Title

What New Modeling Approaches Will Help Us Identify Promising Drug Treatments?

Authors

Baraban, Scott C
Löscher, Wolfgang

Publication Date

2014

DOI

10.1007/978-94-017-8914-1_23

Peer reviewed

What New Modeling Approaches Will Help Us Identify Promising Drug Treatments?

23

Scott C. Baraban and Wolfgang Löscher

Abstract

Despite the development of numerous novel antiepileptic drugs (AEDs) in recent years, several unmet clinical needs remain, including resistance to AEDs in about 30 % of patients with epilepsy, adverse effects of AEDs that can reduce quality of life, and the lack of treatments that can prevent development of epilepsy in patients at risk. Animal models of seizures and epilepsy have been instrumental in the discovery and preclinical development of novel AEDs, but obviously the previously used models have failed to identify drugs that address unmet medical needs. Thus, we urgently need fresh ideas for improving preclinical AED development. In this review, a number of promising models will be described, including the use of simple vertebrates such as zebrafish (*Danio rerio*), large animal models such as the dog and newly characterized rodent models of pharmacoresistant epilepsy. While these strategies, like any animal model approach also have their limitations, they offer hope that new more effective AEDs will be identified in the coming years.

Keywords

Zebrafish • Epileptic dogs • Epileptic rodents • Pharmacoresistant epilepsy • Antiepileptic drugs • Epilepsy syndromes

S.C. Baraban (✉)
Epilepsy Research Laboratory,
Department of Neurological Surgery,
University of California,
San Francisco, CA 94143, USA
e-mail: Scott.Baraban@ucsf.edu

W. Löscher (✉)
Department of Pharmacology,
Toxicology and Pharmacy,
University of Veterinary Medicine,
Hannover 30559, Germany

Center for Systems Neuroscience,
Hannover 30559, Germany
e-mail: Wolfgang.Loescher@tiho-hannover.de

H.E. Scharfman and P.S. Buckmaster (eds.), *Issues in Clinical Epileptology: A View from the Bench*,
Advances in Experimental Medicine and Biology 813, DOI 10.1007/978-94-017-8914-1_23,

23.1 Introduction

Rodent models of seizures and epilepsy have played a fundamental role in advancing our understanding of basic mechanisms underlying ictogenesis and epileptogenesis. They have also been instrumental in the discovery and preclinical development of novel antiepileptic drugs (AEDs) [12]. Indeed, animal models with a similarly high predictive value do not exist for other neurological disorders, such as bipolar disease or migraine [62]. Despite the availability of predictive rodent models, at least 30 % of epilepsy patients are not controlled by currently available AEDs. One reason is that, with few exceptions, most AED candidates were identified in simple evoked seizure models in otherwise healthy rodents such as the maximal electroshock seizure (MES) or acute pentylenetetrazole (PTZ; metrazol) tests [48]. In these traditional models, in use since the 1940s, successful AED treatments suppress acute seizure events, but effects on drug-resistant seizure events or chronic spontaneous seizures are not routinely evaluated. Thus, we urgently need fresh ideas for improving preclinical AED development. Here, a number of promising models will be described, including the use of simple vertebrates such as zebrafish (*Danio rerio*), large animal models such as the dog and newly characterized rodent models of pharmacoresistant epilepsy. We will not discuss *in vitro* brain slice models or neurons derived from patients using induced pluripotent stem cell technology, because the network complexity of the brain and its alterations by seizure activity are difficult to recapitulate in the dish.

23.2 Zebrafish-Based Approaches to Epilepsy and Drug Discovery

Traditionally used as a model organism to study vertebrate development and embryogenesis, zebrafish only recently emerged as an important model for epilepsy research [5, 17, 27, 29, 53, 65, 70]. The rapid ex vivo development, genetic tractability and transparency of larval zebrafish make them ideally suited to these types of studies (Fig. 23.1). Because zebrafish are vertebrates with a fairly complex nervous system [2, 21, 61] recording electroencephalographic activity is also possible [7], and with exposure to standard convulsant manipulations (e.g., PTZ, pilocarpine, 4-aminopyridine, heat) abnormal electrical discharge with brief high-frequency small amplitude (interictal-like) and longer duration, complex multi-spike large amplitude (ictal-like) events can be readily observed. Sophisticated imaging approaches, taking advantage of the transparency of larval zebrafish and genetic modification to express calcium or bioluminescence indicators, provide additional evidence that central nervous system (CNS)-generated seizure-like activity is robust in response to PTZ. This is an important advantage of zebrafish as a model organism for epilepsy research as CNS-generated abnormal electrical events are often considered a hallmark feature of this disease. In the original description of the acute PTZ seizure model in wild-type zebrafish at 6 or 7 days post-fertilization (dpf), Baraban et al. [5] provided a framework for characterizing epilepsy in zebrafish: (i) evidence for seizure-induced gene (*c-Fos*) expression, (ii) a scoring system for seizure-like behaviours, (iii) electrophysiological examples of abnormal electrographic burst discharge and (iv) sensitivity to common AEDs (valproate, ethosuximide, carbamazepine, phenytoin, phenobarbital and diazepam). As expected from similar PTZ testing in rodents [71], valproate and diazepam were the most effective at inhibiting electrographic seizure events with approximate ED_{50}s of 1 mM and 5 μM, respectively. Using this same model, Berghmans et al. [11] extended this dataset to include 14 standard AEDs. These follow-up experiments used an assay where wild-type larvae were "incubated" in a test compound for 24 h prior to acute PTZ administration and monitoring of seizure-like behaviour exclusively in a locomotion-based tracking assay. These studies confirmed the results of Baraban et al. [5] but also highlight the limitations of a behaviour-only assay as two drugs that failed to alter electrographic burst discharge amplitude (ethosuximide

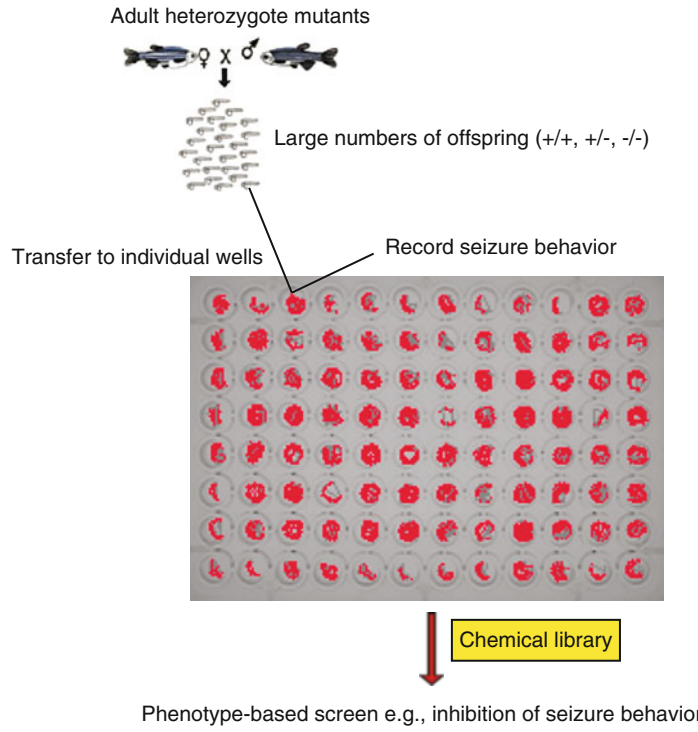

Fig. 23.1 Schematic illustration of the zebrafish assay

and carbamazepine) were identified as "anticonvulsant" as measured by a reduction in swim activity. A likely explanation is that overnight exposure to these AEDs was either toxic or sedative to developing zebrafish, as both possibilities would appear as suppressed locomotion in motion-based tracking assay. More recently, Afrikanova et al. [1] revisited this overnight exposure-PTZ challenge assay and evaluated a similar list of 13 AEDs using a combination of locomotion tracking followed by electrophysiology on agar-immobilized larvae. These latter studies aligned most closely with the original PTZ findings, identifying valproate and diazepam, while also showing that ethosuximide altered burst frequency but not amplitude. Maximum-tolerated drug concentrations were studied in both papers highlighting an additional advantage of the zebrafish platform for simultaneous in vivo evaluation of drug toxicities e.g., one of the primary reasons that most compounds identified in preclinical trials ultimately fail to reach the clinic. In a recent paper by Baxendale et al. [10] also using PTZ, a high-throughput screen of a ~2,000 bioactive small molecule library was reported. These studies used a first-pass assay based on increased *c-Fos* mRNA expression (as measured by in whole-mount situ hybridization) following PTZ exposure at two dpf and a secondary locomotion-based assay at four dpf for additional concentration-response studies. Unfortunately, it is unclear whether the 46 compounds identified using this approach are antiepileptic as previous studies indicate the earliest possible developmental stage where confirmed electrographic seizures could be observed in zebrafish larvae is three dpf [6, 27]. Before this age, larvae are still in chorion and do not swim freely. Furthermore, these non-physiological assays should be interpreted with caution as the Baxendale et al. [10] study identified several candidate compounds with known neurotoxicity profiles e.g., lindane, rotenonic acid, deguelin, endrin and propanil.

Although seizures can be easily induced, drug discovery using acute seizure models, even in zebrafish, are prone to the same limitations as in rodents. Namely, these approaches use healthy animals, the seizure-events are acute and evoked

using potentially non-physiological stimuli such as a stimulation electrode or convulsants, and most importantly they do not model spontaneously occurring seizure events. Zebrafish diverged from humans roughly 450 million years ago but recent genome sequencing revealed that the similarity between the zebrafish and human genome is ~70 % [28]. This fact, coupled with the fecundity of adult zebrafish (producing 100–200 offspring per week from a single adult breeding pair), the permeability of larvae to drugs placed in the bathing media, and ability to thrive in volumes as small as 100 µl make zebrafish an attractive model for a drug discovery program targeted to genetic forms of epilepsy. In the Baraban laboratory, we have focused on zebrafish designed to mimic monogenic epilepsy disorders of childhood as they offer the advantages of spontaneous seizure activity and a genetic basis mimicking the human condition. In this approach, one can model specific forms of pediatric epilepsy – Type I Lissencephaly (*Lis1*), Angelman syndrome (*Ube3A*), Tuberous Sclerosis Complex (*Tsc*) or Dravet syndrome for example (*Scn1a*) – then design drug screening programs targeted to that patient population. In some cases these are stable mutations carried in the zebrafish germline, where other models involve acute antisense knockdown of gene expression in immature zebrafish. Thus, a form of "personalized medicine" aimed at identifying new therapeutic options for relatively rare, but catastrophic, forms of epilepsy. Our recent studies are based on a two-stage screening process. First, zebrafish mutants are placed in individual wells and behaviour (locomotion) is tracked using a 96-well format. Once a baseline level of spontaneous seizure activity is established a test compound is added, and then a second locomotion assay is performed to evaluate the effect on seizure behaviour (with distance travelled and mean velocity of swim movement used as surrogate markers) [5, 16]. As freely behaving larvae can simultaneously be observed for heart rate, edoema or touch-sensitivity, in vivo toxicity is also determined with this strategy. Using a 96-well format it is relatively easy to power this research for statistical analysis and multiple drug concentrations can be assessed in a given plate. The same fish can subsequently be used for electrophysiological analysis, which allows a determination of "false positives" in the locomotion assay that are lethal, sedative or paralyzing. With even a modest zebrafish facility, this approach can easily be used to screen 20–50 drugs per week. The disadvantage of this strategy is that it is not well-suited to acquired forms of epilepsy that develop more slowly over time or in the adult nervous system, or compounds that are not easily dissolved in embryo media. It is also difficult to directly translate concentrations that are effective via bath application in larval zebrafish to those that may be useful clinically in humans.

23.3 Rodent Models of Pharmacoresistant Seizures

The concept of developing rodent seizure or epilepsy models that do not respond to clinically approved AEDs and then using such models for the discovery of novel more effective AEDs is not new but, to our knowledge, was first proposed by Löscher in 1986 [38]. Since then, several models of pharmacoresistant seizures have been developed, including the phenytoin-resistant kindled rat [40], the lamotrigine-resistant kindled rat [68], and the phenobarbital-resistant epileptic rat [14]. In all these models, resistance to one AED extends to other AEDs (cf., [49]), thus fulfilling the criterion of pharmacoresistant epilepsy [32]. By using two of these models, Löscher and colleagues described several factors that differentiated AED-resistant from AED-responsive rats, including the extent of neurodegeneration in the hippocampus, genetic factors, AED target alterations, alterations in drug efflux transporters, and intrinsic severity of the epilepsy as a determinant of AED refractoriness [49]. Similar factors have been described for AED-resistant human epilepsy, so that the rat models obviously reflect clinically important mechanisms of refractoriness. The next logical step was to use such models for new treatment discovery. One example here is that inhibiting the drug efflux transporter P-glycoprotein (Pgp), which is increased at

Table 23.1 A comparison of elimination half-lives of antiepileptic drugs in humans, dogs and rats

	Half-life (h)		
AED	Human	Dog	Rat
Carbamazepine	25–50[a,b]	1–2[a,b]	1.2–3.5[a]
Clobazam	16–50	~1.5	1
Clonazepam	18–50	1–3	?
Diazepam	24–72[a] (DMD = 40–130)	1–5[a] (DMD = 4)	1.4[a] (DMD = 1.1)
Ethosuximide	40–60	11–25	10–16
Felbamate	14–22	4–8	2–17[c]
Gabapentin	5–7	3–4	2–3
Lacosamide	13	2–2.5	3
Lamotrigine	21–50	2–5	12 to >30
Levetiracetam	6–11	4–5	2–3
Oxcarbazepine	1–2.5[a] (MHD = 8–14)	~4[a] (MHD = 3–4)	?[a] (MHD = 0.7–4)
Perampanel	70	5	2
Phenobarbital	70–100[b]	25–90[b]	9–20[b]
Phenytoin	15–20[b,c]	2–6[b,c]	~1–8[b,c]
Potassium bromide	~300	~600	72–192
Pregabalin	6	6–7	2.5
Primidone	6–12[a] (PB = 70–100)	4–12[a,b] (PB = 25–90)	5[a] (PB = 9–20)
Tiagabin	5–8	1–2	1
Topiramate	20–30	3–4	2–5
Valproate	8–15[a]	1–3[a]	~1–5[a,c]
Vigabatrin	5–7[d]	?[d]	~1[d]
Zonisamide	60–70	~15	8

Data are from previous reviews of Löscher [44, 46] and have been revised and updated for the present study. Note that rats and dogs eliminate most AEDs more rapidly than humans, which has to be considered when using such drugs for chronic studies in experimental animals

DMD desmethyldiazepam, *MHD* monohydroxy derivative, *PB* phenobarbital, ? indicates that no published data were found

[a]Active metabolites; [b]shortens on continuing exposure to the drug (because of enzyme induction); [c]non-linear kinetics (half-life increases with dose); [d]duration of action independent of half-life because of irreversible inhibition of GABA degradation

the blood–brain barrier of AED-resistant rats, counteracted resistance to phenobarbital in epileptic rats [15]. The increased Pgp functionality in epileptic rats can be visualized in vivo by positron emission tomography [4]. By using Pgp imaging, Feldmann et al. [19] demonstrated that about 40 % of AED-resistant patients exhibit increased brain functionality of Pgp and could potentially benefit from Pgp inhibition. This example illustrates that chronic rodent models of pharmacoresistant seizures are helpful to discover new strategies for treatment of medically intractable epilepsy.

The disadvantage of the described chronic epilepsy models is that they are not suited for large-scale testing of novel compounds but rather for evaluation of selected treatment strategies as illustrated by the example of Pgp inhibition. Kindling models such as the phenytoin-resistant kindled rat [40] or the lamotrigine-resistant kindled rat [68] have the advantage that seizures can be induced at will, so that chronic drug administration is not needed, whereas models with spontaneous recurrent seizures (SRS) such as the phenobarbital-resistant epileptic rat [14] necessitate continuous (24/7) EEG/video recording for assessing drug efficacy. When testing drug effects on SRS in such rat models, the rapid elimination of most drugs, including AEDs, in rats (Table 23.1) necessitates the use of an adequate dosing regimen during prolonged drug administration to

avoid false negative results [46]. The same is true when administering potential antiepileptogenic drugs in the latent period following epileptogenic brain insults in rats [46]. Mice developing SRS after intrahippocampal injection of kainate have been proposed as a model of pharmacoresistant seizures; these mice have the advantage that the frequency of SRS is so high that drug efficacy can be determined after single dose administration [54, 66]. However, as yet this model has only rarely been used for investigating the antiepileptic efficacy of novel compounds [54].

Based on the logistical problems associated with drug testing in chronic models, models such as the zebrafish or acute rodent seizure models are indispensable when testing large numbers of investigational compounds before evaluating the most interesting compounds in chronic models. One of these acute seizure models, the 6-Hz model of partial seizures in mice, was initially proposed to provide a useful model of therapy-resistant limbic seizures [9], but more recent studies have not confirmed this idea [49]. Rather, the 6-Hz model is a valuable part of a preclinical test battery to further differentiate compounds. Also, a more recent genetic mouse model of Dravet syndrome, in which clinical symptoms of this syndrome occur after Scn1a heterozygous knockout, may be an interesting possibility for testing drugs or drug combinations for treatment of as yet pharmacoresistant types of seizures [59, 60]. Furthermore, a zebrafish Scn1a mutant, such as the one recently described by the Baraban laboratory [8] would be an efficient first pass high-throughput approach to identify potential candidate compounds that can be further investigated in chronic rodent models of pharmacoresistant seizures.

23.4 Naturally Occurring Epilepsy in Dogs as a Translational Model

The dog is an important large animal model in various fields of biomedical research and fills a crucial step in the translation of basic research to new treatment regimens. For instance, because of the relative large body size of dogs and many similarities in physiology and pharmacology between dogs and humans, scaling doses from dogs to humans is much easier than using rodents in selecting doses for clinical trials in humans. To our knowledge, Löscher et al. [37] were the first to propose naturally occurring canine epilepsy as a translational model of human epilepsy. The prevalence and phenomenology of epilepsy in dogs are very similar to human epilepsy. Indeed, epilepsy is the most common chronic neurological disease in dogs, affecting about 0.6–1 % of the dog population [64, 69]. Furthermore, causes of canine epilepsy are similar to those in humans (Fig. 23.2) except that cerebrovascular disease does not play any significant role, because it is rare in dogs [69]. About 50 % of dogs with partial and generalized convulsive seizures are not controlled by treatment with AEDs, so that epileptic dogs have been proposed as a valuable model of pharmacoresistant epilepsy that can be used to unravel mechanisms of resistance and evaluate new strategies for treatment [44, 64]. However, clinical trials on new AEDs in epileptic dogs are as laborious and time-consuming as clinal trials in human patients, necessitating randomized trial designs in which the new drug is compared with either placebo or a standard comparator [57, 58]. Recently, different treatments, including AEDs, vagal stimulation, and ketogenic diet were compared with placebo in epileptic dogs, and an unexpectedly high placebo rate was found, which was similar to that known from controlled clinical trials in humans with epilepsy [57, 58]. In contrast to humans, the placebo effect has been largely disregarded in veterinary medicine. In humans, a placebo response seems to require a recognition by the patient of the intent of treatment efforts. Because it is generally presumed that animals lack certain cognitive capacities, e.g. the ability to comprehend the intent of the veterinarian's manipulations, the power of suggestion, and expectations of recovery and healing, the existence of a placebo effect in animals seems counterintuitive [55]. However, in veterinary studies, the placebo response may be a result of expectations of the pet owner regarding treatment in studies as those conducted by Munana et al.

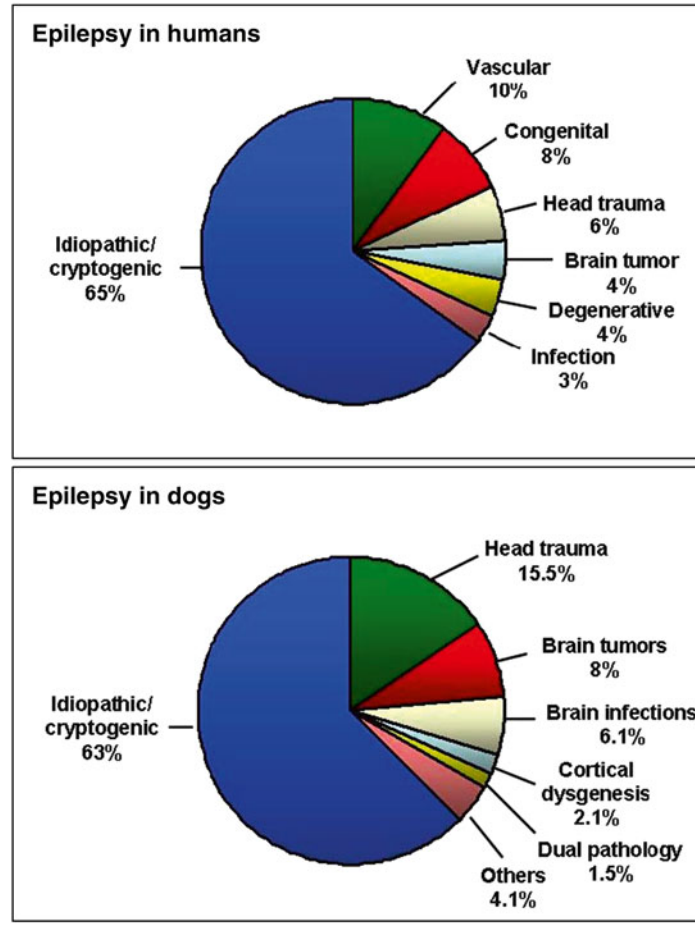

Fig. 23.2 A comparison of the presumed causes of recurrent epileptic seizures in humans and dogs. The graph on humans illustrate the proportion of incidence cases of epilepsy by etiology in Rochester, Minnesota, U.S.A., 1935–1984 [24]; a similar graph was initially shown by Lowenstein [35]. The graph on dogs illustrates data from a recent epidemiologic study on canine epilepsy [69]

[57, 58] in epileptic dogs, where the owners are responsible for administration of treatment and outcome measures (i.e., seizure frequency) are derived solely from owner observations. Other factors that may be included in placebo responses in veterinary studies include regression to the mean, investigator bias, client bias, the potential for a higher level of care during the study, and improved adherence to treatment with active medication that is being administered in addition to the placebo during the study (for details see [57]). Furthermore, the placebo response can be because of effects of placebo administration on the animal, which is well documented in laboratory animals and may involve conditioned responses among others [55]. As a consequence, studies on new treatments in laboratory animals (or pets) should always include a "placebo" group receiving all manipulations (e.g., handling, injections, electrode implantation, seizure recording etc.) that are used for the new treatment.

In addition to chronic epilepsy, naturally occurring canine status epilepticus (SE) has been proposed as a translational platform for evaluating investigational compounds for eventual use in human trials [34] and a controlled study on i.v. levetiracetam for treatment of SE in dogs has been published recently [23].

One important caveat that has to be considered when using dogs for long-term studies on AEDs is that dogs, similar to rodents, eliminate many drugs, including most AEDs, much more rapidly than humans (Table 23.1). Thus, when using AEDs such as phenytoin, carbamazepine or valproate with too low half-lifes for maintenance treatment in epileptic dogs, no sufficient drug

levels and, hence, no antiepileptic effects are obtained in this species [20, 36, 37]. The few AEDs with sufficiently long half-lives for maintenance treatment include phenobarbital, primidone (because of its metabolism to phenobarbital), and potassium bromide, which is the reason why until recently only these old drugs were approved for treatment of canine epilepsy in the US or Europe. This situation has changed by the recent approval of imepitoin for treatment of dogs with newly diagnosed epilepsy (see below). Furthermore, several newer AEDs, including levetiracetam, felbamate, zonisamide, topiramate, gabapentin, and pregabalin are used as add-on treatment in dogs with pharmacoresistant seizures [64]. It has been tried to overcome the problem of too rapid elimination of most AEDs by dogs by using sustained-release formulations; however, sustained-release preparations developed for use in humans are not suited for dogs because of the much higher gastrointestinal passage rate in dogs (~24 h) vs. humans (~65–100 h) [36, 44]. Thus, AED formulations that exhibit retarded release of the drug in the gastrointestinal tract have to be adapted to the dog to overcome problems associated with too rapid drug elimination in this species. For phenytoin, a slow-release preparation has been developed for dogs, by which therapeutic plasma levels could be maintained despite the rapid elimination of this drug in dogs [18], but, to our knowledge, no clinical experience with this preparation has been published. Vigabatrin has been evaluated for control of epilepsy in dogs, because its mechanism of action (irreversible inhibition of GABA degradation) allows an effective treatment which should be independent of species differences in drug elimination. Vigabatrin proved to be effective in epileptic dogs with phenobarbital-resistant seizures, but at least in part vigabatrin had to be withdrawn because of development of severe adverse effects, such as haemolytic anaemia [67].

Löscher's group has used dogs as a translational model over the recent 25 years in the development of a new category of AEDs, i.e., drugs that act as partial agonists at the benzodiazepine (BZD) site of the $GABA_A$ receptor. Such drugs have the wide spectrum of antiepileptic activity against diverse types of seizures as the traditional full BZD agonists such as diazepam, clonazepam or clobazam, but are much better tolerated and lack the tolerance and abuse liability of the full agonists [22, 41]. In our studies, we either used a canine seizure model, in which seizures are induced by i.v. infusion of pentylenetetrazole, or epileptic dogs. The first partial BZD agonist that was characterized in dogs (and compared with full BZD agonists) was the β-carboline abecarnil, providing proof-of-concept that partial BZD agonists are advantageous for treatment of seizures compared to traditional, full-agonist BZDs [39, 41]. More recently, the low-affinity partial BZD agonist imepitoin, an imidazolin derivative, was evaluated in the dog seizure model and epileptic dogs and reported to provide efficacious antiepileptic activity without the known disadvantages of full BZD agonists [45, 51]. Based on several randomized controlled clinical trials in epileptic dogs, imepitoin was recently approved in Europe for treatment of canine epilepsy [13, 51]. That imepitoin is an effective and safe AED in epileptic dogs indicates that low-affinity partial BZD agonists may offer a new mechanistic category of useful AEDs.

23.5 Network Approaches for Development of Novel Treatments

Several of the models described in this review may be particularly interesting for evaluating a novel strategy of AED development, the network approach [3, 26, 50]. One of the dominant strategies in drug discovery is designing maximally selective ligands to act on individual drug targets [26]. However, many effective drugs act via modulation of multiple targets rather than single proteins. Furthermore, most epilepsies develop not from alterations of a single target but rather from complex alterations resulting in an epileptic network in the brain. The only existing cure of epilepsy is resective surgery in which the regional epileptic network or part of this network is removed. Thus, treatments focusing exclusively

on a single protein or individual biochemical pathway may be less effective than treatments targeting different proteins or pathways involved in the network. The latter approach has been recently termed "network pharmacology" and relates to principles of systems biology [3, 26]. The principle of network pharmacology is to develop combinations of existing drugs, which regulate activity via different targets within a biological network, for diseases that do not sufficiently respond to single drug treatment or for which no treatment exists. Integrating network biology and polypharmacology holds the promise of expanding the current opportunity space for druggable targets [26]. However, the rational design of polypharmacology faces considerable challenges in the need for new methods to validate target combinations and optimize multiple structure-activity relationships while maintaining drug-like properties. The advances in zebrafish chemical screening technologies may allow rapid identification of the most interesting drug combinations resulting from network approaches, followed by evaluating these combinations in chronic models of epilepsy.

Some examples for interesting network approaches include combinations of glutamate receptor antagonists that target different glutamate receptor subtypes. We reported that extremely low doses of the NMDA (N-methyl-D-aspartate) receptor antagonist MK-801 (dizocilpine) markedly potentiated the anticonvulsant effect the AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline) without increasing its adverse effects [42]. Similar over-additive effects were seen when NBQX was combined with the competitive NMDA antagonist CGP39551 (the carboxyethylester of DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid) or the low-affinity, rapidly channel blocking NMDA receptor antagonist memantine [42, 43]. We are currently evaluating combinations of clinically approved NMDA antagonists (ketamine, memantine) and the novel AMPA antagonist perampanel in models of difficult-to-treat seizures. Another interesting example is the combination of phenobarbital with the diuretic bumetanide, which is currently evaluated clinically following promising preclinical data [31, 52]. The biologically plausible idea behind this combination is that a shift from inhibitory to excitatory GABA may be involved in difficult-to-treat neonatal and adult seizures [30, 56]. GABA-mediated excitation has been observed when expression of the chloride importer NKCC1 is higher than expression of the chloride exporter KCC2; e.g., early during development and in the hippocampus of adults with temporal lobe epilepsy [30, 56]. Bumetanide inhibits the neuronal chloride cotransporter NKCC1, thereby reverts the GABA shift and enables GABAmimetic drugs such as phenobarbital to potentiate inhibitory GABAergic transmission [52]. This recent work builds on an earlier demonstration from the Schwartzkroin laboratory that furosemide, another chloride cotransporter inhibitor, exhibits powerful anticonvulsant activity across a range of *in vitro* and in vivo seizure models [25]. Further examples for interesting network approaches include combined targeting of different inflammatory pathways, which are involved in seizure generation [33]. These examples strongly indicate that combinatorial treatment strategies offer new options for epilepsy therapy.

23.6 Conclusions

Models for the discovery of drugs with antiepileptic activity have traditionally relied on a relatively small number of acute seizure models employed in otherwise healthy rodents. While useful in the discovery of most drugs currently available in the clinic, more resistant types of epilepsies including temporal lobe epilepsy patients who are unresponsive to available AEDs and catastrophic, often genetically-based, types of epilepsies seen in children necessitate alternative drug discovery strategies. Zebrafish, canine and novel rodent approaches are described here and offer several unique advantages over these traditional models. While these strategies, like any animal model approach also have their limitations, they offer hope that new classes of

AEDs will be identified in the coming years. Furthermore, animal models in which epilepsy develops after brain insults or gene mutations are essential in the search for novel antiepileptogenic treatments that prevent or modify the development of epilepsy in patients at risk [47, 63]. Previously, this field was dominated by studies in SE models in rats, although SE is only rarely a cause of symptomatic epilepsy [47]. Thus, models of more common causes of acquired epilepsy, such as traumatic brain injury, and models in which epilepsy develops after gene mutations should be used more extensively in research on antiepileptogenesis. We have started to use the zebrafish and canine approaches to identify molecular pathways that may be involved in the epileptogenic process and may offer new targets for antiepileptogenic treatments.

Acknowledgements With gratitude and special thanks to Scott Baraban's postdoctoral mentor Phil Schwartzkroin. Scott's years in Seattle were rich in scientific interactions and opportunities. The environment created by Phil and Scott's fellow trainees (Daryl Hochman, Jim Owens, Catherine Woolley and Jurgen Wenzel) was conducive to open discussion, lively debate and exciting discoveries. Phil's scholarly approach to science and passion for epilepsy research was a guiding force in Scott's career. With the laboratory at UCSF, Scott strives to carry on some of these same principles. Wolfgang Löscher acknowledges the many thoughtful and constructive discussions with Phil that he had as an author of invited reviews in Epilepsia during the many years that Phil acted as a Managing Editor for this journal.

Other Acknowledgements Scott Baraban acknowledges funding from the National Institutes of Health, Citizens United for Research in Epilepsy and Dravet Syndrome Foundation, and Wolfgang Löscher funding from the German Research Foundation, the FP7 program of the European Commission and the National Institutes of Health.

References

1. Afrikanova T, Serruys AS, Buenafe OE, Clinckers R, Smolders I, de Witte PA et al (2013) Validation of the zebrafish pentylenetetrazol seizure model: locomotor versus electrographic responses to antiepileptic drugs. PLoS One 8(1):e54166
2. Ahrens MB, Li JM, Orger MB, Robson DN, Schier AF, Engert F et al (2012) Brain-wide neuronal dynamics during motor adaptation in zebrafish. Nature 485(7399):471–477
3. Ainsworth C (2011) Networking for new drugs. Nat Med 17(10):1166–1168
4. Bankstahl JP, Bankstahl M, Kuntner C, Stanek J, Wanek T, Meier M et al (2011) A novel positron emission tomography imaging protocol identifies seizure-induced regional overactivity of P-glycoprotein at the blood-brain barrier. J Neurosci 31(24):8803–8811
5. Baraban SC, Taylor MR, Castro PA, Baier H (2005) Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. Neuroscience 131(3):759–768
6. Baraban SC, Dinday MT, Castro PA, Chege S, Guyenet S, Taylor MR (2007) A large-scale mutagenesis screen to identify seizure-resistant zebrafish. Epilepsia 48(6):1151–1157
7. Baraban SC (2013) Forebrain electrophysiological recording in larval zebrafish. J Vis Exp (71). pii: 50104
8. Baraban SC, Dinday MT, Hortopan GA (2013) Drug screening and transcriptomic analysis in Scn1a zebrafish mutants identifies potential lead compound for Dravet Syndrome. Nat Commun 4:2410
9. Barton ME, Klein BD, Wolf HH, White HS (2001) Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy. Epilepsy Res 47:217–228
10. Baxendale S, Holdsworth CJ, Meza Santoscoy PL, Harrison MR, Fox J, Parkin CA et al (2012) Identification of compounds with anti-convulsant properties in a zebrafish model of epileptic seizures. Dis Model Mech 5(6):773–784
11. Berghmans S, Hunt J, Roach A, Goldsmith P (2007) Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants. Epilepsy Res 75(1):18–28
12. Bialer M, White HS (2010) Key factors in the discovery and development of new antiepileptic drugs. Nat Rev Drug Discov 9(1):68–82
13. Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS (2013) Progress report on new antiepileptic drugs: a summary of the Eleventh Eilat Conference (EILAT XI). Epilepsy Res 103(1):2–30
14. Brandt C, Volk HA, Löscher W (2004) Striking differences in individual anticonvulsant response to phenobarbital in rats with spontaneous seizures after status epilepticus. Epilepsia 45:1488–1497
15. Brandt C, Bethmann K, Gastens AM, Löscher W (2006) The multidrug transporter hypothesis of drug resistance in epilepsy: proof-of-principle in a rat model of temporal lobe epilepsy. Neurobiol Dis 24:202–211
16. Cario CL, Farrell TC, Milanese C, Burton EA (2011) Automated measurement of zebrafish larval movement. J Physiol 589(Pt 15):3703–3708
17. Chege SW, Hortopan GA, Dinday T, Baraban SC (2012) Expression and function of KCNQ channels in larval zebrafish. Dev Neurobiol 72(2):186–198
18. Derkx-Overduin LM (1994) Slow-release phenytoin in canine epilepsy. Thesis, Faculty of Veterinary Medicine, Utrecht, the Netherlands

19. Feldmann M, Asselin MC, Liu J, Wang S, McMahon A, Anton-Rodriguez J et al (2013) P-glycoprotein expression and function in patients with temporal lobe epilepsy: a case-control study. Lancet Neurol 12:777–785
20. Frey H-H, Löscher W (1985) Pharmacokinetics of anti-epileptic drugs in the dog: a review. J Vet Pharmacol Ther 8:219–233
21. Friedrich RW, Genoud C, Wanner AA (2013) Analyzing the structure and function of neuronal circuits in zebrafish. Front Neural Circuits 7:71
22. Haefely W, Facklam M, Schoch P, Martin JR, Bonetti EP, Moreau JL et al (1992) Partial agonists of benzodiazepine receptors for the treatment of epilepsy, sleep, and anxiety disorders. Adv Biochem Psychopharmacol 47:379–394
23. Hardy BT, Patterson EE, Cloyd JM, Hardy RM, Leppik IE (2012) Double-masked, placebo-controlled study of intravenous levetiracetam for the treatment of status epilepticus and acute repetitive seizures in dogs. J Vet Intern Med 26:334–340
24. Hauser WA, Annegers JF, Kurland LT (1993) Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935–1984. Epilepsia 34:453–468
25. Hochman DW, Baraban SC, Owens JW, Schwartzkroin PA (1995) Dissociation of synchronization and excitability in furosemide blockade of epileptiform activity. Science 270:99–102
26. Hopkins AL (2008) Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol 4(11): 682–690
27. Hortopan GA, Dinday MT, Baraban SC (2010) Spontaneous seizures and altered gene expression in GABA signaling pathways in a mind bomb mutant zebrafish. J Neurosci 30(41):13718–13728
28. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M et al (2013) The zebrafish reference genome sequence and its relationship to the human genome. Nature 496(7446):498–503
29. Hunt RF, Hortopan GA, Gillespie A, Baraban SC (2012) A novel zebrafish model of hyperthermia-induced seizures reveals a role for TRPV4 channels and NMDA-type glutamate receptors. Exp Neurol 237(1):199–206
30. Kahle KT, Staley KJ, Nahed BV, Gamba G, Hebert SC, Lifton RP et al (2008) Roles of the cation-chloride cotransporters in neurological disease. Nat Clin Pract Neurol 4(9):490–503
31. Kahle KT, Staley KJ (2008) The bumetanide-sensitive Na-K-2Cl cotransporter NKCC1 as a potential target of a novel mechanism-based treatment strategy for neonatal seizures. Neurosurg Focus 25(3):1–8
32. Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen HW, Mathern G et al (2010) Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia 51(6):1069–1077
33. Kwon YS, Pineda E, Auvin S, Shin D, Mazarati A, Sankar R (2013) Neuroprotective and antiepileptogenic effects of combination of anti-inflammatory drugs in the immature brain. J Neuroinflammation 10:30
34. Leppik IE, Patterson EN, Coles LD, Craft EM, Cloyd JC (2011) Canine status epilepticus: a translational platform for human therapeutic trials. Epilepsia 52(Suppl 8):31–34
35. Lowenstein DH (2009) Epilepsy after head injury: an overview. Epilepsia 50(Suppl 2):4–9
36. Löscher W (1981) Plasma levels of valproic acid and its metabolites during continued treatment in dogs. J Vet Pharmacol Ther 4:111–119
37. Löscher W, Schwartz-Porsche D, Frey H-H, Schmidt D (1985) Evaluation of epileptic dogs as an animal model of human epilepsy. Arzneim-Forsch (Drug Res) 35:82–87
38. Löscher W (1986) Experimental models for intractable epilepsy in nonprimate animal species. In: Schmidt D, Morselli PL (eds) Intractable epilepsy: experimental and clinical aspects. Raven Press, New York, pp 25–37
39. Löscher W, Hönack D, Scherkl R, Hashem A, Frey H-H (1990) Pharmacokinetics, anticonvulsant efficacy and adverse effects of the β-carboline abecarnil, a novel ligand for benzodiazepine receptors, after acute and chronic administration in dogs. J Pharmacol Exp Ther 255:541–548
40. Löscher W, Rundfeldt C (1991) Kindling as a model of drug-resistant partial epilepsy: selection of phenytoin-resistant and nonresistant rats. J Pharmacol Exp Ther 258:483–489
41. Löscher W (1993) Abecarnil shows reduced tolerance development and dependence potential in comparison to diazepam: animal studies. In: Stephens DN (ed) Anxiolytic β-carbolines. From molecular biology to the clinic. Springer, Berlin, pp 96–112
42. Löscher W, Rundfeldt C, Hönack D (1993) Low doses of NMDA receptor antagonists synergistically increase the anticonvulsant effect of the AMPA receptor antagonist NBQX in the kindling model of epilepsy. Eur J Neurosci 5:1545–1550
43. Löscher W, Hönack D (1994) Over-additive anticonvulsant effect of memantine and NBQX in kindled rats. Eur J Pharmacol 259:R3–R5
44. Löscher W (1997) Animal models of intractable epilepsy. Prog Neurobiol 53:239–258
45. Löscher W, Potschka H, Rieck S, Tipold A, Rundfeldt C (2004) Anticonvulsant efficacy of the low-affinity partial benzodiazepine receptor agonist ELB 138 in a dog seizure model and in epileptic dogs with spontaneously recurrent seizures. Epilepsia 45(10):1228–1239
46. Löscher W (2007) The pharmacokinetics of antiepileptic drugs in rats: consequences for maintaining effective drug levels during prolonged drug administration in rat models of epilepsy. Epilepsia 48:1245–1258
47. Löscher W, Brandt C (2010) Prevention or modification of epileptogenesis after brain insults: experimental approaches and translational research. Pharmacol Rev 62:668–700
48. Löscher W, Schmidt D (2011) Modern antiepileptic drug development has failed to deliver: ways out of the current dilemma. Epilepsia 52(4):657–678

49. Löscher W (2011) Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 20:359–368
50. Löscher W, Klitgaard H, Twyman RE, Schmidt D (2013) New avenues for antiepileptic drug discovery and development. Nat Rev Drug Discov 12:757–776
51. Löscher W, Hoffmann K, Twele F, Potschka H, Töllner K (2013) The novel antiepileptic drug imepitoin compares favourably to other GABA-mimetic drugs in a seizure threshold model in mice and dogs. Pharmacol Res 77:39–46
52. Löscher W, Puskarjov M, Kaila K (2013) Cation-chloride cotransporters NKCC1 and KCC2 as potential targets for novel antiepileptic and antiepileptogenic treatments. Neuropharmacology 69:62–74
53. Mahmood F, Mozere M, Zdebik AA, Stanescu HC, Tobin J, Beales PL et al (2013) Generation and validation of a zebrafish model of EAST (epilepsy, ataxia, sensorineural deafness and tubulopathy) syndrome. Dis Model Mech 6(3):652–660
54. Maroso M, Balosso S, Ravizza T, Iori V, Wright CI, French J et al (2011) Interleukin-1beta biosynthesis inhibition reduces acute seizures and drug resistant chronic epileptic activity in mice. Neurotherapeutics 8(2):304–315
55. McMillan FD (1999) The placebo effect in animals. J Am Vet Med Assoc 215(7):992–999
56. Miles R, Blaesse P, Huberfeld G, Wittner L, Kaila K (2012) Chloride homeostasis and GABA signaling in temporal lobe epilepsy. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV (eds) Jasper's basic mechanisms of the epilepsies, 4th edn. Oxford University Press, New York, pp 581–590
57. Munana KR, Zhang D, Patterson EE (2010) Placebo effect in canine epilepsy trials. J Vet Intern Med 24(1):166–170
58. Munana KR, Thomas WB, Inzana KD, Nettifee-Osborne JA, McLucas KJ, Olby NJ et al (2012) Evaluation of levetiracetam as adjunctive treatment for refractory canine epilepsy: a randomized, placebo-controlled, crossover trial. J Vet Intern Med 26:341–348
59. Oakley JC, Kalume F, Catterall WA (2011) Insights into pathophysiology and therapy from a mouse model of Dravet syndrome. Epilepsia 52(Suppl 2):59–61
60. Oakley JC, Cho AR, Cheah CS, Scheuer T, Catterall WA (2013) Synergistic GABA-enhancing therapy against seizures in a mouse model of Dravet syndrome. J Pharmacol Exp Ther 345(2):215–224
61. Panier T, Romano SA, Olive R, Pietri T, Sumbre G, Candelier R et al (2013) Fast functional imaging of multiple brain regions in intact zebrafish larvae using Selective Plane Illumination Microscopy. Front Neural Circuits 7:65
62. Perucca E, French J, Bialer M (2007) Development of new antiepileptic drugs: challenges, incentives, and recent advances. Lancet Neurol 6(9):793–804
63. Pitkänen A, Lukasiuk K (2011) Mechanisms of epileptogenesis and potential treatment targets. Lancet Neurol 10(2):173–186
64. Potschka H, Fischer A, von Rüden EL, Hülsmeyer V, Baumgärtner W (2013) Canine epilepsy as a translational model? Epilepsia 54(4):571–579
65. Ramirez IB, Pietka G, Jones DR, Divecha N, Alia A, Baraban SC et al (2012) Impaired neural development in a zebrafish model for Lowe syndrome. Hum Mol Genet 21(8):1744–1759
66. Riban V, Bouilleret V, Pham L, Fritschy JM, Marescaux C, Depaulis A (2002) Evolution of hippocampal epileptic activity during the development of hippocampal sclerosis in a mouse model of temporal lobe epilepsy. Neuroscience 112(1):101–111
67. Speciale J, Dayrell-Hart B, Steinberg SA (1991) Clinical evaluation of gamma-vinyl-gamma-aminobutyric acid for control of epilepsy in dogs. J Am Vet Med Assoc 198:995–1000
68. Srivastava AK, White HS (2013) Carbamazepine, but not valproate, displays pharmacoresistance in lamotrigine-resistant amygdala kindled rats. Epilepsy Res 104:26–34
69. Steinmetz S, Tipold A, Löscher W (2013) Epilepsy after head injury in dogs: a natural model of posttraumatic epilepsy. Epilepsia 54(4):580–588
70. Teng Y, Xie X, Walker S, Saxena M, Kozlowski DJ, Mumm JS et al (2011) Loss of zebrafish lgi1b leads to hydrocephalus and sensitization to pentylenetetrazol induced seizure-like behavior. PLoS One 6(9):e24596
71. Watanabe Y, Takechi K, Fujiwara A, Kamei C (2010) Effects of antiepileptics on behavioral and electroencephalographic seizure induced by pentetrazol in mice. J Pharmacol Sci 112(3):282–289