Epileptic Disord 2005; 7 (Suppl. 1): S3-S9

Mechanisms of drug resistance

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ABSTRACT – Despite the use of new antiepileptic drugs, approximately one third of patients with epilepsy have seizures that cannot be controlled satisfactorily by medical treatment. Drug resistance may exist at the time of the first seizure or may develop later as result of the disease process. The mechanisms of these different scenarios are likely to be multifactorial, and may include alterations in brain uptake or brain targets of antiepileptic drugs. Such alterations may be constitutive (intrinsic), thus underlying *de novo* drug resistance in epilepsy, or induced, *e.g.*, as a consequence of recurrent seizures or disease progression. Alterations in drug efflux ("multidrug") transporters and drug targets, such as voltage-gated sodium channels, have been found in epileptogenic brain tissue from both patients with epilepsy, and rodent models of epilepsy. However, although the multidrug transporter and target hypotheses are biologically plausible, proof-of-principle is lacking for these hypotheses. An advantage of the multidrug transporter hypothesis is that it can be validated both experimentally and clinically by combining antiepileptic drugs with inhibitors of such transporters. Selective inhibitors of the major efflux transporter P-glycoprotein are currently in clinical trials for reversing chemotherapy resistance in oncology and may soon be used to determine whether such inhibitors can prevent or reverse drug resistance in epilepsy.

Key words: epilepsy, antiepileptic drug, multidrug transporters, P-glycoprotein, sodium channel, GABA receptors

Uncontrolled (drug-resistant, pharmacoresistant, intractable) epilepsy is a considerable burden for the patient and may be associated with severe consequences, including shortened lifespan, excessive bodily injury, neuropsychological and psychiatric impairment, and social disability (Sperling 2004). Thus, it is important to determine the reasons for AED failures and to develop novel treatment strategies to overcome obstacles to seizure control. Although different criteria for intractability are used, most authors agree that approximately 30% of the patients with epilepsy do not enter remission for more than 5 years, despite appropriate treatment with medication (Schmidt and Löscher 2005). The prospect of successful or improved seizure control depends on the type, etiology and duration of epilepsy or epilepsy syndrome, the type and frequency of seizures, the number of previously failed medications at appropriate doses, the degree of mental delay, and a number of other predictors (Regesta and Tanganelli 1999, Kwan and Brodie 2002, Schmidt and Löscher 2005). For instance, several studies have demonstrated that idiopathic epilepsy is less likely to be intractable than symptomatic or probably symptomatic (cryptogenic) epilepsy (Schmidt and Löscher 2005). Furthermore, initial seizure frequency has been a strong predictor of intractability in several prospective

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Presented at the 26th International Epilepsy Congress, Paris 2005

population-based studies (Schmidt and Löscher 2005). Probably, the most powerful prognostic factor for refractoriness is the initial response to antiepileptic drug (AED) therapy (Kwan and Brodie 2000). Most patients with refractory epilepsy will undergo multiple drug trials, most often without any noteworthy reduction in seizure frequency (Kwan and Brodie 2000). There seems to be a growing consensus that failure of two AEDs places a patient in a category where it becomes highly unlikely that further AEDs will successfully control the seizures, even when AEDs with different mechanisms of action are used. The mechanisms underlying this multidrug resistance in epilepsy are not well understood, but a number of hypotheses exist which will be discussed in this review.

A challenge to our understanding of the mechanisms of pharmacoresistance is posed by the different patterns of drug-resistant epilepsy. Although in most patients, drug resistance seems to have been present *de novo*, even before the first AED was started, this is not always the case. In other patients with easily treatable epilepsy, drug resistance seems to develop later, possibly because of progression of the disease, and in a third group of patients there is an intermittent (wax-and-wane) pattern in which active epilepsy is interrupted by periods of remission (Schmidt and Löscher 2005).

Because as many as 75% of patients with mesial temporal lobe epilepsy are considered to have drug-resistant epilepsy (Schmidt and Löscher 2005), most of the data that are discussed in this review will be related to this most common type of drug-resistant epilepsy in patients undergoing surgery. Based on observations in surgically resected brain specimens from patients with drug resistant TLE and data from animal models of TLE, two major hypotheses are currently being discussed as to the possible mechanisms underlying medical refractoriness of seizures, the multidrug transporter hypothesis and the drug target hypothesis. These hypotheses, which are both plausible and based on a reasonable body of evidence, will be discussed in this review.

The multidrug transporter hypothesis

The importance of (multi-)drug efflux transporters such as P-glycoprotein (P-gp) in disease processes and treatment has become increasingly evident in recent years (Taylor 2002, Lage 2003, Lee and Bendayan 2004, Löscher and Potschka 2005). Drug efflux transporters have a major impact on the pharmacological behavior of most drugs used clinically, critically affecting drug absorption, distribution, and elimination in the body (Schinkel and Jonker 2003). Furthermore, such transporters are involved in the emergence of "multidrug resistance" (MDR), which plays an important role in the failure of treatments of tumors, infectious diseases and several brain disorders, including epilepsy (Taylor 2002, Lage 2003, Lee and Bendayan 2004, Löscher and Potschka 2005). P-gp, the encoded 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 (Fromm 2004). Furthermore, P-gp is expressed by tissues with excretory function (small intestine, liver and kidney) and at blood-tissue barriers (blood-brain barrier [BBB], blood-testis barrier and placenta), thus limiting drug entry into the body after oral administration, promoting drug elimination into bile and urine, and limiting drug penetration into sensitive tissues such as the brain (Fromm 2004).

In the BBB, multidrug transporters such as P-gp, 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 *(figure 1)* and combine to reduce the brain penetration of many drugs (Sun *et al*. 2003, Begley 2004). This phenomenon of multidrug resistance is a major hurdle when it comes to the delivery of therapeutics to the brain, including brain cancer chemotherapy. Therefore, the development of strategies for bypassing the influence of these drug efflux transporters, for the design of effective drugs that are not substrates, and for the development of inhibitors for the transporters has become a high priority for the pharmaceutical industry (Begley 2004).

Tishler *et al*. (1995) were the first to report that brain expression of *MDR1*, which encodes P-gp in humans, is markedly increased in the majority of patients with medically intractable partial (mostly temporal lobe) epilepsy. *MDR1* mRNA levels were determined by RT-PCR in brain specimens removed from patients during resective surgery for intractable epilepsy, and were compared with normal brain control specimens obtained from patients undergoing removal of arteriovenous malformations. In line with enhanced *MDR1* expression in epileptogenic brain tissue, immunohistochemistry for P-gp showed increased staining in capillary endothelium and astrocytes. Tishler *et al*. (1995) proposed that P-gp may play a clinically significant role by limiting access of AEDs to the brain parenchyma *(figure 1)*, so that increased P-gp expression may contribute to the refractoriness of seizures in patients with treatment-resistant epilepsy.

Following the report by Tishler *et al*. in 1995, the finding of *MDR1*/P-gp overexpression in epileptogenic brain tissue of patients with drug-refractory epilepsy was confirmed by several other groups (Kwan and Brodie 2005, Löscher and Potschka 2005, Schmidt and Löscher, 2005). Furthermore, it was shown that, in addition to P-gp, several MRPs, but not BCRP, are overexpressed in brain capillary endothelial cells and/or astrocytes of pharmacoresistant patients (Löscher and Potschka 2005, Schmidt and Löscher 2005). 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

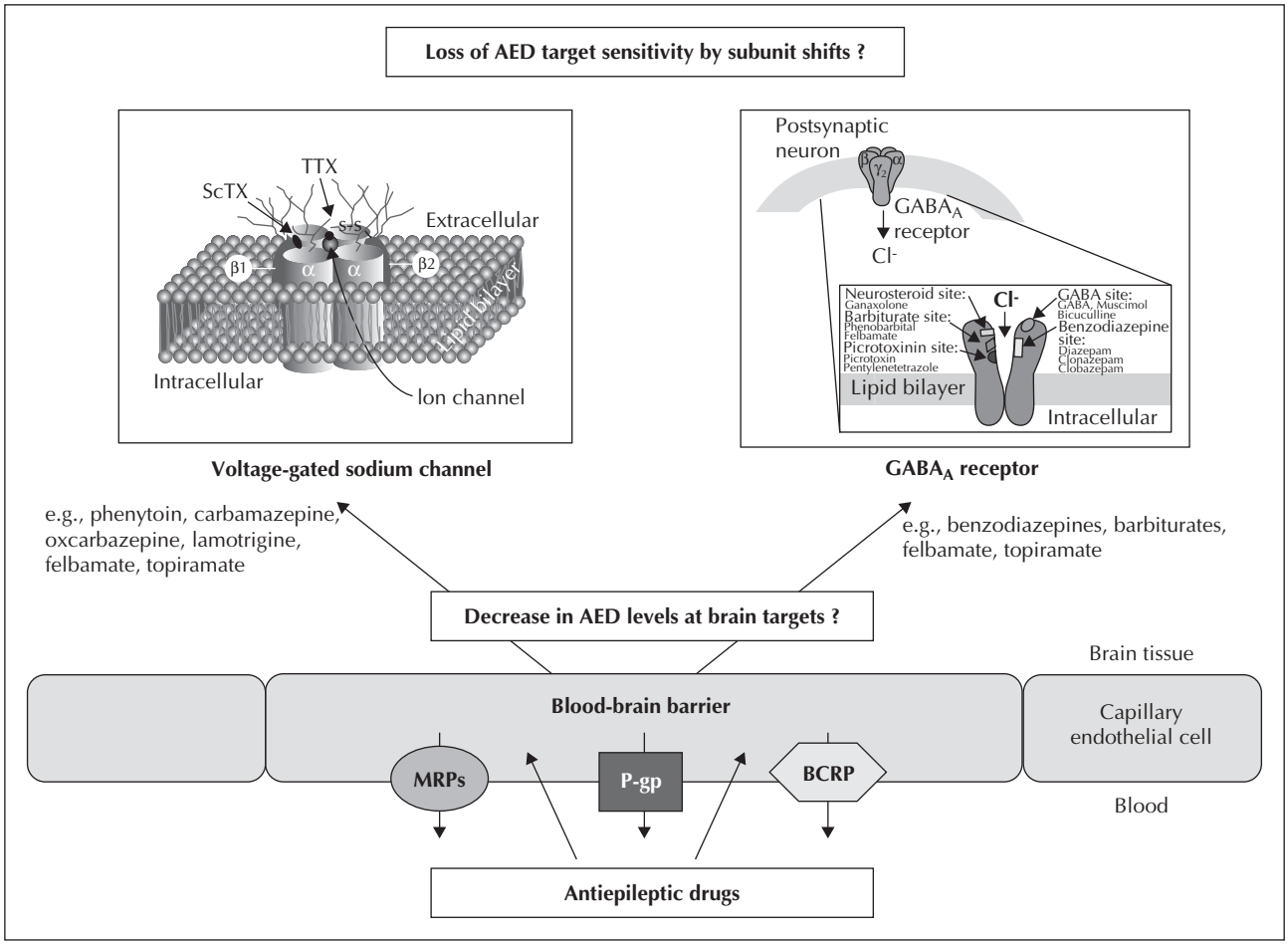

Figure 1. A schematic illustration of the multidrug transporter and drug target hypotheses of drug resistance in epilepsy. In order to reach their targets in the brain, antiepileptic drugs (AEDs) must penetrate through the blood-brain barrier, which is formed by capillary endothelial cells as illustrated in the figure. Numerous drug efflux transporters are located at the luminal (blood-directed) membrane of these cells, aiming to protect the brain from intoxication by lipophilic xenobiotics, including numerous drugs, which otherwise would penetrate from the blood into the brain by passive diffusion, without limitation. These efflux transporters include P-glycoprotein (P-gp), multidrug resistance proteins (MRPs), and breast cancer-related protein (BCRP). These transporter are locally over-expressed in epileptogenic tissue, thereby reducing the amount of AEDs that reaches brain targets. Apart from this transporter-based loss of efficacy, AEDs may lose efficacy because of alterations in brain targets, including ion channels or neurotransmitter receptors. As shown in the figure, most AEDs act by either modulating voltage-gated sodium channels or $GABA_A$ receptor-associated chloride channels (Rogawski and Löscher 2004). The subunit composition and/or expression of sodium channels and $GABA_A$ receptors seem to change in epileptogenic tissue, thereby leading to alterations in the efficacy of many AEDs that act *via* these targets (Schmidt and Löscher 2005).

endothelial barrier function of the BBB is transiently disrupted during seizures (Duncan and Todd 1991, Cornford 1999), overexpression of multidrug transporters in astroglial end-feet covering the blood vessels may represent a "second barrier" under these conditions (Abbott *et al*. 2002, Sisodiya *et al*. 2002). Sisodiya *et al*. (2002) proposed that overexpressed multidrug transporters lower the extracellular concentration of AEDs in the vicinity of the epileptogenic pathology and thereby rendering the epilepsy, caused by these pathologies, resistant to AED treatment.

An open question is whether the overexpression of P-gp 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 AEDs, or of combinations of these factors. Because treatment-resistant patients have the same extent of neurotoxic side effects under AED treatment as patients who are controlled by AEDs, the overexpression of drug transporters in treatment-resistant patients is most likely restricted to the epileptic focus or circuit. This is substantiated by a previous study of Sisodiya *et al*. (2002), in which overexpression of P-gp and MRP1 was found in epileptogenic tissue but not in adjacent normal tissue of the same patients.

In animal models of temporal lobe epilepsy, such as the kindling and kainate models, a transient overexpression of

P-gp was found in brain capillary endothelial cells, astroglia and neurons following seizures (Löscher and Potschka 2005, Schmidt and Löscher 2005), indicating that seizures themselves can induce overexpression of drug transporters. This could explain why one of the major predictors of pharmacoresistance is high seizure frequency prior to initiation of treatment (Regesta and Tanganelli 1999). 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, functional polymorphisms of these transporters may play a role in pharmacoresistance (Kerb *et al*. 2001, Siddiqui *et al*. 2003). Furthermore, both overexpression and functional polymorphisms 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 P-gp 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*. (1999) have reported persistent subtherapeutic plasma levels of AEDs (including phenytoin and phenobarbital) despite aggressive and continuous AED administration in a patient with refractory epilepsy associated with overexpression of *MDR1*.

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 of major clinical interest to evaluate whether AEDs are substrates for these transporters. Only then could overexpression of P-gp or MRPs critically contribute to pharmacoresistance in epilepsy. The first indication that AEDs are substrates for P-gp came from experiments of Tishler *et al*. (1995), who found that intracellular phenytoin levels in a *MDR1*-expressing neuroectodermal cell line were only a quarter that in *MDR1*-negative cells, suggesting that P-gp contributes significantly to cell export of phenytoin. Phenytoin transport by P-gp was also demonstrated in a kidney epithelial cell line transfected with the rodent *mdr1a* cDNA, which could be blocked by the P-gp inhibitor PSC833 (Schinkel *et al*. 1996).

More recently, Rizzi *et al*. (2002) demonstrated that *mdr1a/b* knockout mice, which lack P-gp, exhibit a significant, 50% increase in phenytoin levels in the hippocampus compared to wild type mice. *Mdr1* knockout mice were also used to demonstrate P-gp transport of carbamazepine (Rizzi *et al*. 2002) and topiramate (Sills *et al*. 2002). By using a rat microdialysis model with microdialysis probes in both brain hemispheres and local (cerebral) inhibition of multidrug transporters in one hemisphere, we have previously demonstrated that several major AEDs are substrates for either P-gp or MRPs or both (Löscher and Potschka 2005). Overall, current data from these different experimental approaches described above indicate that at least eight major AEDs (phenytoin, phenobarbital, carbamazepine, oxcarbazepine, lamotrigine, gabapentin, felbamate, topiramate) are substrates for P-gp and some of them (phenytoin, carbamazepine, valproate) also seem to be transported by MRPs at the BBB (Löscher and Potschka 2005).

In view of the overexpressed ABC transporters found in epileptogenic brain tissue of patients with pharmacoresistant epilepsy and animal models of epilepsy, another important question is whether this overexpression lowers brain uptake of AEDs. By using the kainate model of temporal lobe epilepsy in mice, Rizzi *et al*. (2002) demonstrated that the significant increase in *mdr1* mRNA expression measured by RT-PCR in the hippocampus after kainate-induced seizures was associated with a 30% decrease in the brain/plasma ratio of phenytoin, thus substantiating the idea that P-gp alterations significantly affect concentrations of AEDs in the brain. A decrease in phenytoin concentrations of similar magnitude was also determined in the hippocampus of amygdala-kindled rats (Potschka and Löscher 2002).

A further important step in the evaluation of the multidrug transporter hypothesis of drug resistant epilepsy was the demonstration that rats that do not respond to AEDs exhibit significantly higher expression levels of P-gp in brain capillary endothelial cells of the BBB than AED-responsive rats (Potschka *et al*. 2004a, Volk and Löscher 2005). This was demonstrated for two different rat models of TLE (Potschka *et al*. 2004a, Volk and Löscher 2005).

With respect to the ultimate proof for the hypothesis of drug resistance, *i.e.*, demonstration that inhibition or avoidance of the resistance-mediating mechanism counteracts drug resistance in epilepsy, we have some indirect, correlative evidence from experiments with diverse AEDs in pharmacoresistant rats selected from the kindling model of temporal lobe epilepsy (Löscher 2002). All AEDs that were substrates for P-gp showed absent or low anticonvulsant efficacy in nonresponders that had been selected by repeated testing with the AED phenytoin (Löscher 2002, Löscher and Potschka 2002). The only exception was the novel AED levetiracetam, which was as efficacious in both responders and nonresponders (Löscher 2002). Recent data from our group showed that levetiracetam is the first AED tested in our laboratory that is not a substrate for P-gp (Potschka *et al*. 2004b). This drug shows an impressive efficacy in patients with epilepsy that did not respond to previous treatments with AEDs (Betts *et al*. 2003). For direct proof of principle, it should be examined whether P-gp inhibitors can be used to counteract multidrug resistance as recently shown for brain cancer (Fellner *et al*. 2002). That such a strategy may be functioning in patients with epilepsy is suggested by a recent report by Summers *et al*. (2004) on a patient with intractable epilepsy and in whom the P-gp inhibitor verapamil was added to the AED

regimen. This addition greatly improved overall seizure control and subjective quality of life (Summers *et al*. 2004). However, the practical importance of the multidrug transporter hypothesis in the treatment of epilepsy needs to be validated further.

The drug target hypothesis

To exhibit antiepileptic activity, a drug must act on one or more target molecules in the brain. These targets include voltage-dependent ion channels, neurotransmitter receptors, and transporters or metabolic enzymes involved in the release, uptake and metabolism of neurotransmitters (Rogwaski and Löscher 2004). The target hypothesis is primarily based on studies with carbamazepine on voltage-gated sodium channels in hippocampal neurons. To our knowledge, Wytse Wadman's group was the first to report a loss of carbamazepine's modulatory effects on sodium channels in hippocampal neurons of patients with intractable epilepsy (Vreugdenhil *et al*. 1998). The latter group found that the modulation of sodium current inactivation by carbamazepine in hippocampal CA1 neurons from patients with TLE and mesial temporal lobe sclerosis was only half of that encountered in neocortical neurons from the same patients, and only half of that encountered in CA1 neurons from patients without mesial temporal lobe sclerosis (Vreugdenhil *et al*. 1998). Similar observations were obtained in the kindling model of TLE in that the carbamazepine-response of sodium channels of CA1 neurons isolated from the epileptic focus of fully kindled rats was only half of that in control rats (Vreugdenhil and Wadman 1999).

More recently, Heinz Beck's group substantiated and extended these data by showing 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 AED (Remy *et al*. 2003a). 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 (Remy *et al*. 2003a). Consistent with these data from patients with intractable TLE, Remy *et al*. (2003a) also showed that use-dependent block of Na^+ channels by carbamazepine is absent in the pilocarpine rat model of TLE. Based on these data, the authors suggested that a loss of Na^+ channel drug sensitivity may explain the development of drug-resistant epilepsy. In a subsequent study in the rat pilocarpine model in 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, although not as much 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 inter-group difference (Remy *et al*. 2003b).

In order to evaluate which molecular and functional changes in voltage-dependent Na^+ channels underlie the lost or reduced pharmacosensitivity of these channels in the pilocarpine model of TLE, Ellerkmann et al. (2003) studied the expression of Na^+ channel subunits. Both the β1 and β2 subunits were down-regulated, indicating that Na^+ channel subunit composition changes may explain the altered pharmacosensitivity of Na^+ channels *(figure 1)*.

Apart from voltage-dependent Na^+ channels, other drug targets, such as GABA-mediated inhibition, may be altered in patients with intractable epilepsy *(figure 1)*. Using the rat pilocarpine model of TLE, Brooks-Kayal *et al*. (1998) demonstrated that the expression of $GABA_A$ receptor subunit mRNAs is substantially altered in hippocampal dentate granule cells. These changes in $GABA_A$ receptor subunit expression correlated with profound alterations in receptor function and pharmacology (Brooks-Kayal *et al*. 1998, Coulter 2000, Coulter 2001). In normal granule cells, $GABA_A$ receptors of dentate granule cells are insensitive to zinc that is released from mossy fibers, and functions as a negative allosteric modulator of $GABA_A$ receptors. This zinc insensitivity of normal $GABA_A$ receptors is a result of high levels of expression of the alpha1 subunit in these cells (Coulter 2000). In epileptic rats, expression of the alpha1 subunit decreases, and expression of alpha4 and delta 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 loose their sensitivity to augmentation by the benzodiazepine-type site I modulator zolpidem (Cohen *et al*. 2003). Coulter (2000, 2001) has proposed that the temporal and spatial juxtaposition of these pathophysiological alterations may compromise the normal 'gatekeeper' function of the dentate gyrus through dynamic, zinc-induced failure of inhibition, predisposing the hippocampal circuit to generate seizures. Assuming that similar alterations in $GABA_A$ receptor function and pharmacology also take place in the epileptogenic human hippocampus, this could lead to reduced efficacy of AEDs acting via GABA-mediated inhibition *(figure 1)*.

The best evidence that changes in $GABA_A$ receptors occurring during epileptogenesis can lead to drug resistance comes from a series of studies by Bob Macdonald's group using the pilocarpine model (Kapur and Macdonald 1997, Macdonald and Kapur 1999, Jones *et al*. 2002). The latter group demonstrated that during a pilocarpine-induced status epilepticus, there is a substantial reduction of potency for termination of seizures by AEDs that enhance

$GABA_A$-mediated inhibition, such as benzodiazepines and phenobarbital. This progressive development of pharmacoresistance during a sustained status epilepticus is paralleled by alterations in the functional properties of dentate granule cell $GABA_A$ receptors. The authors concluded that rapid modulation of $GABA_A$ receptors during status epilepticus may result in pharmacoresistance to AEDs that enhance $GABA_A$ receptor-mediated inhibition (Jones *et al.* 2003).

As a proof-of-principle for the target hypothesis, it will be important to demonstrate that AED-resistant subgroups of patients differ from AED-responsive subgroups in their AED-target sensitivity. Such a proof-of-principle is difficult to obtain in patients, because, in contrast to patients with intractable epilepsy, patients responding to AEDs in general do not undergo surgical treatment for 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 AED treatment and other variables may present a bias for such a comparison. Animal models of TLE allowing selection of age-matched AED responders and nonresponders may be useful to further evaluate the target hypothesis. Such a study has recently been published involving the rat kindling model (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 Na^+ and Ca^{2+} channels of hippocampal CA1 neurons (Jeub *et al.* 2002). 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 studied.

Although the target hypothesis is a novel and biologically plausible theory to explain drug resistance, the fact that most patients resistant to AED treatment, are resistant to a broad range of AEDs with different mechanisms of action suggests that other, less specific mechanisms contribute to drug resistance. As discussed earlier, the most prominent hypothesis in this respect, the multidrug transporter hypothesis, which was first explored in chemotherapy-resistant cancer, is currently attracting growing interest as a putative mechanism to explain drug resistance in epilepsy by reduced penetration of AEDs into the brain.

Conclusion

Although AEDs are very useful in blocking seizures, many patients do not respond adequately to these agents. In order to enhance our understanding of the mechanisms of pharmacoresistance in epilepsy and thereby develop new strategies for more efficacious treatments, studies on brain tissue from drug-resistant patients and suitable experimental models of intractable epilepsy are mandatory. There is increasing evidence from studies on epileptic brain tissue that overexpression of multidrug transporters and AED target alterations may be important mechanisms of pharmacoresistance, and both mechanisms of refractoriness may coexist in the same epileptogenic brain tissue. In addition, the long-term, progressive changes in neural networks during development and progression of epilepsy may lead to reduced pharmacosensitivity. However, none of these hypotheses has been verified as yet, but much of the evidence is correlative in nature. Furthermore, there are certainly other mechanisms contributing to pharmacoresistance and have to be dealt with when thinking about effective therapeutic agents for hitherto intractable types of epilepsy. Development of novel pharmacological strategies for improved treatment of drug-refractory epilepsy will be a complex venture. □

Acknowledgements. The author's own research was supported by a grant (Lo 274/9) from the Deutsche Forschungsgemeinschaft (Bonn, Germany) and a grant (1 R21 NS049592-01) from the National Institutes of Health (NIH; Bethesda, MD, USA).

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