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Review

Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs

Wolfgang Löscher [a,b,*]

[a] Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Hannover, Germany
[b] Center for Systems Neuroscience, Hannover, Germany

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ABSTRACT

Animal models for seizures and epilepsy have played a fundamental role in advancing our understanding of basic mechanisms underlying ictogenesis and epileptogenesis and have been instrumental in the discovery and preclinical development of novel antiepileptic drugs (AEDs). However, there is growing concern that the efficacy of drug treatment of epilepsy has not substantially improved with the introduction of new AEDs, which, at least in part, may be due to the fact that the same simple screening models, i.e., the maximal electroshock seizure (MES) and s.c. pentylenetetrazole (PTZ) seizure tests, have been used as gatekeepers in AED discovery for >6 decades. It has been argued that these old models may identify only drugs that share characteristics with existing drugs, and are unlikely to have an effect on refractory epilepsies. Indeed, accumulating evidence with several novel AEDs, including levetiracetan, has shown that the MES and PTZ models do not identify all potential AEDs but instead may fail to discover compounds that have great potential efficacy but work through mechanisms not tested by these models. Awareness of the limitations of acute seizure models comes at a critical crossroad. Clearly, preclinical strategies of AED discovery and development need a conceptual shift that is moving away from using models that identify therapies for the symptomatic treatment of epilepsy to those that may be useful for identifying therapies that are more effective in the refractory population and that may ultimately lead to an effective cure in susceptible individuals by interfering with the processes underlying epilepsy. To realize this goal, the molecular mechanisms of the next generation of therapies must necessarily evolve to include targets that contribute to epileptogenesis and pharmacoresistance in relevant epilepsy models.

1. Introduction

Despite the successful development of various new antiepileptic drugs (AEDs) in recent decades, the search for new therapies with better efficacy and tolerability remains an important goal.[1] The discovery and development of a new AED relies heavily on the preclinical use of animal models to establish efficacy and safety prior to first trials in humans.[2] This approach has been very successful and crucially contributed to the development of numerous clinically effective AEDs. In the discovery and development of new AEDs, animal models of seizures or epilepsy serve a variety of purposes (Fig. 1). First, they are used for identifying novel AEDs. Second, once the anticonvulsant activity of a novel compound has been detected, animal models are used to evaluate the possible specific efficacies of the compound against different types of seizures or epilepsy. Third, specific models of AED-resistant seizures are used to investigate whether the novel drug has advantages towards clinically established AEDs for therapy of difficult-to-treat types of seizures or epilepsies. Fourth, animal models are used to characterize the preclinical efficacy of novel compounds during chronic administration. Such chronic studies can serve different objectives, for instance evaluation of whether drug efficacy changes during prolonged treatment, e.g. because of development of tolerance. Fifth, in view of the possibility that chronic brain dysfunctions, such as epilepsy, might lead to altered sensitivity to drug adverse effects, models with epileptic animals are useful to study whether epileptogenesis alters the adverse effect potential of a given drug. Sixth, animal models can be used to estimate effective plasma concentrations of new AEDs for first clinical trials. And finally, seventh, animal models are crucial in discovering therapies that may prevent or modify the development of epilepsy after brain

* Correspondence address: Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany. Tel.: +49 511 856 8721; fax: +49 511 953 8581.
E-mail address: wolfgang.loescher@tiho-hannover.de.

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Animal models of seizures or epilepsy in AED development

- **Discovery of new AEDs**
- **Characterization of spectrum of anticonvulsant activity of new AEDs**
- **Specific models for pharmacoresistant seizures**
- **Evaluation whether efficacy of new AEDs changes during chronic treatment**
- **Comparison of adverse effects of new AEDs in epileptic vs. nonepileptic animals**
- **Estimation of effective plasma concentrations of new AEDs for first clinical trials**
- **Discovery of antiepileptogenic or disease-modifying treatments**

Fig. 1. The main purposes of animal models of seizures or epilepsy in the discovery and development of new AEDs. For details see text.

insults. I will discuss each of these purposes in more details later in this review.

Not all animal models of seizures and/or epilepsy can be used for all of the above described purposes. Furthermore, the intention of the experiment is essential for selection of a suitable animal model. For instance, simple seizure models such as the maximal electroshock seizure (MES) test, allowing to test high numbers of compounds for anticonvulsant activity in relatively short time, will be preferred above more complex models in screening approaches of anticonvulsant drug development.

Most animal models used in epilepsy research are models of epileptic seizures rather than models of epilepsy. Since epilepsy is characterized by spontaneous recurrent seizures (SRS), a test such as the MES test, in which an acute seizure is electrically induced in a normal non-epileptic animal, cannot represent a model of epilepsy. On the other hand, there are true models of epilepsy, for instance animal mutants or transgenic animals with spontaneously recurrent seizures, which are obviously more closely related to human epilepsy than mere seizure models (Fig. 2). Furthermore, epileptogenesis resulting in SRS can be induced by chemical or electrical means (Fig. 2). Unfortunately, researchers often do not differentiate between animal models of epilepsy and animal models of epileptic seizures although the difference may be important in interpretation of data obtained with such models. Of course, models of epilepsy, e.g. mutant animals with inherent epilepsy, can be used as models of seizures, e.g. in anticonvulsant drug potency studies, whereas a pure seizure model in a non-epileptic animal can not be used as a model of chronic epilepsy.

Innumerable models of epilepsy and epileptic seizures have been described.[3] The various animal models can be assigned to different categories, e.g. models with spontaneously occurring seizures versus chemically or electrically induced seizures, models with recurrent seizures vs. models with single seizures (i.e., chronic versus acute models), models with partial seizures versus models with generalized seizures, models with convulsive seizures versus models with nonconvulsive seizures, screening models versus models for a more advanced phase of the screening procedure (“secondary screening”), mechanism-related models (i.e., with seizure induction by a known mechanism) versus models without a specific (or known) mechanism, and seizure threshold models versus models with (supra)maximal or suprathreshold induction of seizures.[3,4]

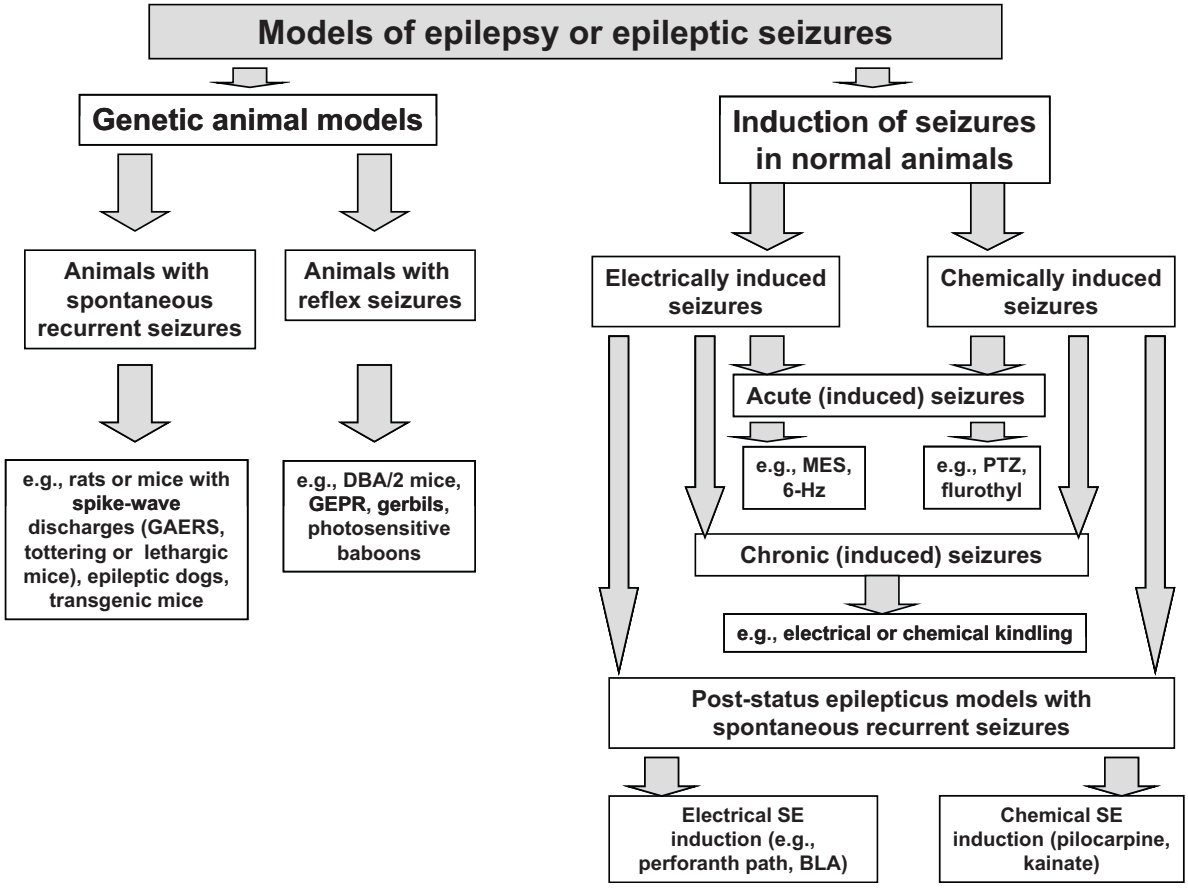

Fig. 2. An overview of models of epilepsy or epileptic seizures. Note that there are numerous models not shown in this figure, including chronic epilepsy models in which spontaneous recurrent seizures develop after traumatic brain injury, ischemic brain damage, or febrile seizures. For more details see Löscher[3] and Pitkänen et al.[8]

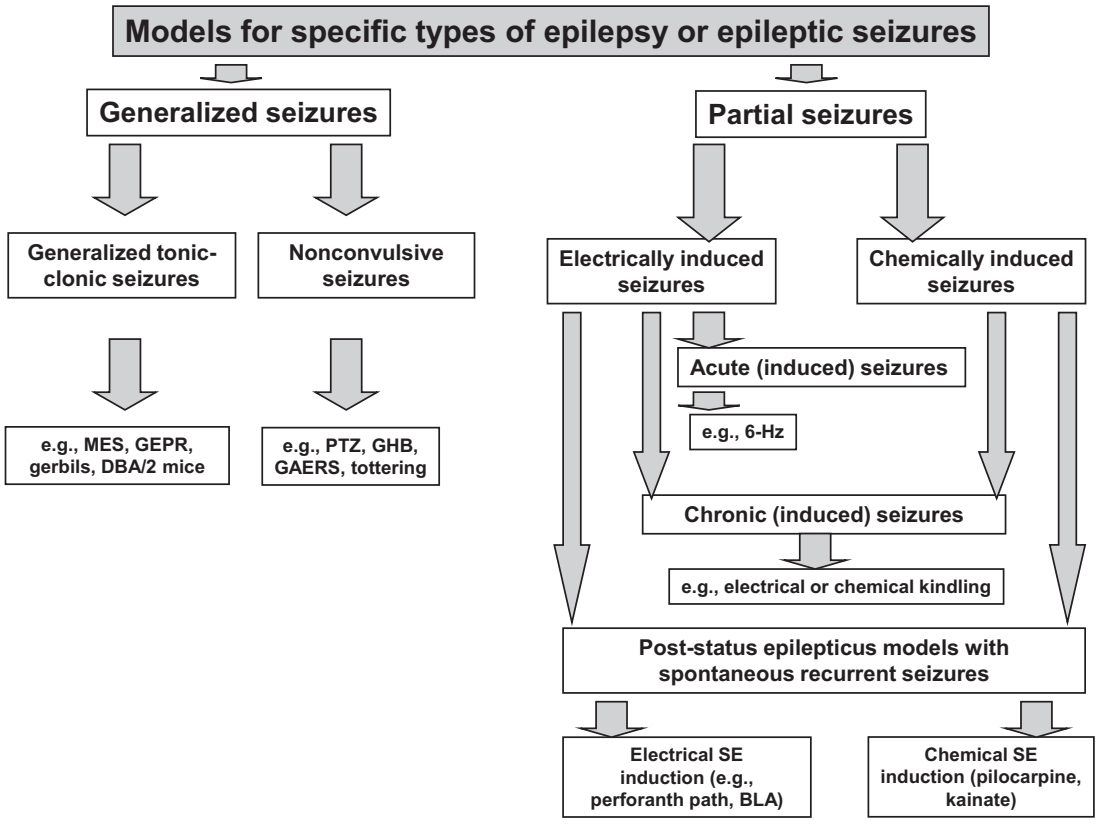

Fig. 3. An overview of models for specific types of epilepsy or epileptic seizures. As already noted in Fig. 2 legend, there are numerous models not shown in this figure, including chronic epilepsy models in which spontaneous partial seizures develop after traumatic brain injury, ischemic brain damage, or febrile seizures. For more details see Löscher[3] and Pitkänen et al.[8]

One simple scheme of a classification of experimental animal models of epilepsy and epileptic seizures is shown in Fig. 2. However, the clinical selection of an AED is based primarily on its efficacy for specific types of seizures and epilepsy, so that for the purpose of preclinical drug evaluation, it may be more appropriate to classify models on the basis of type of seizure or epilepsy as shown in Fig. 3. This should also allow a more precise interpretation of data from investigations into mechanisms of any of these models and facilitate comparisons between experimental and clinical data. This review will only deal with models that are commonly used in AED discovery and development. For a more detailed review of animal models of seizures or epilepsy and their use in studying mechanisms underlying epileptogenesis and ictogenesis, the interested reader is referred to several previous reviews.[3,5–9]

2. Animal models used in AED discovery

For AED discovery, which necessitates screening of large numbers of compounds, animal models should be easy-to-perform, time- and cost-efficient, and predictive of clinical activity. This explains that two simple seizure models in mice and rats, the MES and s.c. pentylenetetrazole (PTZ) tests, which have been developed >60 years ago, are still the most widely used animal seizure models employed in the search for new AEDs.[1,2] In the MES test, tonic–clonic seizures are induced by transcorneal or, less often, transauricular application of a short (0.2 s) suprathreshold electrical stimulus in normal mice (50 mA) or rats (150 mA). The endpoint in this test is tonic hindlimb extension, and the test is thought to be a predictive model for generalized tonic–clonic seizures.[10] In addition, it was proposed that the MES test may predict AEDs with efficacy against partial seizures,[10] but the lack of anti-MES efficacy of several novel AEDs (e.g., levetiracetam, tiagabine, vigabatrin) that subsequently were shown to suppress partial seizures in epilepsy patients (Table 1) strongly argues against this idea. Thus, true models of partial seizures have to be included at subsequent stages of preclinical drug development (see below).

In the s.c. PTZ (or metrazol) seizure test, the convulsive dose of PTZ inducing a clonic seizure of at least 5 s duration in 97% of the animals (CD_{97}) is subcutaneously injected and animals are observed for a post-injection period of usually 30 min for the occurrence of such a "threshold" seizure. The test is thought to be predictive of anticonvulsant drug activity against nonconvulsive (absence or myoclonic) seizures.[10] However, as shown in Table 1, various AEDs that protect against nonconvulsive seizures in epilepsy patients failed in the PTZ test, so that other models of nonconvulsive seizures, including genetic rat mutants with spontaneous nonconvulsive seizures, are needed for correct prediction of AED efficacy against this seizure type.[4]

3. Animal models used to assess the spectrum of anticonvulsant activity of new AEDs

Once the efficacy of an investigational AED is established using simple screening models such as the MES or PTZ tests, a battery of additional models is used to characterize further the anticonvulsant potential and spectrum of activity of this compound. The most often used model in this respect is the kindling model of temporal lobe epilepsy (TLE). Whereas the MES and PTZ models induce seizures in healthy, neurologically intact rodents, kindling is a chronic model in which the repeated application of electrical stimuli via a depth electrode in the limbic system (amygdala or hippocampus) of rats induces permanently enhanced seizure

Table 1
Anticonvulsant spectrum of AEDs in models and man. "NE" = not effective.

Drug	Anticonvulsant effect in rodent models			Clinical efficacy (seizure suppression)		
	MES (mice/rats)	s.c. PTZ (mice/rats)	Amygdala-kindling (rats, focal seizures)	Partial seizures	Generalized seizures	
					Convulsive	Nonconvulsive
Predominant Na^+ (and Ca^{2+}) channel activity						
Phenytoin	+	NE	+	+	+	NE
Carbamazepine	+	NE	+	+	+	NE
Oxcarbazepine	+	NE	+	+	+	NE
Lamotrigine	+	±	+	+	+	+
Zonisamide	+	±	+	+	+	+
Predominant Ca^{2+} channel activity						
Ethosuximide	NE	+	NE	NE	NE	+
GABA systems						
Benzodiazepines	+	+	+	+	+	+
Vigabatrin	NE	+	+	+	+	NE
Tiagabine	NE	+	+	+	+	NE
Mixed						
Valproate	+	+	+	+	+	+
Felbamate	+	+	+	+	+	+
Topiramate	+	NE	+	+	+	+
Phenobarbital	+	+	+	+	+	±
Novel targets						
Gabapentin	±	±	+	+	+	NE
Pregabalin	+	NE	+	+	+	NE
Levetiracetam	NE	NE	+	+	+	±
Lacosamide	+	NE	+	+		
Retigabine	+	+	+	+		

Adapted from Rogawski and Löscher[45] and Bialer et al.[41]

susceptibility and other enduring brain alterations that are similar to those occurring in human TLE.[11] The kindling model is the only chronic model that is currently used by most AED discovery programs, including the NIH/NINDS-sponsored anticonvulsant drug development (ADD) program in the U.S.[1] It is the only model that adequately predicted the clinical utility of novel AEDs against partial seizures in patients with epilepsy (Table 1). Approaches to replace the classical kindling model, which is costly and laborious, by easier models such as corneal kindling have not been successful, because the predictivity of such models is not clear.

Various other models of seizures or epilepsy are employed in subsequent steps of preclinical AED development, including genetic models such as DBA/2 mice with audiogenic seizures and rats mutants with spontaneously occurring spike-wave absences in the EEG, such as the Strasbourg rat.[2,4] Such models are useful to elucidating the potency and spectrum of anticonvulsant activities against different types of epileptic seizures. They do, however, not allow evaluating whether the new drug possesses a higher *efficacy* for suppressing seizures compared to clinically established AEDs, particularly in difficult-to-treat types of epilepsy.

4. Animal models for pharmacoresistant seizures

Despite the development of various new AEDs over the recent 20 years, the available evidence indicates that the efficacy of drug treatment of epilepsy has not substantially improved, but that still about 30–40% of patients suffer from AED-resistant seizures.[1,12,13] Thus, there is a need to identify and incorporate models of refractory epilepsy into development of new AEDs.[2,14] This idea is not new[15] but, surprisingly, has not been fully appreciated for almost two decades. Based on the operational definition of AED resistance in patients with epilepsy,[16] the term "pharmacoresistant" applied in the context of animal models can be defined as persistent seizure activity not responding or with very poor response to monotherapy with at least two current AEDs at maximum tolerated doses.[17] Several models which fulfill this definition have been developed in the last 20 years.[14] In this respect, two different approaches have been employed (Fig. 4). One is to use models of seizures or epilepsy that *per se* are resistant to antiepileptic effects of AEDs. An example is the 6-Hz psychomotor seizure model in mice as a potential screen for therapy-resistant epilepsy. In this model, which is used in the early drug identification studies by the ADD program at the University of Utah (Fig. 5), electrical stimulation by low-frequency (6-Hz) rectangular pulses of 0.2-ms duration delivered through corneal electrodes for 3 s induces seizures that are reminiscent of "psychomotor seizures" occurring in human limbic epilepsy.[18] At 22 mA, the convulsant current in 97% of the mice tested (CC_{97}), all AEDs examined in this model block the seizures, demonstrating that the model does not discriminate between clinical classes of AEDs at this current. However, when the current is increased to twice the CC_{97} (i.e., 44 mA), most AEDs loose their efficacy, and only few AEDs, including levetiracetam (at high doses), valproate, and novel AEDs such as brivaracetam and retigabine, allow complete protection against the 6-Hz seizures (Fig. 4). Based on these observations, it was suggested that the 6-Hz stimulation may provide a useful and rather inexpensive model of therapy-resistant limbic seizures.[18]

Another, much more labor-intensive and expensive approach is the use of chronic epilepsy models such as kindling (Fig. 4). In the kindling model of TLE, repeated excitatory stimuli induce partial and, later, secondarily generalized seizures that increase in length and severity with continued stimulation. In the amygdala-kindling model in rats, in which kindling is produced by repeated electrical stimulation of the amygdala, exposure to low doses of lamotrigine during kindling development leads to a reduced subsequent anticonvulsant response to the drug in fully kindled animals.[19] The same observation was reported for kindling with pentylenetetrazole.[20] Lamotrigine-refractory kindled rats are also resistant to carbamazepine, phenytoin and topiramate, but not valproate, felbamate and retigabine.[21] It was

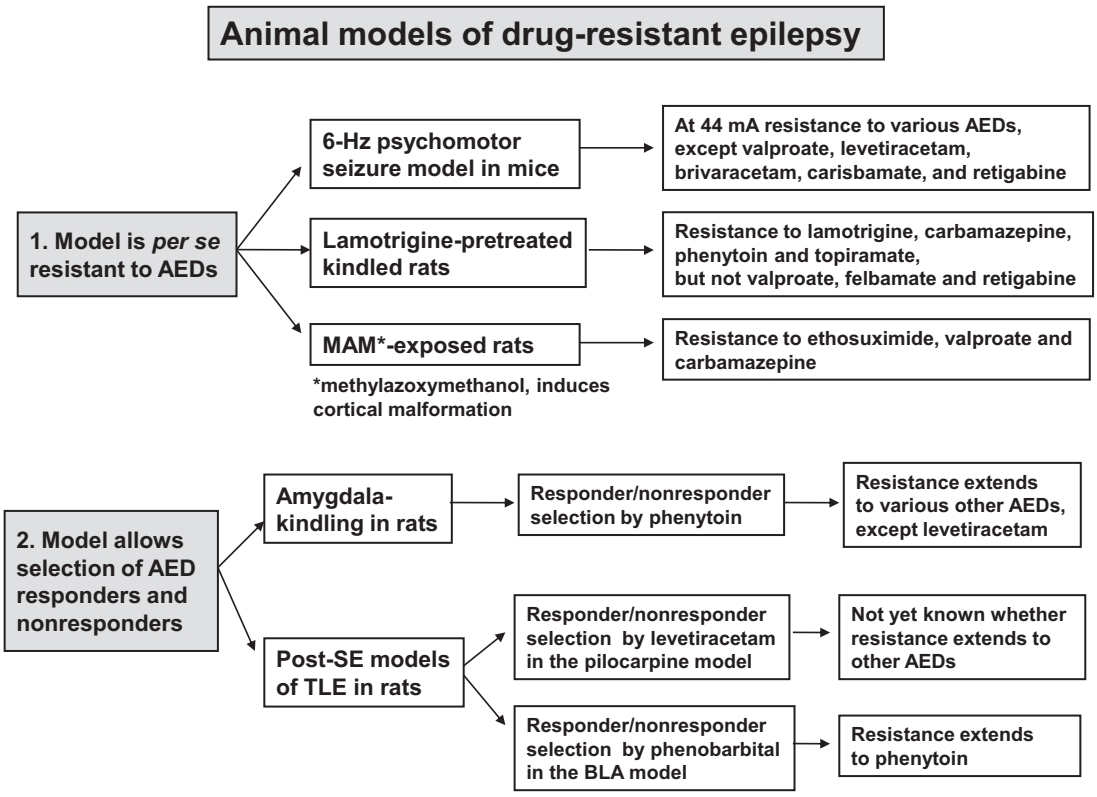

Fig. 4. Different categories of mouse and rat models of AED-resistant seizures and epilepsy. For details see text and Löscher[14,21] and Bialer et al.[41]

suggested that lamotrigine-resistant kindled rats may serve as a model of drug-refractory epilepsy.[2] However, in view of the fact that loss of efficacy (tolerance) develops upon prolonged exposure to several AEDs, including lamotrigine, the loss of efficacy of several AEDs in lamotrigine-resistant rats could be a consequence of cross-tolerance between lamotrigine and AEDs that act, at least in part, by the same mechanism, i.e., modulation of voltage-dependent sodium channels.[22] Whereas cross-tolerance between AEDs acting by similar mechanisms has been demonstrated previously in animal models such as kindling, it is

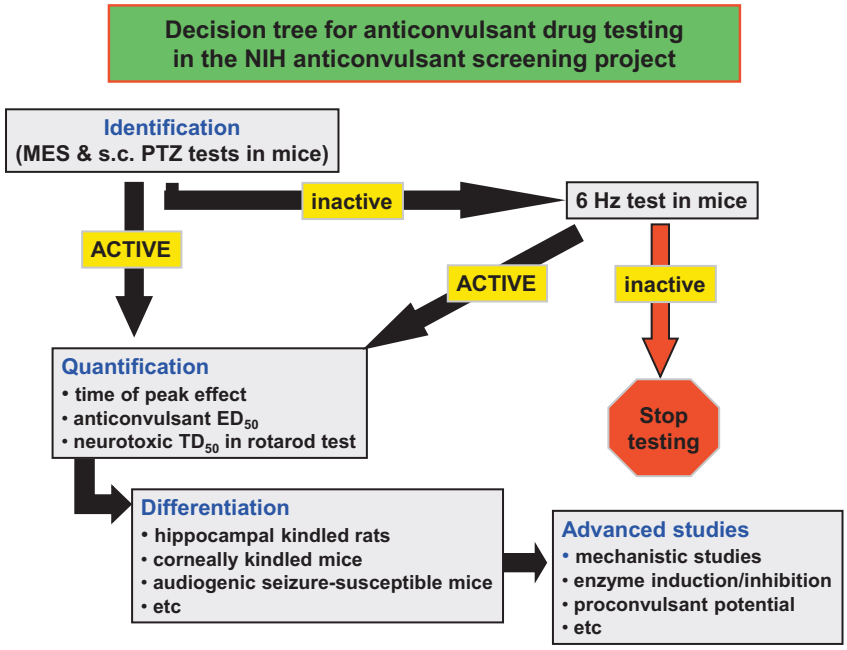

Fig. 5. Schematic diagram illustrating the initial screen of the NINDS-sponsored University of Utah Anticonvulsant Drug Development (ADD) Program. An investigational compound is initially screened for efficacy in the MES and s.c. PTZ tests. The activity of those compounds with demonstrated efficacy and minimal behavioral toxicity is subsequently quantitated (ED50 and TD50) at the time of peak anticonvulsant effect. Compounds found *inactive* in the MES and s.c. PTZ tests are evaluated in the LEV-sensitive 6-Hz seizure test in mice. For those compounds that are found to be active in the 6-Hz test, their activity is quantitated at their respective time of peak effect. All compounds found to be active in one or more of these three identification screens are then differentiated on the basis of their activity in additional seizure models, including the hippocampal kindled rat model of TLE.Adapted from White et al.[2]

not clear whether cross-tolerance plays a role in AED resistance in patients with epilepsy.[22]

In patients, seizure activity associated with cortical dysplasia is often resistant to commonly used AEDs.[23] In rats, exposure to treatment with the antimitotic agent methylazoxymethanol acetate (MAM) *in utero* produces a neuronal migration disorder similar to the cortical dysplasias seen in human brain.[24] In such MAM-exposed rats, seizures induced by either kainate or the cholesterol biosynthesis inhibitor AY-9944 are refractory to valproate, ethosuximide or carbamazepine (Fig. 4). Thus, these rats seem to provide a two-hit model of refractory seizures.

An alternative approach to develop animal models of drug-resistant epilepsy is the treatment of large group of kindled or epileptic rats with AEDs and subsequent selection of subgroups of animals that either respond or do not respond to this treatment (Fig. 4). An animal model of epilepsy allowing selection of subgroups of animals with drug-refractory and drug-responsive seizures could be a valuable tool to study why and how seizures become intractable and to develop more effective treatment strategies. Two models that allow such subgroup selection have been developed and characterized by my group [cf., 14,25].

We became interested in developing such animal models >20 years ago,[15] leading to the discovery of phenytoin-resistant subgroups of amygdala-kindled Wistar rats (Fig. 4). By repeated testing of the anticonvulsant effect of phenytoin in large groups of rats of the Wistar outbred strain, we found that the individual response of fully kindled rats to phenytoin differs, that is that kindled seizures in some animals consistently respond and others never respond to phenytoin.[26] In recent years, we have repeated the selection of phenytoin responders and nonresponders in kindled Wistar rats several times, using either phenytoin or its prodrug fosphenytoin for selection. Average data from more than 200 rats show a consistent anticonvulsant response to phenytoin in only about 20% of the animals, no anticonvulsant response in another 20%, and a variable response in the remaining 60%. Based on our data, we suggest that the three subgroups of amygdala-kindled rats model three different clinical scenarios.[14] The nonresponder subgroup models drug-refractory patients with TLE in which AED treatment does not significantly reduce seizure frequency. The variable responder group model patients in which AED treatment reduces seizure frequency but does not achieve complete control of seizures. The responder subgroup models patients which achieve complete control of seizures during AED treatment.

Following the identification of phenytoin resistant kindled Wistar rats, most clinically available AEDs were tested in such animals. Except the novel drug levetiracetam, all examined AEDs were significantly less efficacious or not efficacious at all in phenytoin nonresponders compared to phenytoin responders, demonstrating that the phenytoin resistance of a subgroup of kindled Wistar rats extends to various other old and new AEDs.[14] This reflects the clinical situation in patients with TLE, because most patients who are refractory to one AED are also resistant to other AEDs, including newly developed drugs.

Stimulated by the findings in the kindling model, we started studying whether pharmacoresistant rats can also be selected from TLE models with SRS. In post-status epilepticus (post-SE) models of TLE, a chemically or electrically induced SE is followed, after a latent period, by SRS.[17] This group of models is often considered to have the greatest parallels with human TLE, but until recently only few studies examined the pharmacological responsiveness of the spontaneous seizures in these models. One obvious reason is that drug efficacy testing in rats with SRS is technically difficult, expensive, and time-consuming, because AEDs have to be administered over a prolonged period, taking into account the marked differences in elimination kinetics between rodents and humans,[27] and rats have to be recorded continuously by video/EEG monitoring during this period for the occurrence of seizures, to allow comparison of seizure frequency during treatment with seizure frequency in control periods.

In the pilocarpine model of TLE in Wistar rats, prolonged administration of levetiracetam via osmotic minipumps resulted in a large inter-individual variation in drug response.[28] About 40% of the epileptic rats were responders with complete or almost complete control of spontaneous seizures, another 40% were nonresponders, and the remaining rats could not clearly be included in either group because of variation between pre- and postdrug control seizure frequency. Prompted by these promising data from the pilocarpine model, we investigated whether responders and nonresponders also occur in another post-SE model of TLE.[29] In this model, SE is induced by prolonged electrical stimulation of the basolateral amygdala (BLA), which leads to development of SRS in >90% of the animals. Prolonged treatment of epileptic Sprague–Dawley rats with phenobarbital at maximal tolerated doses resulted in two subgroups, responders and nonresponders.[29] In two independent studies in 11 and 15 epileptic rats, 36 and 40% of the rats were resistant to treatment with phenobarbital, demonstrating the reproducibility of this model.[14] When the phenobarbital-resistant rats were subsequently treated with phenytoin, 83% of these rats were also resistant to the latter drug (Fig. 4), thus fulfilling the minimum requirements for a model of drug-resistant epilepsy described above. Plasma drug levels and adverse effects of phenobarbital and phenytoin were comparable in responders and nonresponders, demonstrating that the resistance is restricted to the anticonvulsant effect of these AEDs. The severity or duration of the initial brain insult (the SE) did not differ between responders and nonresponders, indicating that the different AED response in the two subgroups is genetically determined.

Epilepsy models allowing selection of AED responders and nonresponders are an ideal tool to investigate mechanisms of AED resistance.[14] Fig. 6 summarizies the most important differences between AED responders and nonresponders in the kindling and post-SE TLE models that we determined in recent years.[14,21] Several of our findings are in line with clinical findings in patients with AED resistant seizures, including high frequency of SRS, psychopathology, and hippocampal damage as poor prognostic factors for treatment, alterations in AED targets in resistant individuals, and a role of genetic factors (Fig. 6). Interestingly, the only common finding in both models was increased expression of the efflux ("multidrug") transporter P-glycoprotein in brain capillary endothelial cells that form the blood–brain barrier, which has also been described in epileptogenic brain tissue of patients undergoing resective surgery, and may be associated with lower brain concentrations of various AEDs by increased efflux from the brain.[30]

5. Evaluation whether efficacy of new AEDs changes during chronic treatment

In most seizure models, investigational drugs are tested after administration of a single dose and the drug effect is then determined at one fixed time point (e.g., 30 min) following drug administration. However, treatment of patients with epilepsy is typically by chronic, daily drug administration, which may change drug efficacy. There are different scenarios in the respect. (1) With some drugs the anticonvulsant efficacy increases during prolonged treatment[4]; examples are primidone (due to accumulation of phenobarbital), valproic acid (reasons are unknown), and vigabatrin (due to accumulation of GABA by irreversible inhibition of its degradation). Consequently, determination of acute potency of

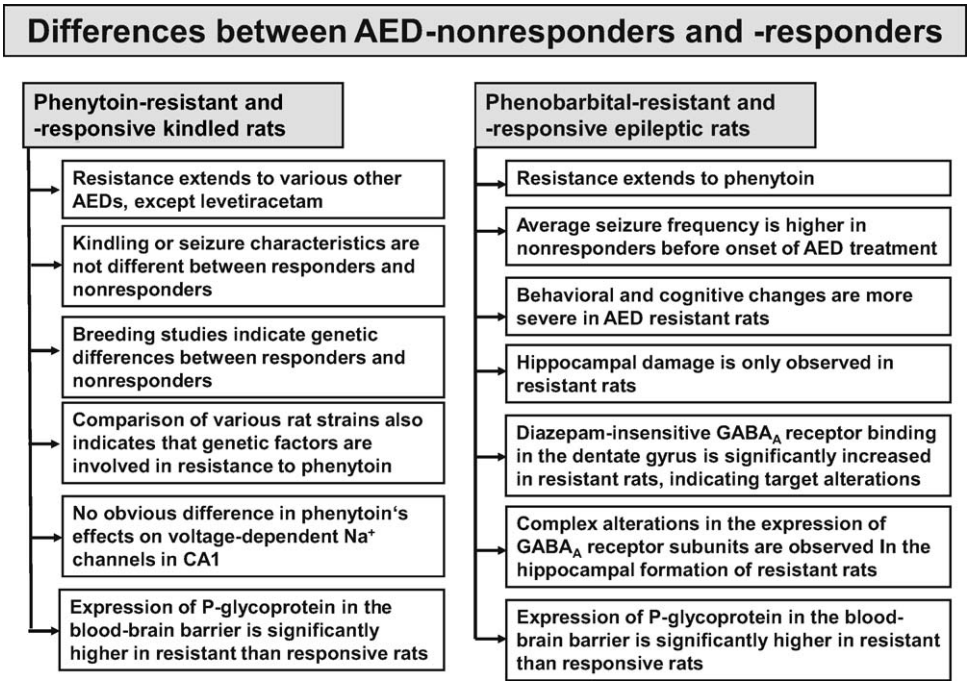

Fig. 6. Differences between AED-responders and–nonresponders in animal models of drug-resistant epilepsy. For details see Löscher.[14,21]

such drugs underestimates their potency during prolonged treatment and, in case of new compounds, may thus lead to false decisions with respect to further preclinical or clinical development.[27]

(2) With several AEDs, particularly benzodiazepines, the anticonvulsant efficacy decreases during prolonged treatment due to development of adaptive processes ('functional tolerance') in the brain.[22] With some older AEDs, such as phenobarbital, carbamazepine or phenytoin, also "metabolic tolerance" may occur due to enhanced drug elimination by induction of AED metabolizing enzymes. Tolerance is clinically advantageous when it concerns the adverse effects of AEDs but disadvantageous when it involves the antiepileptic efficacy itself. In mice and rats, tolerance to the anticonvulsant and adverse effect of benzodiazepines and various other AEDs can be demonstrated in a variety of models of seizures or epilepsy with 1–4 weeks of daily drug administration, provided that effective drug concentrations are maintained during treatment.[22] In addition to benzodiazepines, evidence for loss of efficacy of those old and new AEDs for which functional tolerance was shown in animal models has also been reported in a small portion of patients with epilepsy, which should be taken into account when considering mechanisms of AED resistance.[22]

6. Comparison of adverse effects of new AEDs in epileptic vs. nonepileptic animals

A crucial parameter deciding the clinical utility of new AEDs during preclinical development is the therapeutic (or protective) index expressing the margin between anticonvulsant and adverse effects.[4,10,31] "Neurotoxic" adverse effects such as motor impairment or sedation are commonly quantified during preclinical testing by simple models such as the rotarod test in normal, healthy rodents. However, the validity of using normal animals for adverse effect predictions in epilepsy patients is questionable, because the chronic brain alterations associated with epilepsy may affect the tolerability of AEDs. Limbic kindling of rodents induced by corneal kindling of mice and amygdala kindling of rats confirm that animals with epileptogenic brain alterations are more susceptible to the behavioral and cognitive alterations following acute administration of certain established AEDs and investigational drugs, such as N-methyl-D-aspartate (NMDA) receptor antagonists.[31–33] Similar findings have been reported for genetic absence epilepsy-prone rats, in which certain AEDs also are associated with a more marked deterioration of motor function than in normal animals.[31] This appears in line with several complications with AED use in man being linked to an interaction with the dysfunction of the brain imposed by the epileptic condition.[31] Furthermore, adverse effect testing in kindled rats correctly predicted the unexpected serious adverse effects associated with competitive NMDA antagonists in epilepsy patients but not healthy volunteers.[34] Thus, it is important to involve epileptic animals in preclinical adverse effect testing, in particular when evaluating new AED candidates with novel or unknown mechanisms. In that respect, limbic kindling appears to represent a sensitive and relevant approach.

7. Estimation of effective plasma concentrations of new AEDs for first clinical trials

Because rodents eliminate most drugs much more rapidly than humans, anticonvulsant doses of AEDs (in mg/kg body weight) are usually much higher in rodent models of seizures or epilepsy than effective doses in epilepsy patients.[27] However, determination of effective AED plasmas levels in rodents after acute or chronic drug administration has demonstrated that effective plasma AED levels are remarkably similar in humans and rats.[27] Thus, plasma levels determined at time of anticonvulsant effect in rodent models can be used for selecting adequate doses of a new AED for first clinical trials by calculating the doses that will produce such plasma levels in humans. Of course, many other details, including toxicity, have to be dealt with when selecting doses of an investigational drug for first use in humans, but, in view of the critical problems associated with dose finding in epilepsy patients, the information obtained by plasma level determinations in preclinical seizure models should at least be considered.

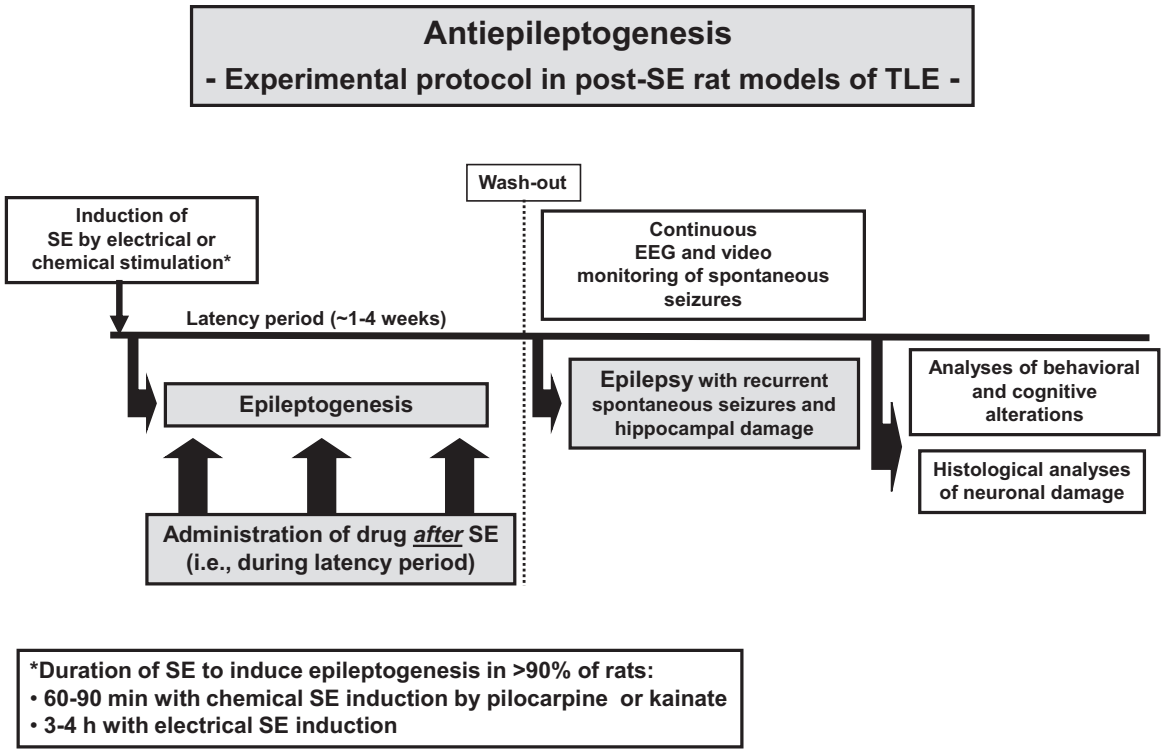

Fig. 7. Schematic illustration of an experimental protocol to evaluate antiepileptogenic (or disease-modifying) drug effects by prophylactic drug treatment *after* a status epilepticus. For details see Löscher and Brandt.[38]

8. Discovery of antiepileptogenic or disease-modifying treatments

The search for new AEDs has traditionally been directed to compounds that suppress seizures in a symptomatic fashion. There is no clinical evidence that any AED is capable of preventing or modifying epilepsy after brain insults, such as traumatic brain injury (TBI).[35] In view of the complex molecular, morphological and functional alterations that are induced by brain insults and thought to be involved in the epileptogenic process leading to epilepsy, drugs that interfere with these alterations will most likely act by other mechanisms than AEDs that suppress seizures. As yet, prevention of epilepsy in patients at risk is an unmet clinical need, but various strategies for epilepsy prevention or disease-modification are evaluated in animal models. The most widely used models in this respect are kindling, post-SE models of TLE, and models of TBI.[36–38] Drug testing in such models sharply differs from testing of novel AEDs as illustrated in Fig. 5, in that drugs with potential anti-epileptogenic efficacy are tested immediately after the brain insult, before SRS occur.[39] A typical protocol for antiepileptogenesis testing in post-SE rats models of TLE is shown in Fig. 7. There is an enormous effort by several groups in the field to develop new strategies for antiepileptogenesis, and the progressively enhanced understanding of mechanisms underlying epileptogenesis will hopefully soon lead to effective treatments.[38]

9. Why has testing in animal models not provided more effective AEDs?

There is growing concern that the efficacy of drug treatment of epilepsy has not substantially improved with the introduction of new AEDs.[12,13,40] This current dilemma of AED development has led to increasing disappointment among clinicians, basic scientists, and industry and may halt any further improvement in the treatment of epilepsy unless we find ways out of this dilemma. What are the reasons for this apparent failure of modern AED development to discover drugs with higher efficacy? One major reason is certainly the fact that, with few exceptions, all AEDs have been discovered by the same conventional animal models, particularly the MES test in rodents, which served as a critical gatekeeper. These tests have led to useful new AEDs, but obviously did not help developing AEDs with higher efficacy in as yet AED-resistant patients. This concern is not new but, surprisingly, has largely been unappreciated for several decades. A logical consequence would be to include models of AED resistant seizures, such as the 6-Hz test in mice or phenytoin-resistant kindled rats, in AED development, but this has started only recently.[2] For instance, as illustrated in Fig. 5, the 6-Hz test is now included in the initial drug screening of the ADD program to avoid that effective drugs such as levetiracetam, which do not act in the MES and PTZ models but show efficacy in the 6-Hz model, are falsely considered inactive in the early evaluation process. However, whether this problem can be minimized by the 6-Hz test is currently not known, because levetiracetam was only retrospectively identified by this test. Furthermore, the fact that novel AEDs such as brivaracetam and carisbamate are highly effective in the 6-Hz test[41] but recently failed to exhibit any robust efficacy in phase III clinical trials casts doubt on whether the 6-Hz test is really the best available model in the search for more effective AEDs. Rather a battery of models of AED resistant seizures as illustrated in Fig. 4 should be included in the development of novel AEDs to concentrate on drugs that exhibit clear advantages in efficacy towards established compounds.

Another argument that has recently been raised is that the seizure types used as endpoints in the MES, kindling and other models included in current AED screening programs may primarily result in the development of new, but redundant drugs that primarily target convulsive (e.g., tonic–clonic) seizures.[42] This is a result of current definitions of experimental seizures that often

focus on specific types of motor seizures with a defined minimum duration, but tend to ignore short nonconvulsive seizures, which often resemble human complex-partial seizures more than those seizure types used as endpoints for drug testing. Thus, during screening of potential AEDs, new agents that may control human complex-partial seizures more effectively than existing AEDs might be missed.[42]

Another important point is that the typical approach of AED testing in animal models primarily focuses on drug *potency* and not efficacy. Thus, different investigational drugs are compared in terms of their anticonvulsant ED_{50}s, i.e., the dose suppressing seizures in 50% of the animals, which is calculated from dose–response curves, testing one group of animals per dose. The lower the ED_{50}, the more potent is the drug, and high potency is often an important argument for selecting drugs for further development. However, it is the antiepileptic *efficacy* which finally determines the clinical usefulness of a new AED and should be considered during preclinical drug testing.

Finally, current strategies of AED development search for drugs that symptomatically suppress seizures by diverse mechanisms. It is unlikely that anticonvulsant efficacy can be markedly enhanced by any of the new mechanisms of seizure suppression of those numerous investigational drugs that are currently in the AED pipeline.[1,13,41] Instead, one may argue that progress in the efficacy of AEDs, particular with regard to pharmacological treatment of drug-resistant epilepsy, will not be made unless and until we develop drugs that specifically target the underlying disease. Indeed, already in 2001, a workshop organized by the NINDS to explore the current problems, needs, and potential usefulness of existing methods of discovery of new therapies to treat epilepsy patients concluded that the epilepsy research community should undergo a conceptual shift to move away from using models that identify therapies for the symptomatic treatment of epilepsy to those that may be useful for identifying therapies that are more effective in the refractory population and that may ultimately lead to an effective cure in susceptible individuals.[36] To realize the goal of a cure, the molecular mechanisms of the next generation of therapies must necessarily evolve to include targets that contribute to epileptogenesis and pharmacoresistance in relevant epilepsy models.

10. Conclusions

The new, third generation AEDs, which have been discovered by testing of large numbers of investigational compounds in animal models over the last 20 years, have undoubtedly expanded the therapeutic options, in particular for those in need for a change in medical regimen.[1] However, the efficacy of these new AEDs for treatment of new-onset epilepsy is at best similar to that of older AEDs.[13,40] New AEDs have other benefits over some of the older drugs for epilepsy in that treatment with some of the new AEDs avoids adverse drug interactions and hypersensitivity reactions[43] and some new AEDs have clinically important utility for disorders other than epilepsy.[44] However, the major goal of AED discovery and development should be more effective treatments for the AED-resistant epilepsy patients. For this goal, we need new concepts and fresh thinking about how to radically change and improve AED discovery and development. Studies in animal models have significantly contributed to our understanding of ictogenesis and epileptogenesis and how AEDs act to suppress seizures.[8] Furthermore, they are indispensable in the preclinical discovery and development of novel AEDs.[1] However, the current strategies and concepts of preclinical AED development need to be radically overhauled.

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