subgroups of patients differ from ASD-responsive subgroups in their ASD-target sensitivity. Such a proof-of-principle is difficult to obtain because, in contrast to patients with intractable epilepsy, patients responding to ASDs in general do not undergo surgical treatment of their epilepsy. Although Remy et al. (2003a) obtained surgical “reference” specimens from two patients who responded well to treatment with carbamazepine for comparison with 10 patients with carbamazepine-resistant TLE, differences in age, gender, history of epilepsy and ASD treatment, and various other variables could bias this comparison. As illustrated by our previous studies, animal models of TLE that permit the selection of age-matched ASD responders and nonresponders could be useful in further evaluating the target hypothesis. Furthermore, mechanisms leading to target changes (transcriptional or posttranslational or both, role of seizures and cell loss, etc.) need to be further explored. Although the target hypothesis is a biologically
plausible theory to explain drug resistance, the fact that most drug-resistant patients are resistant to several ASDs acting on different therapeutic targets undermines the general utility of the target hypothesis and instead supports the existence of a mechanism nonspecific to individual ASDs (Tang et al., 2017).

In summary, clinical evidence for the target hypothesis is restricted to loss of carbamazepine’s effect on voltage-dependent Na+ channels in carbamazepine-resistant patients, whereas such data are lacking for ASD nonresponders in animal models. Instead, alterations in the expression and pharmacological sensitivity of GABA A receptors have been described in ASD-resistant epileptic rats, which would be in line with the target hypothesis. Thus, although at first glance the target hypothesis appears scientifically plausible, the available evidence is quite limited (Table 3).

B. Alteration of Drug Uptake into the Brain

The “transporter hypothesis” suggests resistance is due to inadequate penetration of ASDs across the BBB because of increased expression of multidrug efflux transporters (Lösch er and Potschka, 2005; Tang et al., 2017). Multidrug resistance (MDR) because of efflux transporters, which was first demonstrated in chemotherapy-resistant cancer, has attracted significant interest as a putative mechanism to explain resistance to multiple ASDs, irrespective of their mechanism of action (Tang et al., 2017). However, since it was first postulated by Tishler et al. (1995), the transporter hypothesis has also been a matter of debate.

The role of multidrug efflux transporters, such as P-glycoprotein (Pgp), at the BBB in restricting brain entry of multiple drugs is widely accepted (Giacomini et al., 2010; Neuwelt et al., 2011; Abbott, 2013; König et al., 2013; Saunders et al., 2016). Indeed, such transporters are involved in the emergence of MDR, which plays an important role in the failure of treatments of brain tumors, brain infections, and several other brain disorders (Lösch er and Potschka, 2005; Mahringer and Fricker, 2016). Pgp, the product of the human multidrug-resistance-1 (MDR1; ABCB1) gene is of particular clinical relevance in that this transporter has a broad substrate specificity (which led to the term “multidrug transporter”), including a variety of structurally divergent drugs in clinical use today (König et al., 2013; Saunders et al., 2016).

In the BBB, multidrug transporters such as Pgp, members of the multidrug resistance protein (MRP) family and breast cancer–related protein (BCRP) are located in brain capillary endothelial cells that form the BBB and act in concert to reduce the brain penetration of many drugs to protect the brain from intoxication by lipophilic xenobiotics that otherwise would cross the BBB by passive diffusion (König et al., 2013).

Using brain specimens removed from patients during surgery for intractable epilepsy, Tishler et al. (1995) were the first to report that brain expression of MDR1, which encodes Pgp in humans, is markedly increased in the majority of patients. Based on their findings, Tishler et al. (1995) proposed that increased Pgp expression may play a clinically significant role by limiting access of ASDs to the brain parenchyma, thereby contributing to the refractoriness of seizures. Following the report by Tishler et al. (1995), the finding of MDR1 (or Pgp protein) overexpression in epileptogenic brain tissue of patients with DRE was confirmed by several other groups (Tang et al., 2017). Furthermore, it was shown that in addition to Pgp, several MRPs are overexpressed in brain capillary endothelial cells and/or astrocytes of pharmacoresistant patients, whereas data on BCRP were inconsistent (Tang et al., 2017). In some of these studies, the overexpression of drug efflux transporters in astrocytes appeared most marked around blood vessels. In view of data indicating that the endothelial barrier function of the BBB is transiently and locally disrupted during seizures (van Vliet et al., 2015), overexpression of multidrug transporters in astroglial end-feet covering the blood vessels may represent a “second barrier” under these conditions. As a consequence, overexpressed multidrug transporters may lower the extracellular concentration of ASDs in the vicinity of the epileptogenic pathology and thereby render the epilepsy caused by these pathologies resistant to ASD treatment.

An open question is whether the overexpression of Pgp and MRPs in epileptogenic brain tissue of patients with intractable epilepsy is intrinsic (constitutive) or acquired, i.e., a consequence of epilepsy, of uncontrolled seizures, of chronic treatment with ASDs, or combinations of these factors. Because treatment-resistant patients have no fewer neurotoxic side effects under ASD treatment as patients who are controlled by ASDs, the overexpression of drug transporters in treatment-resistant patients is most likely restricted to the epileptic focus or circuit. This has been substantiated by Sisodiya et al. (2002), in that overexpression of Pgp and MRP1 was found in epileptogenic tissue but not in adjacent normal tissue of the same patients. Furthermore, the same group studied the expression of Pgp in postmortem brains from patients with drug-sensitive or drug-resistant chronic epilepsy and nonepileptic controls (Liu et al., 2012). They found that: 1) there is a highly localized overexpression of Pgp in the epileptogenic hippocampus of patients with DRE; 2) this overexpression appears specific to Pgp and does not affect other transporters; and 3) Pgp is expressed on the vascular endothelium and end-feet of vascular glia (forming a “double cuff”) in drug-resistant epileptic cases but not in drug-sensitive patients or postmortem controls. In another study using positron imaging tomography (PET) with the Pgp substrate (R)-[11C]verapamil to study the functionality of Pgp in the brain of patients with ASD-resistant and -responsive epilepsy.
and controls, data demonstrated higher Pgp functionality in epileptogenic brain regions of drug-resistant patients (Feldmann et al., 2013), which is consistent with the Pgp expression data reported by Liu et al. (2012). Interestingly, in the ASD-resistant patients, higher seizure frequency was significantly correlated with higher Pgp activity in the hippocampus, maybe suggesting that increased Pgp plays a role in the “intrinsic severity hypothesis” of Rogawski and Johnson (2008), which will be discussed below. In a subsequent PET study, it was shown that the increased Pgp function in the temporal lobe of patients with drug-resistant TLE was reduced after epilepsy surgery in patients responding to surgery compared with nonoptimal surgery outcome (Bauer et al., 2014).

In animal models of TLE, such as the kindling and kainate models, a transient overexpression of Pgp was found in capillary endothelial cells, astroglia, and neurons of limbic brain regions following seizures (Löscher and Potschka, 2005; Tang et al., 2017), indicating that seizures themselves can induce overexpression of drug transporters. This could explain that one of the major predictors of drug resistance is high seizure frequency (or density) prior to initiation of treatment (Regesta and Tanganelli, 1999; Hitiris et al., 2007; Dalic and Cook, 2016). However, constitutive rather than induced or acquired overexpression of multidrug transporters has been reported in patients with malformations of cortical development (Sisodiya et al., 1999). In addition to intrinsic or acquired overexpression of multidrug transporters in the BBB of patients with epilepsy, polymorphisms in transporter genes may play a role in drug resistance, which will be discussed in a separate section on the gene variant hypothesis.

In view of the emerging evidence that multidrug transporters are overexpressed in epileptogenic brain tissue, particularly in capillary endothelial cells and astrocytes contributing to BBB permeability, it is obviously important to know whether ASDs are substrates for these transporters. The first indication that ASDs are substrates for Pgp came from experiments of Tishler et al. (1995), who found that intracellular phenytoin levels in a MDRI-expressing neuroectodermal cell line were only one-fourth that in MDRI-negative cells, suggesting that human Pgp significantly contributes to cell export of phenytoin. Various subsequent in vitro and in vivo studies have indicated that many ASDs are substrates of Pgp, whereas only few are substrates for MRPs or BCRP (Löschner et al., 2011; Zhang et al., 2012; Tang et al., 2017).

However, data on transport of ASDs by BBB efflux transporters such as Pgp are controversial, which is a key reason why the transporter hypothesis is a matter of debate (Löschner et al., 2011). The difficulty in determining which ASDs are substrates of human Pgp is mainly a consequence of the fact that ASDs are highly permeant (lipophilic and small) compounds, which are not easily identified as Pgp substrates in in vitro models of the BBB, such as monolayer (Transwell) efflux assays. By using a modified assay (concentration equilibrium transport assay), which minimizes the influence of high transcellular permeability, two groups have independently demonstrated that most major ASDs are transported by human Pgp (Löschner et al., 2011; Zhang et al., 2012). Importantly, it was demonstrated in these studies that Pgp-mediated transport highly depends on the ASD concentration and may not be identified if concentrations below or above the therapeutic range are used. In line with these findings, by using an in vitro BBB model with human capillary endothelial cells from either normal brain or drug-resistant epileptic brain, Cucullo et al. (2007) reported a dramatically reduced permeability of phenytoin across the in vitro BBB formed from endothelial cells of patients with DRE, which could be partially counteracted by the selective Pgp inhibitor tariquidar, substantiating transport of ASDs by human Pgp.

By using either mdr1a/b knockout mice or brain microdialysis with Pgp inhibitors, Pgp-mediated efflux of ASDs at the BBB was also demonstrated in vivo (Löschner et al., 2011). In view of the overexpressed efflux transporters found in epileptogenic brain tissue of patients with pharmacoresistant epilepsy and animal models of epilepsy, another important question was whether this overexpression indeed lowers brain uptake of ASDs, as suggested. By using the intraperitoneal kainate model of TLE in mice, Rizzi et al. (2002) demonstrated that the significant increase in mdr1 mRNA expression measured by reverse-transcription polymerase chain reaction in the hippocampus after kainate-induced seizures was associated with a 30% decrease in the brain/plasma ratio of phenytoin, thus substantiating the view that Pgp alterations significantly affect concentrations of ASDs in the brain. Comparing phenytoin brain/plasma ratio in mdr1 knockout mice with this ratio in mice with kainate-induced overexpression of Pgp indicated that Pgp can affect up to about 70% of phenytoin brain uptake (Löschner et al., 2011). In epileptic rats, van Vliet et al. (2007) reported decreased brain levels of phenytoin that were restricted to brain regions with increased expression of Pgp, which could be counteracted by inhibiting Pgp. In patients with oxcarbazepine (OXC)-resistant epilepsy, the brain tissue expression of ABCB1 mRNA was found to be inversely correlated with brain levels of 10,11-dihydro-10-hydroxy-5H-dibenzo[b,f]azepine-5-carboxamide, the active metabolite of OXC, indicating that Pgp may play a role in the pharmacoresistance to OXC by causing insufficient concentrations of its active metabolite at neuronal targets (Marchi et al., 2005).

A further important step in the evaluation of the multidrug transporter hypothesis of DRE was the
demonstration that rats that do not respond to ASDs exhibit significantly higher expression levels of Pgp in brain capillary endothelial cells of the BBB than ASD-responsive rats (Potschka et al., 2004; Volk and Löscher, 2005). This was demonstrated for two different rat models of TLE: phenytoin-resistant kindled rats and phenobarbital-resistant rats with spontaneous recurrent seizures (Fig. 4).

If drug resistance is due to increased transporter expression and functionality, it should be possible to demonstrate that the inhibition or avoidance of the resistance-mediating mechanism counteracts drug resistance in ASD nonresponders. This was approached by coadministration of phenobarbital (PB) and the Pgp inhibitor tariquidar in PB nonresponders. Three different doses of tariquidar (10, 15, and 20 mg/kg) were used, demonstrating a dose-dependent effect. Data are from Brandt et al. (2004), Volk and Löscher (2005), Brandt et al. (2006), Bethmann et al. (2007), and Brandt and Löscher (2014).

Fig. 5. Selection and characterization of ASD responders and nonresponders by phenobarbital in a rat model of TLE in which spontaneous recurrent seizures (SRS) develop following sustained electrical stimulation of the basolateral amygdala. (A) Schematic illustration of selection of drug-resistant and drug-responsive epileptic rats by prolonged administration of phenobarbital. (B) Effect of phenobarbital (PB) on SRS. About 5 months after the electrically induced SE, SRS were recorded over a period of 2 weeks before onset of PB treatment (predrug control), followed by drug treatment of 2 weeks and then a 2-week postdrug control period. All data are shown as means ± S.E.M. The graphs in (B) show 1) average seizure data from 33 epileptic rats from three prospective experiments, 2) respective data from 20 responders, 3) data from 13 nonresponders, and 4) average plasma concentration of PB from the blood samples taken at the end of the treatment period. The shaded area indicates the therapeutic plasma concentration range of PB. In the responder group, PB significantly suppressed SRS compared with the pre- and postdrug periods (*P < 0.001). Note the higher average frequency of SRS in nonresponders versus responders. (C) Pgp expression in brain capillary endothelial cells of responders and nonresponders. Significant differences are indicated by asterisk (*P < 0.05). (D) Coadministration of PB and the Pgp inhibitor tariquidar lead to a significant (*P < 0.05) suppression of SRS in PB nonresponders. Three different doses of tariquidar (10, 15, and 20 mg/kg) were used, demonstrating a dose-dependent effect. Data are from Brandt et al. (2004), Volk and Löscher (2005), Brandt et al. (2006), Bethmann et al. (2007), and Brandt and Löscher (2014).
overexpression at the BBB (Tang et al., 2017). These phenytoin-resistant rats have an increased expression of Pgp in focal epileptogenic brain tissue (Potschka et al., 2004). All ASDs that were substrates for Pgp showed absent or low antiseizure efficacy in phenytoin nonresponders (Löschker and Potschka, 2002). More importantly, we examined whether Pgp inhibitors counteract multidrug resistance. For this purpose, we used epileptic rats that were either responsive or resistant to phenobarbital (Brandt et al., 2006). As shown in Fig. 5, in resistant animals, coadministration of the selective Pgp inhibitor tariquidar together with phenobarbital reversed resistance, leading to seizure control in animals that were resistant to phenobarbital alone. That such a strategy may be relevant in patients with epilepsy is suggested by several recent promising reports in whom the nonselective Pgp inhibitor verapamil was added to the ASD regimen (section V. D. Targeting of Transporter Function and Expression). In addition to inhibiting Pgp, the recently clarified signaling cascade that explains seizure-induced overexpression of Pgp raises the possibility of direct manipulation of this overexpression, e.g., by inhibiting N-methyl-D-aspartate (NMDA) glutamate receptors or cyclooxygenase (COX) 2 (Potshcka, 2010). Indeed, both NMDA antagonists and COX-2 inhibitors, such as celecoxib, have been shown to prevent the seizure-induced increase in Pgp expression and functionality, and celecoxib reversed ASD-resistance in rats (Potschka, 2010). Details on the link between neuroinflammation and Pgp overexpression are provided in the section on neuroinflammation below.

In summary, the available clinical evidence for the transporter hypothesis includes numerous reports that Pgp and other efflux transporters are overexpressed in epileptogenic brain regions of patients with intractable epilepsy and that various ASDs are transported by human Pgp. The experimental evidence fulfills all of the criteria described in Table 3; i.e., ASD nonresponders exhibit higher expression of Pgp at the BBB than responders in rat models, various ASDs are transported by rodent Pgp, overexpression of Pgp is associated with lower brain levels of ASDs in rodents, and, most importantly, inhibition of Pgp by tariquidar counteracts resistance to ASDs in a rat model of TLE. However, at present, aspects of the transporter hypothesis are still controversial, and further research is needed to determine the clinical relevance of efflux transporter overexpression at the BBB (Tang et al., 2017).

C. Alterations of Pharmacokinetics in the Periphery

The “pharmacokinetic hypothesis” proposes that overexpression of efflux transporters in peripheral organs such as intestine, liver, and kidney decreases ASD plasma levels in DRE patients, thereby reducing the amount of ASD available to cross the BBB (Tang et al., 2017). Indeed, alterations in expression and functionality of multidrug transporters in patients with intractable epilepsy need not necessarily be restricted to the brain but could also occur in other tissues, such as the small intestine, where Pgp is thought to form a barrier against entrance of drugs from the intestinal lumen into the bloodstream, thereby limiting their oral bioavailability (Fromm, 2004). In this respect, it is interesting to note that Lazarowski et al. (2007) have reported persistent subtherapeutic plasma levels of ASDs (including phenytoin and phenobarbital) despite aggressive and continuous ASD administration in patients with DRE that was associated with overexpression of MDR1. However, the pharmacokinetic hypothesis suggested by Lazarowski et al. (2007) is based on only two case studies, and it is unclear at this point if their observation is limited to these cases or is a wider phenomenon. Support for the pharmacokinetic hypothesis comes from studies showing persistently low ASD levels in patients with DRE, which, however, relate to drug metabolizing enzymes rather than to efflux transporters such as Pgp (Tang et al., 2017). In this respect, it is important to note that cytochrome P450 metabolic enzymes not only occur in the periphery but also in the brain parenchyma and endothelial cells of the BBB, thus adding to the barrier function (Ghosh et al., 2011). Changes in the cerebrovascular hemodynamic conditions can affect expression of cytochrome P450 enzymes and multidrug-resistance transporters, leading to a synergistic role in drug resistance (Ghosh et al., 2011).

Animal studies do not support the pharmacokinetic hypothesis. In these studies, ASD plasma concentrations were not different between ASD responders and nonresponders (Löschker, 2017c) (Fig. 5B), although, in rare instances, single ASD-resistant rats exhibited lower plasma levels, necessitating increased ASD dosing. However, these experiments were done with intraperitoneal not oral administration. Overall, the evidence for the pharmacokinetic hypothesis of Lazarowski et al. (2007) is quite limited (Table 3).

D. Neural Network Hypothesis

The “neural network hypothesis,” first proposed by Fang et al. (2011), suggests that epilepsy-associated structural alterations (including neurodegeneration, axonal sprouting, synaptic reorganization, neurogenesis, and gliosis) contribute to the formation of an abnormal neural network, thereby reducing ASD efficacy. Hippocampal sclerosis is a common finding in patients with pharmacoresistant TLE, so it had often been suggested that hippocampal sclerosis plays a causal role in the mechanisms underlying ASD resistance long before the neural network hypothesis was proposed (Schmidt and Löschker, 2005). Indeed, following resection of the affected temporal lobe, ~60% of patients with formerly drug-resistant TLE become
seizure free with continued medical treatment (Wiebe and Jette, 2012), thus providing a proof-of-concept of the neural network hypothesis. Furthermore, malformations in cortical development are often associated with pharmacoresistant epilepsy (Barkovich et al., 2015). However, the major weakness of this hypothesis is that alterations in the neural network do not lead to refractoriness in all epilepsy patients (Tang et al., 2017). Furthermore, not all ASD-resistant patients become ASD responders following epilepsy surgery, although this may be due, at least in part, to incomplete resection of affected tissue such as the piriform cortex (Galovic et al., 2019).

To address directly whether hippocampal sclerosis is causally related to ASD resistance, we compared hippocampal damage in epileptic rats that either responded or did not respond to ASD treatment (Volk et al., 2006; Bethmann et al., 2008). As shown in Fig. 4, in most (>90%) nonresponders of this model, we determined a significant loss of neurons in the CA1, CA3c/CA4, and dentate hilus, whereas most (>90%) responders did not differ in hippocampal morphology from nonepileptic controls, which was a highly significant difference. Based on these observations, it appears that the functional alterations in hippocampal pyramidal neurons and in the dentate gyrus developing as a response to hilar cell loss are critically involved in the mechanisms underlying the refractoriness of seizures to ASD treatment. Such structural and functional network changes will also affect ASD targets, as discussed in the section on target alterations.

In summary, clinical evidence for the neural network hypothesis is convincing in that, in TLE, hippocampal sclerosis is often associated with drug resistance and resection of the affected tissue often reverses resistance (Table 3). Experimental evidence includes the finding that hippocampal damage is associated with ASD resistance in a rat model of pharmacoresistant epilepsy.

E. Intrinsic Severity Hypothesis

Rogawski and Johnson (2008) proposed that ASD resistance is not due to specific pharmacoresistance factors but rather that epilepsy severity exists on a continuum and that more severe epilepsies are more difficult to treat. This “intrinsic severity hypothesis” was subsequently updated by Rogawski (2013), who postulates that pharmacoresistance is an inherent property of the epilepsy related to disease severity. Seizure frequency is one marker of severity, and high seizure frequency or density before onset of ASD therapy is the single most important factor associated with a low chance of longterm remission of seizures on treatment (Rogawski and Johnson, 2008). Interestingly, as shown in Fig. 4, similar observations were made in the rat model of basolateral amygdala stimulation that allows differentiating rats with different ASD responses (Löschler and Brandt, 2010). Epileptic rats that responded to treatment exhibited a relatively low, uniform seizure frequency; none of the responders had a high seizure frequency. In contrast, many nonresponders exhibited very high seizure frequencies. However, there were some nonresponders who also exhibited low seizure frequencies comparable to those of ASD-responsive animals. As in the clinical situation (Sillanpää and Schmidt, 2009), although high seizure frequency is a reliable predictor of pharmacoresistance, it is clearly not the only determinant of pharmacoresistance. Rogawski (2013) mentioned other measures of epilepsy severity, such as the extent of structural lesions (e.g., hippocampal damage) or the behavioral phenotype, that also predict ASD resistance.

Although the intrinsic severity hypothesis appears biologically plausible, it does not adequately apply to epilepsy types that demonstrate a fluctuating or evolving pattern of ASD resistance (Löschler and Schmidt, 2016). In addition, there is little evidence supporting a direct mechanistic link between the severity of epilepsy and ASD response (Schmidt and Löschler, 2009). Thus, overall, the evidence for this hypothesis is quite limited (Table 3).

F. Gene Variant Hypothesis

A strong candidate mechanism for the target hypothesis of drug resistance, and a plausible reason for drug resistance on first principles, is that there is endogenous variation in people with epilepsy that, through whatever mechanism, reduces the chances of ASDs controlling seizures. The best understood source of internal variation in people with epilepsy is the genome. Variation in the genome may be classified as rare or common. There are around 10,000,000 single nucleotide polymorphisms (SNPs) in the human genome, nucleotides that differ between people, with a minor allele frequency of >5%; this class of variant is the best studied type of common variation in the genome. Such variation can be neutral or can affect the function of the gene in some way. Thus, for example, an SNP in the gene SCN1A might affect conformation, binding or other activity of the encoded protein, or stability of the transcribed mRNA, leading eventually to reduced response to sodium channel–blocking ASDs. Studies of common variation may focus on a single SNP, SNPs in a gene or set of genes, or across the entire genome and typically seek to test the hypothesis that there is an association between the distribution of the genetic variation and the phenotype in question. There have been many association studies published in epilepsy, and a good proportion address aspects of drug resistance. The difficulty with most is the study design; the fundamental hypothesis is based on weak evidence, sample sizes are small, and there are methodological failings and a lack of replication, among other issues (for a review, see Gambardella et al., 2017; Balestrini and Sisodiya, 2018). These issues limit the value of many
association studies undertaken in epilepsy, including those addressing the phenotype of drug resistance.

The Epilepsy Genetic Association Database (epiGAD; www.epigad.org) is an online repository of data related to association studies in the epilepsies and is supported by the ILAE Genetics Commission. It is regularly updated and is a good source of information, obviating the need to reproduce a rapidly out-of-date list of such studies here. Moreover, so far, there have been no robust, generally accepted genetic associations for drug resistance across the spectrum of the epilepsies to support the model of broad, syndrome-independent mechanisms of drug resistance driven by genetic variation. Published studies are, on the whole, of limited size, differing patient groups and definitions, and selected SNPs and are lacking replication. Genes of particular interest in this area have included SCN1A and ABCB1, encoding Pgp (Lösch et al., 2009; Orlandi et al., 2018). Evaluation of the latter is instructive. Deriving justification from work in cancer, animal models, and tissue studies in human epileptogenic brain tissue, the first association of ABCB1 was a single SNP study in 2003 (Siddiqui et al., 2003) of a cohort considered small by today’s standards. Many further single and multiple SNP-based associations were published of different patient groups, varying case and control definitions, and, generally, small sample sizes. Some studies supported the original association, whereas others did not; meta-analyses of many such studies remain equivocal (Haerian et al., 2011; Sun et al., 2014; Chouchi et al., 2017), but it is still the case that the correctly designed, adequately controlled, and credibly powered study has not yet been undertaken. There are no common genetic variants associated with drug resistance currently in use to predict this phenotype. However, the search should continue; in the absence of selection pressure acting against such variants (because exposure of the human species to medication has been short in comparison with the duration of exposure to other environmental agents), there is no a priori reason to think that common genetic variation does not contribute to drug resistance.

Consideration of the contribution of rare variation to drug resistance opens up new problems and questions. Until recent large-scale efforts (Epi25 Collaborative. Electronic address: s.berkovic@unimelb.edu.au; Epi25 Collaborative, 2019), sample sizes from most studies were simply too small to even consider rare variant association studies, especially as most such studies were skewed toward people with epilepsies at the more severe end of the spectrum. But, in addition, rare variation has more typically been considered in the context of genetic causation of the epilepsy itself rather than a facet of the epilepsy such as drug resistance, but this raises interesting questions. For example, a hypothetical patient may have epilepsy because of a rare deleterious variant in a gene encoding a neuropeptide receptor; currently used ASDs do not target this system, as far as we know, and their actions on the downstream pathophysiological processes shared across the epilepsies, such as disordered inhibition, may not be sufficiently potent to prevent seizures from occurring. In this scenario, the receptor gene mutation might be considered causal for the epilepsy and responsible for the drug resistance it shows. Then, if rare variant studies have to be large to have meaningful power, there is a risk that such causes of drug resistance might be lost in the heterogeneity of rare variant causes likely to comprise the large study sample in the first place. Here, drug resistance and “precision medicine” can be considered to overlap, and this is considered further below. Moreover, rare variations may have additional complexities to be considered, such as organ-specific genomic change (somatic mutation) and organ-specific regional variation (spatial microheterogeneity), areas we have barely begun to explore.

G. The Epigenetic Hypothesis

The genome is one source of endogenous variation, contributing to different disease risks between different people. There are, however, other sources of variation, such as “omes” beyond the genome: the epigenome, transcriptome, proteome, microbiome, and so on. Some of these “omes” have been interrogated for their role in drug resistance in epilepsy, but it must be acknowledged at this point that the data available are even more sparse than for most genome-based studies and that, currently, none of these “omes” have been proven to influence drug resistance.

The epigenome is a set of molecules that regulates gene expression across the genome. In contrast to the genome, which is considered for the purposes of epilepsy to be largely (but not completely) fixed over time and across tissues, the epigenome can be very dynamic, varying even during short time periods (Guo et al., 2011) and across (and potentially within) organs. Studying epigenomic contribution to drug resistance in epilepsy (Kobow et al., 2013; Kobow and Blümcke, 2018), which is likely to be due to processes in the brain, is therefore very challenging. Among classes of molecules constituting the epigenome are histones and noncoding RNAs, both long and shorter, including microRNAs. The latter contribute to RNA silencing and post-transcriptional regulation of gene expression, altering expression levels of multiple proteins. A central problem in studying the epigenome in humans is to disentangle cause from effect and relevance either way from epiphenomena. Thus, although a series of microRNAs have been shown to associate with human TLE (Miller-Delaney et al., 2015), the studied tissue had been surgically resected from people with drug resistance, and cause and effect (for either disease susceptibility or drug resistance) could not be distinguished. In animal models, manipulation of specific microRNAs can...
influence seizures and disease (Morris et al., 2019), though some data are less supportive (Haenisch et al., 2016); however, whether this would be the case in human epilepsy, and specifically whether this approach would counter drug resistance, remains unknown.

The microbiome has also attracted much interest recently in neuropsychiatric diseases (Iannone et al., 2019). Studies of the microbiome may seem simple and enticing, but there are many complexities and pitfalls. Nevertheless, potential interaction between the gut microbiota and human disease is an intriguing one, with unreplicated early studies suggesting links through the microbiome between the effect of the ketogenic diet and disease (Peng et al., 2018). In all these conditions, BBB dysfunction is associated with Pgp induction in brain vessels and astrocytes as well as with a concomitant neuroinflammatory response in the same tissue districts. There is experimental evidence that neuroinflammation may be a causative factor both for inducing a dysfunctional BBB and an upregulation of Pgp in DRE, as reported below. The main mechanisms discussed in the following are illustrated in Fig. 6.

1. Pathophysiological Link between Neuroinflammation and Blood-Brain Barrier Dysfunction in Epilepsy.

Neuroinflammation and BBB dysfunction are hallmarks of human epileptogenic foci in various forms of DRE as well as in animal models of acquired epilepsies (for comprehensive reviews, see Friedman and Heinemann, 2012; Broekaart et al., 2018; Vezzani et al., 2019; Löscher and Friedman, 2020). These two phenomena are mechanistically linked as shown in vivo and in vitro experimental models. For example, the induction of a neuroinflammatory response caused by an increased brain expression of interleukin (IL-1β or tumor necrosis factor (TNF) in rodents has been shown to result in enhanced BBB permeability to blood macromolecules such as albumin, which is normally excluded from the brain (Yang et al., 1999; Ferrari et al., 2004). Although the underlying mechanisms linking neuroinflammation to BBB dysfunction are not fully elucidated, there is evidence that IL-1β induces breakdown of tight junctions, such as zonula occludens (ZO)-1, by activating IL-1 receptor type 1 (IL-1R1) expressed by endothelial cells (Ferrari et al., 2004; Ravizza and Vezzani, 2006; Morin-Brureau et al., 2011; Librizzi et al., 2012). This signal activation induces, together with vascular endothelial growth factor (VEGF) receptor (VEGFR) 2, the release of ceramide from plasma membrane and the subsequent Src (sarc) kinase protein activation, which is responsible for ZO-1 downregulation (Morin-Brureau et al., 2011). Notably, the ceramide-Src kinase pathway induced by the activation of the IL-1β-IL-1R1 axis in neurons leads to increased excitability and excitotoxicity and promotes seizures mediated by NMDA receptor subunit N2B subunit phosphorylation and enhanced neuronal Ca2+ influx through NMDA receptors (Viviani et al., 2003; Balosso et al., 2008). Therefore, an increase in brain IL-1β couples neuronal hyperexcitability in response to glutamate to BBB dysfunction.

An alternative but not mutually exclusive mechanism links cytokines, e.g., TNF, to BBB permeability changes via endothelial cell transcytosis by enhanced vesicular transport (Abbott, 2000). Additional mechanisms underlying BBB alterations in epilepsy, and in other central nervous system diseases, include direct injury
to endothelial cells such as in stroke and during seizures, edema, oxidative stress, alterations in pericytes, and angiogenesis (Friedman and Heinemann, 2012; Klement et al., 2019; Swissa et al., 2019). In particular, angiogenesis in epileptogenic brain tissue is coupled with overexpression of VEGF in neurons and astrocytes and induction of VEGFR2 in neurons and brain vessels (Rigau et al., 2007). Angiogenesis is induced by epileptic activity and neuroinflammation (Marcon et al., 2009; Morin-Brureau et al., 2011; Dudovska Stankovic et al., 2016). The activation of the VEGF-VEGFR2 axis in astrocytes and vessels alters extracellular matrix and tight junctions, respectively, thereby contributing to BBB dysfunction (reviewed in Sandovol and Witt, 2008).

A direct causative link between seizures, neuroinflammation, and increased BBB permeability to serum albumin was established in the isolated guinea pig brain preparation, in which recurrent seizures were evoked by arterial perfusion of bicuculline (Librizzi et al., 2012). In this in vitro whole brain preparation, seizures triggered the expression of IL-1β in astrocytes, and this phenomenon caused the brain extravasation of arterially perfused albumin, a phenomenon that was prevented by blockade of IL-1R1 with IL-1 receptor antagonist (IL-1Ra). These events were associated with ZO-1 downregulation in endothelial cells and were independent on either circulating leukocytes or blood-borne inflammatory molecules (Librizzi et al., 2012).

Conversely, BBB dysfunction results in pathophysiological changes in perivascular brain tissue mainly involving astrocytes. A sequence of events has been described that involves the brain extravasation of serum albumin, which, in turn, activates the tumor growth factor (TGF)-β receptor type 2-Smad2 mediated signaling in perivascular astrocytes. The activation of this pathway promotes the transcription of inflammatory genes and the downregulation of Kir4.1 and aquaporin 4 channels as well as glutamate transporter 1 and glutamate-aspartate transporter (reviewed in Friedman and Heinemann, 2012). These phenotypic changes in astrocytes impair the glia ability to buffer extracellular K+ and glutamate and to maintain water homeostasis, thus resulting in neuronal hyperexcitability and reducing seizure threshold (Friedman and Heinemann, 2012; Frigerio et al., 2012). Recent evidence has shown that the activation of TGF-β in astrocytes is also involved in remodeling of the extracellular matrix, leading to reactive excitatory synaptogenesis and degradation of perineuronal nets around GABAergic neurons. Overall, these alterations favor pathologic hyperexcitability underlying seizure generation and recurrence (Weissberg et al., 2015; Kim et al., 2017; Vezzani et al., 2019).

Accordingly, animal studies have shown that neuroinflammation, BBB permeability changes, and astrocytic cell dysfunctions contribute to epileptogenesis and epilepsy progression because pharmacological interventions with drugs that prevent or reverse these phenomena reduce the incidence of epilepsy and the frequency or duration of spontaneous seizures in rodents [reviewed in Vezzani et al. (2019) and Bar-Klein et al. (2014, 2017)]. The evidence that neuroinflammation contributes to pathologic hyperexcitability and disease severity raises the possibility that it may contribute to drug resistance according to the “intrinsic severity” hypothesis, as previously proposed.

The following paragraphs report experimental evidence for additional mechanisms that may mediate the role of neuroinflammation in drug resistance.

2. Neuroinflammation and Blood-Brain Barrier Dysfunction: Role in Drug Resistance. The extravasation of serum albumin in the brain parenchyma because of BBB disruption might have functional consequences on the therapeutic effects of ASDs. Evidence from acute rat entorhinal cortex-hippocampal slices has shown that phenytoin and carbamazepine failed to suppress seizure-like events induced by 4-aminopyridine in the presence of tissue perfusion with albumin. This effect was attributed to a “buffering-like” effect of albumin binding to the drugs, which could be overcome by increasing the drug concentration to supratherapeutic doses (Salar et al., 2014).

Release of inflammatory mediators and glutamate by astrocytes and neurons because of brain injury or due to recurrent seizures may increase multidrug transport proteins in the BBB, thereby contributing to resistance to some ASDs in epilepsy, as described above for the transporter hypothesis. More specifically, there is experimental evidence in support of a link between neuroinflammatory molecules and Pgp induction, as shown in studies focusing on COX-2 and IL-1β signals.

3. Cyclooxygenase 2–Prostaglandin E2–Prostaglandin E2 Receptor 1 Axis. BBB dysfunction is associated with Pgp induction in brain vessels and astrocytes and with increased COX-2 protein and prostaglandin (PG) E2 levels in the same epileptogenic regions (Bauer et al., 2008; Zibell et al., 2009; Schlichtiger et al., 2010; van Vliet et al., 2010). A strict association between COX-2 expression in neurons and glia, COX-1 expression in microglia, and increased Pgp and BCRP expression in microvessels was recently reported in surgical brain tissue from patients with drug-resistant mesial TLE (Weidner et al., 2018). The EP1 receptor (EP1R) for PGE2 seems to be a key factor mediating the COX-2 induced upregulation of Pgp. In fact, the increase in Pgp in the hippocampus of a rat model of SE was precluded in animals treated with an EP1R antagonist despite sustained seizures (Pekcec et al., 2009). Consistent with these in vivo data, in vitro experiments showed an increase in both Pgp expression and drug transport activity in isolated rodent brain capillaries exposed to glutamate. The increase in Pgp was dependent on NMDA receptors and Ca2+-dependent activation of phospholipase A2, arachidonic acid release, and COX-2-mediated
triggers that lead to Pgp upregulation in brain vessels. Indeed, cerebral endothelial cells express glutamate receptor subtypes, and brain extracellular glutamate levels raise rapidly during seizures; therefore, the overactivation of endothelial glutamate receptors might be one of the earliest triggers that lead to Pgp upregulation in brain vessels in vivo.

4. Interleukin-1β–Interleukin-1–Receptor Type 1 Axis. A link between the activation of IL-1β–IL-1R1 signaling and Pgp induction was recently provided by a study showing that the expression of Pgp transcript and protein level in the hippocampus and cerebral cortex microvessels of rats exposed to SE was downregulated by a local injection of a synthetic mimic of the microRNA miR146a (Deng et al., 2019). miR146a is a negative regulator of the IL-1β–IL-1R1 signaling, as it reduces the protein levels of IL-1 receptor–associated protein kinases-1 and TNF receptor–associated factor 6, which are pivotal for signal transduction in IL-1R1–expressing cells. The modulation of Pgp expression by IL-1β was dependent on the transcriptional factor NF-kB (Deng et al., 2019).

Notably, miR146a is induced in neurons and astrocytes by SE and in animal models of chronic epilepsy as well as in human TLE. The intracerebroventricular injection of its synthetic mimic was shown to reduce carbamazepine-resistant seizures in a murine model of epilepsy (Iori et al., 2017).

I. Other potential mechanisms of drug resistance. Several other mechanisms (Fig. 3), including disease etiology and progression (Löscher and Schmidt, 2016), psychiatric comorbidities (Hitiris et al., 2007), and loss of drug efficacy (tolerance) during chronic drug exposure (Löscher and Schmidt, 2006), may contribute to ASD resistance in patients with epilepsy, enhancing the complexity of this condition. These mechanisms have been discussed in detail elsewhere.

V. How to Overcome Drug Resistance?

Despite the development of numerous new ASDs in recent decades, still ~30% of people with epilepsy have seizures that remain drug-resistant, even if ASDs with different mechanisms of action are combined (Kwan et al., 2011; Löscher and Schmidt, 2011; Golyala and Kwan, 2017). This would seem to indicate that mechanisms of resistance operative in the brain of patients with DRE are not specific for single ASDs but rather affect a variety of such drugs. In this respect, it is important to note that the hypotheses discussed above are not mutually exclusive, but instead that several mechanisms of resistance may occur in the same patient or group of patients. Thus, overcoming drug resistance is a challenging task. Some of the current approaches of identifying more effective treatments for ASD-resistant epilepsy are discussed in the following sections.

A. Development of New Antiseizure Drugs by Using New Drug-Screening Paradigms

For over 40 years, the NINDS/NIH-funded Anticonvulsant Screening Program (ASP) has provided a preclinical screening service for participants worldwide that helped identify/characterize new ASDs, a number of which advanced to the market for the treatment of epilepsy (Kehne et al., 2017; Löscher, 2017a; Porter and Kupferberg, 2017). However, the identification part of this program relied on simple seizure tests, such as the MES test that are not likely to discover new compounds with higher efficacy against DRE (Löscher and Schmidt, 2011). Therefore, as described above, a revised NINDS/NIH-funded program was implemented in 2016 and termed ETSP (Kehne et al., 2017). Since then, an External Consultant Board provides ongoing individual feedback to the program. In response to the clinical need of more effective therapies for DRE and based on the input of the External Consultant Board and other reviewers, the ETSP has developed a more refined flowchart to evaluate the potential of new compounds for treating DRE (Kehne et al., 2017; Löscher, 2017a). As shown in Fig. 5, in the initial “Identification” phase, the 6-Hz 44 mA mouse test of difficult-to-treat partial seizures has been included to raise the threshold for advancing compounds for the management of pharmaco-resistant epilepsy, thereby increasing the probability that agents with improved efficacy relative to existing agents will be detected. Additional assay options available in the Identification phase include the corneal kindled mouse and a hippocampal/entorhinal cortex-containing brain slice with spontaneous electrical bursting prepared from kainate treated rats. Compounds with good activity in the Identification phase advance into the “Differentiation” phase, which is currently composed of three chronic assays, one in mice and two in rats (Kehne et al., 2017). The Differentiation phase models include the intrahippocampal kainate mouse model of mesial TLE; the lamotrigine-resistant amygdala-kindled rat; and the chronically epileptic rat, in which epilepsy develops after systemic administration of kainate. All three models offer the added advantage of being models of chronic seizure activity induced by chemical or electrical insult. Moreover, all three models replicate numerous clinical aspects of TLE to provide a more etiologically relevant approach to the further characterization of promising investigational ASDs (Kehne et al., 2017). In addition, the ETSP has incorporated into its test battery an etiologically relevant mouse model of epilepsy associated with viral-induced encephalitis. Collectively, the data generated from these models create a pharmaceutical profile that identifies promising investigational compounds for further development and potential treatment of...
pharmacoresistant epilepsy. However, this promise has yet to be realized.

An inherent problem of the ETSP strategy is the focus on TLE-related in vitro and in vivo models and the lack of models that resemble human causes more closely, such as models of acquired epilepsy developing after traumatic brain injury or stroke. Furthermore, for efficacy testing of novel molecular targeting approaches, animal models may not be that relevant because of major species differences in the target molecules and the need for specific modulators (e.g., targeting of species-specific regulatory RNAs). Here, the use of patient-derived iPSCs and other novel approaches, such as genetically engineered zebrafish and mouse models, may be of value (Baraban and Löscher, 2014; Du and Parent, 2015; Grone and Baraban, 2015; Parent and Anderson, 2015; Demarest and Brooks-Kayal, 2018). This, for instance, concerns the targeting of the natural antisense transcript (NAT) class of long noncoding RNAs by antagoNAT oligonucleotides, which can be designed to inhibit cis-acting IncRNA, thereby increasing the expression of a selected protein. This approach is of particular interest for therapeutic management of haploinsufficiencies as a cause of epileptic encephalopathies. Hsiao et al. (2016) recently tested a similar approach in a Dravet mouse model, in African Green

### TABLE 4

Potential etiology-specific drugs ("precision medicine") that are currently used or discussed for treatment of severe pediatric-onset epilepsies

| Mutated gene | Gene name | Encoded protein function | Type of epilepsy | Potentially beneficial therapy | Comments |
|--------------|-----------|---------------------------|------------------|-------------------------------|----------|
| CHRNA4       | Cholinergic receptor nicotinic alpha 4 subunit | Nicotinergic acetylcholine receptor | Nocturnal frontal lobe epilepsy | Zonisamide, acetazolamide, and nicotine patches | Zonisamide and acetazolamide are not really "precision." Nicotinergic agents are theoretically of possible use, but none have been proven to be of value currently |
| GRIN2A       | Glutamate ionotropic receptor N-methyl-D-aspartate (NMDA) type subunit 2A | Glutamate (NMDA) receptor | Focal epilepsy and speech disorder with or without mental retardation | Memantine | Has been proposed on the basis of two studies only, none published since 2015 |
| KCNQ2        | Potassium voltage-gated channel subfamily Q member 2 | Potassium channel | Benign familial neonatal seizures or, in infancy and childhood, EIEE | Retigabine/ezogabine | Has in vitro evidence to support its use in gain-of-function mutants, but prospective controlled trials are still lacking |
| KCNT1        | Potassium sodium-activated channel subfamily T member 1 | Potassium channel | EIEE | Quinidine | The evidence is equivocal, with many negative reports after the initial reports of benefit |
| PCDH19       | Protocadherin 19 | Cell adhesion molecule | EIEE | Potassium bromide, clozabam | Only anecdotal evidence. Better rationale for hormonal treatment with allopregnanolone. |
| PLCB1        | Phospholipase C beta 1 | Enzyme | EIEE | Inositol | Not any evidence for this in humans |
| PRRT2        | Proline-rich transmembrane protein 2 | Unclassified | Benign familial infantile seizures | Carbamazepine, oxcarbazepine | Not really precision (mechanism-based) treatments |
| SCN1A        | Sodium voltage-gated channel alpha subunit 1 | Voltage-gated sodium channel | Dravet syndrome | GABAergic drugs, fenfluramine, cannabidiol | Fenfluramine and cannabidiol cannot be considered precision (mechanism-based) treatments |
| SCN2A        | Sodium voltage-gated channel alpha subunit 2 | Voltage-gated sodium channel | Benign familial infantile seizures or EIEE | High levels of phenytoin; levetiracetam | Not yet clear whether levetiracetam can be considered precision (mechanism-based) treatment |
| SCN2A        | Sodium voltage-gated channel alpha subunit 2 | Voltage-gated sodium channel | EIEE, status epilepticus | Lidocaine, acetazolamide | Evidence for precision (mechanism-based) treatment status limited |
| SCN8A        | Sodium voltage-gated channel alpha subunit 8 | Voltage-gated sodium channel | Benign familial infantile seizures or EIEE | High levels of phenytoin or carbamazepine; amitriptyline, nilvadipine, carvedilol | Based on one study for one mutation in SCN8A |
| SLC2A1       | Solute carrier family 2 member 1 | Transporter | Idiopathic generalized epilepsy | Carbamazepine, divalproex | Bypasses the pathophysiology to provide an alternative energy supply to the brain |
| STXBP1       | Syntaxin-binding protein 1 | Membrane trafficking | EIEE | Levetiracetam, folic acid, vigabatrin | Only anecdotal evidence |
| TSC1         | TSC (tuberous sclerosis complex) subunits 1 and 2 | Unclassified; mutations lead to increased activity of mTOR | Tuberous sclerosis | Everolimus | A precision treatment with support from clinical trials and licensed for particular uses in tuberous sclerosis complex |

EIEE, early infantile epileptic encephalopathy.
Monkeys, and in patient-derived fibroblast lines. As the authors emphasized, primate- and mouse-specific antagoNATs had to be selected for the different experiments. This example nicely illustrates that flexible approaches are necessary for specific indications and molecular targeting approaches. Nevertheless, the major advantage of the ETSP is its capacity to screen hundreds of structurally and mechanistically diverse compounds per year at the major contract site (University of Utah), which increases the chance of identifying a new “next-generation” compound that really makes a difference for drug-resistant patients.

B. Precision Medicine

Precision medicine is a treatment approach in which disease treatment and prevention is tailored to individual variability in genes, environment, and lifestyle for each person (National Research Council US Committee on A Framework for Developing a New Taxonomy of Disease, 2011). Despite much recent discussion about precision medicine, in concept, it is how clinical medicine should always have been practiced at any time in the modern era, taking into consideration all the apparently relevant contemporary information about the patient, their circumstances, and investigations. The advent of potent genomic technologies now allows us to add genomic sequence data to all the other data available for decision-making; in due course, other “omic” data (e.g., epigenomic information) may also be added to the mix. Enthusiasm for precision medicine currently stems largely from discoveries from genetics about the causation of some of the rare, severe, typically early-onset epilepsies, including the developmental and epileptic encephalopathies. The list of genes carrying pathogenic rare variants is growing at a rapid pace. These discoveries have in some cases led to better understanding of disease biology, and, occasionally, rational treatment strategies have been devised, including better selection from existing ASDs or repurposing of drugs licensed but previously not for use in epilepsy, sometimes with dramatic responses (Demarest and Brooks-Kayal, 2018; Møller et al., 2019; Table 4). However, this enthusiasm needs to be tempered by the fact that most such reports are anecdotal and short-term, and for many of the newly explained genetic epilepsies, a precision medicine approach employing a theoretically ideal treatment is not available or, in fact, fails (Sisodiya, 2020). Nevertheless, the approach of identifying the cause of a particular epilepsy and establishing a rational treatment option remains attractive and may offer a novel strategy for epilepsies that were previously resistant to treatment. Perhaps the best example of this is the understanding of disease biology and patterns of response to existing ASDs that has emerged from the now well-established finding that most cases of Dravet syndrome are due to loss-of-function pathogenic variants in SCN1A (Ziobro et al., 2018), though there is still a need for better treatments (Brigo et al., 2018; Mantegazza and Broccoli, 2019). Whether the paradigm that Dravet syndrome so nicely illustrates will be commonly replicated for other such defined epilepsies remains to be seen. The precision medicine approach might also extend to developments that specifically seek to target the underlying pathophysiology, some of which we highlight below.

Though several ASDs targeting sodium channels are already available, the identification and design of selective sodium channel modulators may offer novel therapeutic opportunities for patients with gain- or loss-of-function mutations in genes encoding a specific sodium channel isoform. These compounds include XEN901 and Prax330 as Na1.6-selective sodium channel modulators developed for management of SCN8A-related epileptic encephalopathy caused by gain-of-function mutation (Bialer et al., 2018; Wengert et al., 2019). Another drug discovery program seeks to identify compounds that selectively activate Na1.1 (Frederiksen et al., 2017). In this context, the spider venom peptide heteroscodratoxin-1 (Hm1a) has also been characterized as a Na1.1-selective sodium channel activator (Richards et al., 2018). Drug candidates that target Na1.1 would be of particular interest for people with Dravet syndrome caused by loss-of-function variants in SCN1A.

Other approaches aim to increase expression rates of functional proteins in patients with genetic deficiencies. Readthrough compounds such as ataluren increase the generation of a functional protein despite a nonsense mutation in the encoding gene (Namgoong and Bertoni, 2016). Although originally developed for therapeutic management of Duchenne muscle dystrophy, clinical trials have been registered for Dravet syndrome and cyclin-dependent kinase-like 5 deficiency (https://clinicaltrials.gov/ct2/show/NCT02758626).

Another therapeutic concept for patients with haploinsufficiencies is based on targeting of a cis-acting lncRNA based on administration of an antagoNAT. The antagoNAT binds and inhibits the gene-specific lncRNA, thereby limiting the suppression of gene expression from the intact allele. The antagoNAT CUR-1916 has been shown to increase Scn1a expression in a Dravet mouse model and in patient-derived cells (Hsiao et al., 2016).

Interestingly, success of therapeutic trials in individual patients may also guide the development of precision medicine approaches. Following case reports describing a responsiveness of febrile infection–related epilepsy syndrome (FIRES) with super-refractory SE to the human recombinant form of IL-1Ra, anakinra (Kennedy-Jung et al., 2016; Dilena et al., 2019), a recent study provided evidence for a functional deficiency of the endogenous IL-1Ra in patients with FIRES, and sequencing in one index patient revealed genetic variants...
that might be linked with this functional deficiency (Clarkson et al., 2019). Though this example requires further support linking genetics, pathophysiology, and the therapeutic concept, it nevertheless represents an elegant example of how individual cases can guide research strategies in the development of novel precision medicine approaches. An even more impressive example for precision medicine is everolimus, an inhibitor of the mammalian target of rapamycin (mTOR), which has been approved for treatment of seizures in patients with mutations in tuberous sclerosis complex (TSC) genes, which are causally linked to activation of the mTOR signaling cascade (Table 4). Several other examples of precision (mechanism-based) treatments are shown in Table 4, although the clinical evidence is often limited.

C. Development of More Effective Antiseizure Drugs by Revised Target-Based Drug Discovery

The recent advance of developing etiology-specific drugs (precision medicine) for severe pediatric epilepsies is an excellent example of new target-based drug discovery strategies. However, monogenic epilepsies individually are rare, so more effective ASDs are urgently needed for the more common polygenic and nongenetic (acquired) epilepsies as well. Unfortunately, principles of precision medicine cannot be used yet for therapy selection in common types of epilepsy because resistance or response to a specific ASD cannot be predicted, thus preventing stratification of individuals into subpopulations based on likely differences in treatment response (Tang et al., 2017). Obviously, the various mechanisms by which currently used ASDs act (Table 1) are not effective in at least 30% of patients with such epilepsies. So how do we identify targets for more effective drugs? Here, several lines of evidence and modern technology are relevant, including recent advances in understanding the pathophysiological mechanisms underpinning pharmaco-resistance and the epilepsies; systems-level, multi-omic, and big data approaches; and tissues, cells, or organoids from drug-resistant patients. In addition, iPSCs from individuals with specific genetic variants constitute a promising model for both mechanistic studies at the cellular and network level as well as a potential platform for high-throughput drug development. Importantly, any novel or repositioned candidate ASD, including those identified by “big data” computational approaches, will need to be validated in complex animal models, such as those described above. Two recent examples of how modern technology can provide interesting novel target-based therapies are described in the following.

In the first example, a medicinal chemistry program was initiated to rationally design compounds with high affinity for presynaptic SV2 proteins and low-to-moderate affinity for the postsynaptic benzodiazepine binding site on GABA_A receptors, resulting in the first-in-class compound padsevonil (Wood et al., 2020). The rationale for targeting these proteins was based on observations that the SV2A ligand levetiracetam markedly potentiated the activity of ASDs acting via GABAergic transmission, notably benzodiazepines, in several animal models, resulting in an improved efficacy/safety ratio (Kaminski et al., 2009). In contrast to benzodiazepines, which typically act as high-affinity full agonists at the benzodiazepine binding site on GABA_A receptors, padsevonil acts as a low-affinity partial agonist at this site, with only 40% intrinsic efficacy compared with full agonists (Wood et al., 2020). This profile was intended to reduce tolerance and dependence liability as previously shown for other low-affinity partial agonists (Rundfeldt and Löscher, 2014). Padsevonil also differs from levetiracetam because it acts not only at SV2A but also SV2B and SV2C isotypes of the SV proteins (Wood et al., 2020). As a consequence of this novel profile, padsevonil was more effective in blocking seizures in various animal models, including models of ASD-resistant seizures, than levetiracetam and brivaracetam or combinations of these SV2A ligands with benzodiazepines (Leclercq et al., 2020). A recent clinical proof-of-concept trial confirmed the superior efficacy of padsevonil in 55 patients with frequent multidrug-resistant partial seizures (Bialer et al., 2018). Currently, the drug is undergoing a phase III trial, which will determine whether padsevonil is efficacious in patients with DRE.

In the second example, Srivastava et al. (2018) used a novel gene network approach for identifying mechanistically new drug targets for epilepsy from disease-related gene expression data. Starting from genomewide gene expression profiling of hippocampi from pilocarpine-treated epileptic mice, they first identified coexpression networks (modules) associated with the epileptic condition. Twelve modules were found to be significantly differentially coexpressed between the epileptic and control hippocampus. The cell-type specificity of these modules and their functional processes was assessed using enrichment analyses. Nine of the 12 modules correlated with seizures, and of those, module 18 (enriched for inflammatory processes and expressed in microglia) was the module most significantly positively correlated with seizures. Seven modules, including module 18, were also differentially coexpressed in the hippocampus of humans with TLE, supporting the relevance of the pilocarpine mouse model of TLE to human TLE. From the pragmatic perspective of drug discovery, Srivastava et al. (2018) then set out to identify regulators of each of these modules as potential ASD targets. Of the many membrane receptors predicted to significantly influence the expression of module genes in a direction-specified manner, the tyrosine kinase receptor CSF1R (also known as macrophage colony-stimulating factor receptor) was predicted to be a regulator of two of the seven prioritized candidate epilepsy modules, including module 18. It was hypothesized that blockade of CSF1R should be therapeutic in
epilepsy (i.e., reduce seizures). The availability of the known CSF1R inhibitor PLX3397 provided a tool to experimentally test this hypothesis. PLX3397 and similar CSF1R inhibitors have been shown to deplete brain microglia at high doses (Elmore et al., 2014). However, at the low doses of PLX3397 used by Srivastava et al. (2018), microglia were not depleted, but by downregulating module 18 genes, the epilepsy-induced changes in microglia phenotype were reversed. Next, the antiseizure efficacy of PLX3397 was demonstrated in two mouse models of TLE, the pilocarpine and the intrahippocampal kainate models. In both models, PLX3797 significantly reduced the frequency or duration of spontaneous seizures. In contrast, PLX3397 did not suppress seizures in acute seizure models in non-epileptic mice, i.e., the MES model, the 6-Hz seizure model, and the PTZ model. These data clearly distinguish PLX3397 from most presently used ASDs, which are typically effective in such acute seizure models.

Overall, this is an impressive study, which resulted from a concerted effort between scientists, clinicians, and industry, demonstrating that restoration of disease-related module expression toward health is predictive of therapeutic benefit, allowing “target” validation at the earliest stage of the drug discovery process. It remains to be proven whether the approach used by Srivastava et al. (2018) leads to more effective and tolerable ASDs in people with epilepsy.

Several other novel ASDs with mechanisms different from those illustrated in Table 1 are currently in the preclinical or clinical pipeline (Golyala and Kwan, 2017; Bialer et al., 2018; Löscher and Klein, 2020). Clinical studies of cenobamate, which was recently approved for the treatment of partial-onset seizures in adults (Fig. 1), showed approximately 20% of patients experienced seizure freedom, which is very impressive compared with previous add-on clinical trials with various other novel ASDs in patients with DRE (Krauss et al., 2020). Cenobamate is thought to work through a dual mechanism, enhancing inhibitory currents through GABA<sub>A</sub> receptor modulation and decreasing excitatory currents by inhibiting the persistent component of the sodium current (Golyala and Kwan, 2017). A similar impressive antiseizure effect has been observed with the novel ASD fenfluramine in Dravet syndrome, in which approximately 25% of patients had long-term seizure freedom, suggesting that the long hoped-for breakthrough is a feasible goal (Polster, 2019). In addition to novel ASDs, add-on treatment with drugs that act on mechanisms of ASD resistance, such as coadministration of Pgp inhibitors or anti-inflammatory drugs with ASDs, is a promising therapeutic avenue to overcome drug resistance, which will be discussed in the following.

**D. Targeting of Transporter Function and Expression**

Based on the transporter hypothesis, one strategy to counteract pharmacoresistance in epilepsy is the adjunctive use of Pgp inhibitors (Schmidt and Löscher, 2009; Tang et al., 2017; see also section IV. B. Alteration of Drug Uptake into the Brain). That such a strategy may be relevant in patients with epilepsy is suggested by several anecdotal reports on single patients with intractable epilepsy in whom the nonselective Pgp inhibitor verapamil was added to the ASD regimen (Tang et al., 2017). One pilot non-placebo-controlled open-label study in 19 adult patients with drug-resistant TLE found that adding verapamil (120 mg daily in 13 patients and 240 mg daily in six patients) to the existing ASD treatment improved seizure control in a dose-dependent manner (Asadi-Pooya et al., 2013). However, in a randomized, double-blinded, placebo-controlled trial on once-daily 240 mg verapamil as an add-on therapy in DRE patients with focal onset seizures, no statistically significant decrease in seizure frequency was observed (Borlot et al., 2014). A more recent non-placebo-controlled open-label study, which explored the efficacy of low-dose verapamil (20 mg three times daily) as adjunctive treatment in DRE, reported that 10 out of 19 patients achieved 50% or more seizure reduction (Narayanan et al., 2016). Importantly, in none of these studies was it shown that drug resistance was due to Pgp overactivity in the first place or that verapamil was actually modulating Pgp activity. Clinical proof-of-concept trials with more selective Pgp inhibitors such as tariquidar or elacridar are needed, although the risks of such an approach need to be considered. In this respect, it is important to note that Feldmann et al. (2013) demonstrated that ASD-resistant patients with increased Pgp functionality in epileptogenic brain regions can be identified by PET, thus selecting those patients who may benefit most from add-on treatment with a Pgp inhibitor.

However, as shown in clinical cancer trials, the use of Pgp-specific inhibitors such as tariquidar or elacridar is not without concerns, as systemic inhibition of Pgp could increase plasma and tissue levels of drugs and toxins, potentially leading to systemic toxicity (Chung et al., 2016; Tang et al., 2017). Another approach we and others suggested is modulating transporter regulation in epilepsy without affecting basal transporter expression and function (Bauer et al., 2008; Potschka, 2010; Hartz et al., 2017; Tang et al., 2017). Such strategies aim to target the signaling cascades that upregulate transporter expression in response to seizure activity. As further outlined below, confirmation of the potential of this approach came from experimental studies in rodent models and from assessment in human capillary samples from people with epilepsy undergoing therapeutic surgery (e.g., Potschka, 2012; Avermey et al., 2013).

As discussed above, clinical trials on such strategies would benefit from the use of PET imaging to enrich the trial population with people with increased Pgp function in the brain. Furthermore, developing new ASDs that are not substrates of efflux transporters is an
option. For this option, it is important to note that Pgp is not the only major efflux transporter at the human BBB and that BCRP is even more highly expressed (Uchida et al., 2011). As shown recently, lamotrigine is a substrate of mouse and human BCRP (Römermann et al., 2015), and the same may be true for other ASDs not yet tested in this respect. Furthermore, MRPs may be involved in ASD efflux at the BBB (Potschka et al., 2003a,b).

E. Anti-Inflammatory Drugs and Strategies to Repair the BBB

The causal and reciprocal link between neuroinflammation and BBB dysfunction and their potential involvement in drug-resistant seizure mechanisms has fostered therapeutic interest in developing drugs that target pathologic inflammatory pathways or re-establish the physiologic permeability properties of the BBB.

1. Cyclooxygenase 2-Prostaglandin E2 Signaling. The role of COX-2 in Pgp upregulation in epilepsy was demonstrated by molecular studies (see previous section) and was reinforced by pharmacological data showing that treatments of epileptic rats with selective COX-2 inhibitors could reverse Pgp upregulation (Schlichtiger et al., 2010). Notably, COX-2 inhibitors administered to epileptic rats with enhanced Pgp expression in brain vessels also increased the brain delivery of systemic phenytoin, a substrate of Pgp (van Vliet et al., 2010). Moreover, COX-2 inhibition by celecoxib was able to restore pharmacosensitivity of seizures to phenobarbital in a chronic rat model of DRE and to decrease Pgp expression in hippocampal vessels of drug-resistant rats to control levels (Schlichtiger et al., 2010).

Among the various prostanoids deriving from COX-2 activation, PGE2 appears to be the key molecule modulating Pgp expression though EP1R activation. Pharmacological EP1R blockade in a rat kindling model of epileptogenesis resulted in anticonvulsice effects of a phenobarbital dose that was otherwise ineffective in the absence of EP1R blockade (Peckec et al., 2009). This strategy might also be of value for reducing the risk of developing SE; constitutive EP1R gene knockout mice displayed a reduced likelihood to enter SE, and EP1R knockout mice that did experience SE showed both reduced hippocampal neurodegeneration and neuroinflammatory response (Rojas et al., 2014). Drugs that block EP1R should be safer than COX-2 inhibitors that may be associated with cardiotoxicity, and some COX-2 inhibitors were reported to worsen spontaneous seizures or increase mortality after SE in animal models (Holtman et al., 2009, 2010).

2. Interleukin-1β–Interleukin-1–Receptor Type 1 Signaling. This inflammatory pathway is involved in the generation of the neuroinflammatory cascade in epilepsy and plays a significant role in seizure generation and recurrence in animal models (Vezzani et al., 2011, 2019). In the acute experimental setting, the human recombinant form of IL-1Ra, i.e., anakinra, a drug in medical use for autoinflammatory and autoimmune diseases, was shown to enhance the efficacy of diazepam for reducing duration and severity of benzodiazepine-resistant SE in mice (Xu et al., 2016). The anti-ictogenic activity of IL-1Ra in various experimental models of acute seizures (Vezzani et al., 1999, 2000; Marchi et al., 2009) led to clinical application of anakinra for controlling drug-resistant seizures in children with FIRES and in other DRE forms (Jyonouchi and Geng, 2016; Kenney-Jung et al., 2016; DeSena et al., 2018; Dilena et al., 2019; Sa et al., 2019). Another example of clinical translation is a phase II study in focal onset drug-resistant adult epilepsy with belnacasan (VX-765), an inhibitor of caspase-1, which is the enzyme involved in the biosynthesis of the ictogenic cytokine IL-1β (Bialer et al., 2013). The trial reported a 50% reduction in seizures in 31.3% of subjects in the VX-765 group versus 8.3% in the placebo group; 12.5% of the VX-765 subjects were seizure-free versus 0% in the placebo group. The same drug was proven effective in mice with chronic pharmacoresistant nonconvulsive seizures (Maroso et al., 2011).

3. Other Anti-Inflammatory Strategies. Recent clinical studies have reported therapeutic effects on drug-resistant seizures of various anti-inflammatory drugs that target specific inflammatory pathways activated in human epilepsy and in animal studies [reviewed in Terrone et al. (2017), van Vliet et al. (2018)]. The inflammatory targets include COX-1/2 (aspirin), TNF (adalimumab), IL-6R (tocilizumab), anti-α4 integrin antibody (natalizumab), and the broad-spectrum microglia inhibitor (minocycline) (reviewed in Vezzani et al., 2019).

4. Molecular Mechanisms of Therapeutic Effects. Except for the molecular studies on COX-2-PGE2-EP1R axis and its relationship with Pgp expression and activity, we do not know whether the mechanisms by which anti-inflammatory drugs inhibit drug-resistant seizures involve drug transport proteins. However, experimental evidence has repeatedly shown that neuroinflammation may decrease seizure threshold by a rapid onset and persistent modulation of voltage-gated ion channels as well as by mediating changes in phosphorylation and molecular assembly of both glutamate and GABA receptor–coupled ion channels in neuronal membranes (Roseti et al., 2013, 2015; Vezzani and Viviani, 2015; Frigerio et al., 2018). These neuromodulatory effects of cytokines such as IL-1β and TNF, which are permissive for hyperexcitability, may be involved, for example, in reducing target susceptibility to classic ASDs that act on voltage-gated channels (Rogawski and Löscher, 2004) or play a role in SE refractoriness to benzodiazepines and the associated changes in GABAA receptor subunits (Niquet et al., 2016).

5. Strategies to Repair the BBB. Neuroinflammation contributes to BBB permeability modifications (see
above), which, in turn, may change pharmacokinetics of ASDs by reducing their brain delivery at cellular and molecular targets, thereby decreasing their efficacy (Löschner and Potschka, 2005; Löschner, 2007). Thus, anti-inflammatory drugs that repair BBB permeability dysfunction by reducing cytokine and COX-2 signals may improve seizure response to some therapeutic drugs.

Targeting of the albumin-activated TGF-β signaling in astrocytes is another option for blocking the BBB dysfunction and the potential consequences for pharmacoresistance. In particular, losartan (Bar-Klein et al., 2014, 2017), or the more specific TGF-β–pathway inhibitor SiN2511 (Weissberg et al., 2015), respectively, prevented the microvascular changes and the pathologic consequences of BBB dysfunction, such as excitatory synaptogenesis, in epilepsy models. These treatments also reduced the incidence of epilepsy and the number of spontaneous seizures in the animals and reduced the neuroinflammatory response. However, it remains to be tested whether the epileptic seizures still developing in treated animals were more sensitive to ASDs than seizures occurring in untreated animals.

Finally, broad spectrum immunosuppressive and anti-inflammatory steroids, diet-based treatments such as the ketogenic diet, and neurostimulation such as the vagal nerve stimulation may control drug-resistant seizures in a proportion of epilepsy patients, particularly in the pediatric population (French et al., 2017). These therapeutic approaches are endowed of anti-inflammatory effects, and steroids also repair BBB dysfunction and vessel inflammation by reducing the brain extravasation of leukocytes. Although there is no clear demonstration that these anti-inflammatory and BBB-repairing actions do mediate the therapeutic effects of these interventions, these actions likely contribute to seizure control, as supported by preclinical studies and by evidence based on the use of more specific anti-inflammatory drugs in human DRE.

VI. Conclusions

Despite the introduction of various novel ASDs, drug resistance remains one of the major challenges in epilepsy treatment. In this review, we critically discuss various theories that have been proposed to explain the mechanisms underlying DRE. Furthermore, we discuss several possible strategies to overcome drug resistance. There are various nonpharmacological options, including epilepsy surgery, electrical stimulation, ketogenic diet, and gene therapy, which are not discussed here (for review, see Devisnsky et al., 2018). It is important to consider that ASD resistance is very probably not caused by a single mechanism in all patients but is rather more likely due to several mechanisms, which may even occur together in the same patient. Thus, overcoming ASD resistance is unlikely to be an easy task but will necessitate combined efforts of basic and clinical epileptologists. For improved therapy of seizures in people with DRE, factors specific to individual patients, such as disease etiology, medical history, drug response, temporal patterns of refractoriness, comorbidities, and the multifactorial nature of pharmacoresistance, need to be taken into account, thus placing more emphasis on personalizing the therapy (Tang et al., 2017). Novel imaging techniques might help to increase our understanding of the mechanisms of drug resistance active in people with epilepsy and guide treatment in individual patients. In addition, based on the availability of various new ASDs, there is increasing evidence that the old concept of “rational polytherapy,” combining ASDs that work by different mechanisms, may provide a reasonable approach to managing DRE (Brodie, 2016). The fact that, despite numerous new ASDs, the proportion of patients with epilepsy who do not respond to treatments has changed little should not discourage efforts to develop ASDs with novel mechanisms, as underlined by the promising studies with cenobamate, fenfluramine, or padsevon discussed above. Similarly, the ongoing development of strategies focused on specific disease mechanisms might soon result in a paradigm shift for management of certain genetic epilepsies.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Löschner, Potschka, Sisodiya, Vezzani.

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