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Molecular Targets *Versus* Models for New Antiepileptic Drug Discovery

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Abstract

Animal models have played a key role in the discovery and characterization of all marketed antiepileptic drugs (AED). The conventional wisdom is that the standard animal screening models are becoming obsolete because they fail to identify compounds that act in mechanistically new ways and as a result do not offer therapeutic advantages over presently available agents. In fact, far from only detecting me-too drugs, the models often uncover compounds with distinctive profiles of activity in various types of epilepsy and in addition have unexpected efficacy in non-epilepsy conditions, such as neuropathic pain, bipolar disorder, and migraine. Moreover, the animal models—because they are unbiased with respect to mechanism—provide an opportunity to uncover drugs that act in new ways and through new targets, such as $\alpha 2\delta$ and SV2A. In vitro testing is not likely to replace screening in animal models because in vitro systems cannot model the specific pharmacodynamic actions required for seizure protection, and do not assess bioavailability and brain accessibility.

1. Introduction

Most antiepileptic drugs (AEDs) were discovered through screening in animal models. Such models fall into two main categories: models of acute seizures (non-epileptic animals induced to have a seizure by an electrical or chemical stimulus) and models of chronic epilepsy (animals induced to have enhanced seizure susceptibility or spontaneous seizures). For practical reasons, rodents (rats, mice and Mongolian gerbils) are the most commonly used species, although many mammalian species—including dogs and primates—experience seizures and their brain architecture and physiology are arguably closer to that of humans. Also, for practical reasons, screening is often carried out using acute seizure models, although various types of kindling models, a class of chronic model, are commonly included in the battery of tests to which early stage compounds are subjected (White et al., 2002). Despite the diversity of models that could potentially be used to screen for anticonvulsant activity, the maximal electroshock model (MES) and the subcutaneous pentylenetetrazol model (s.c. MET) remain the “gold standards” in the early stages of testing. An alternative electroshock test—the 6-Hz model, in which the end point is limbic seizure activity rather than tonic hind limb extension—is now also used in initial screening. Other models, including audiogenic seizure susceptible mice, various chemoconvulsant models and either amygdala or corneal kindling are invariably used to further characterize a potential clinical candidate. Compounds active in one or both of the MES and s.c. MET tests have generally been efficacious in clinical trials. A notable exception has been NMDA receptor antagonists, which are highly active in the MES test but did not fare well in the clinic largely because of poor efficacy (Löscher and Rogawski, 2002). It is often stated: “Old models recapitulate old drugs.” Here, I consider two questions. First, is the conventional

wisdom about the acute models true: do they really just discover me-too drugs that act in mechanistically similar ways to drugs already available on the market and offer little clinical benefit? Second, is screening in animal models even necessary at all?

2. Do conventional animal screening models lead to the discovery of me-too drugs?

2.1. “Me-too” drugs are drugs that largely duplicate the actions of existing drugs and offer little or no therapeutic gain. Since the approval of felbamate in August 1993, nine new AEDs have become available: felbamate, lamotrigine, gabapentin, topiramate, oxcarbazepine, vigabatrin, zonisamide, levetiracetam, and pregabalin. There is now over a decade of experience with these second-generation drugs, yet the number of patients who continue to have uncontrolled epilepsy has not been measurably reduced. A reasonable inference is that the screening models used for AED discovery only identify me-too drugs that act by the same old physiological mechanisms and that new models of treatment-resistant epilepsy are needed to identify drugs with different mechanisms of action (Brodie, 2001). Does the evidence support this conclusion?

2.2. To address this question, it is useful to consider the broad range of uses of the major AEDs. As shown in Table 1, the so-called sodium channel blocking AEDs (many of which also block various types of voltage-activated calcium channels) do not all have identical clinical utilities. In fact, while the older sodium channel blockers phenytoin and carbamazepine are inactive—and may in some cases worsen—absence seizures, lamotrigine is effective for absence seizures. The discovery that lamotrigine is effective in the treatment of absence epilepsy was a surprise and clearly distinguished the drug from other sodium channel blocking AEDs. Lamotrigine is also effective in juvenile myoclonic epilepsy (JME; Buchanan, 1996) whereas other sodium channel blocking anticonvulsants are inactive against myoclonic seizures or may worsen them. It was a further surprise that lamotrigine is effective in bipolar disorder, with particular activity in acute bipolar depression and also rapid-cycling bipolar disorder (Goodwin et al., 2004). In contrast, most other AEDs that have been studied in bipolar disorder seem mainly to be useful in the acute treatment of mania. Other new AEDs have also been found to have an enhanced spectrum of activity. For example, valproate and topiramate are probably broadly active in primary generalized epilepsies, including absence and JME. These two drugs are now registered for migraine prophylaxis. Similarly, gabapentin has been found to be very useful for neuropathic pain and migraine prophylaxis; pregabalin is also effective for neuropathic pain. All of these new drugs—lamotrigine, topiramate, gabapentin and pregabalin—are effective in traditional AED screening models. Based upon the results of the preclinical testing, it was assumed that lamotrigine and topiramate would be me-too drugs that would largely duplicate the clinical activities of phenytoin and carbamazepine. As experience with these drugs has accrued, this assumption was found to be incorrect. A lesson learned is that it is not possible to predict the potential clinical uses of AEDs based upon their activities in present-day animal screening models; indeed, the models routinely seem to uncover drugs with unique and unexpected clinical utilities.

2.3. A corollary to the clinical lesson is that it is not unusual for animal screening models to identify antiepileptic drugs with novel molecular mechanisms of action. Thus, novel chemical structures identified in screening models may act on well-recognized targets in novel ways or by novel combinations of actions on well-recognized targets. In some cases, the screening models have revealed entirely new AED targets. For example, lamotrigine and topiramate were identified largely by activity in the MES test, yet their pharmacodynamic actions must be different from the prototypical sodium channel blocking AEDs phenytoin and carbamazepine since they have distinct clinical activities. In fact, while all four drugs do interact with fast transient and persistent sodium channels, lamotrigine at clinically relevant concentrations also has substantial effects on high voltage-activated calcium channels (Stefani et al., 1996).

Topiramate has these activities on sodium and calcium channels, and in addition potentiates a subset of GABA receptors and also blocks GluR5 kainate receptors, a new target (White et al., 1997; Gryder and Rogawski, 2003; Rogawski and Löscher, 2004a).

2.4. Gabapentin and levetiracetam provide examples where the screening models have identified AEDs that act on entirely new molecular targets for AEDs. Evidence from a variety of experimental approaches has revealed that the primary target for gabapentin and pregabalin is the $\alpha 2\delta$ subunit of voltage-activated calcium channels (Gee et al., 1996). Binding at this site leads to a reduction in the release of neurotransmitters, including glutamate (Dooley et al., 2000). Recently, SV2A, a synaptic vesicle protein, has been identified as the likely target for levetiracetam (Lynch et al., 2004) and two more potent follow-on structural analogs seletracetam (ucb 44212) and brivaracetam (ucb 34714). The mechanism whereby binding to SV2A results in anticonvulsant activity is unknown. SV2A is an abundant protein component of synaptic vesicles that is structurally similar to 12-transmembrane domain transporters, although a transporter activity has not yet been identified. SV2A is not essential for synaptic transmission, but knockout of the protein (along with the closely related protein SV2B which appears to be able to substitute for SV2A) in mice leads to seizures (Janz et al., 1999). The effect of levetiracetam may be similar to that of AEDs that target voltage-activated sodium and calcium channels, including $\alpha 2\delta$, which largely act by inhibition of glutamate release at excitatory synapses (Rogawski and Löscher, 2004a).

2.5. Levetiracetam is not active in the traditional MES or s.c. MET AED screening models when conducted according to standard protocols (Klitgaard et al., 1998; Klitgaard, 2001). Nevertheless, the drug was discovered in another common screening model: audiogenic seizures in susceptible mice, and subsequently found to have activity in a range of chemoconvulsant models, including seizures induced by sub-maximal pentylenetetrazol doses (Gower et al., 1992), the 6-Hz model, and also various kindling models, including amygdala kindled rats, where it potently inhibits fully kindled seizures (Löscher and Hönack, 1993). Levetiracetam is active in a rat genetic model of absence epilepsy (Gower et al., 1995), which predicts its likely clinical activity in human absence epilepsy (Table 1). Levetiracetam also seems to have “antiepileptogenic” activity, in that it retards the development of pentylenetetrazol—kindled seizures (Gower et al., 1992) as well as conventional amygdala-kindled seizures (Löscher et al., 1998). Gabapentin and levetiracetam are novel AEDs with distinct clinical profiles that were identified by presently available epilepsy screening models. Remarkably, these compounds have, in turn, led to the discovery of two entirely new drug targets for AEDs— $\alpha 2\delta$ and SV2A. These targets can now be used to screen for congeners with improved properties. Indeed, this is how seletracetam and brivaracetam were identified.

3. Is screening in animal models necessary?

3.1. Modern cellular neurophysiological and biochemical approaches have made it possible to identify the likely molecular targets of AEDs, which include voltage-activated sodium and calcium channels, GABA_A receptors, ionotropic glutamate receptors, GABA transporters, GABA transaminase and now the synaptic vesicle protein SV2A (Rogawski and Löscher, 2004a). Given this growing list of protein targets, do we still need to screen compounds for anticonvulsant activity using low-throughput animal models? Would screening of chemical libraries against the recombinant proteins be a more efficient approach to identifying new AEDs? There are two key reasons why this strategy is not likely to yield new clinically useful AEDs. First, for ion channel targets—the predominant class of molecules through which AEDs act to exert their therapeutic effects—it is not possible to predict anticonvulsant activity simply on the basis of binding affinity or even on the results of more specific biophysical studies examining the functional effects of the drug (for example, voltage-clamp studies assessing the ability of the drug to modify channel conductance and gating). In considering anticonvulsant

activity, we always assess protection against seizure activity in the context of a drug's propensity to produce neurobehavioral side effects. Optimizing anticonvulsant activity is not a sufficient goal. We must optimize inhibition of seizure-related neuronal firing while the drug at the same time preserves normal physiological activity, a far more challenging task. AEDs that are capable of protecting against seizure activity at doses that do not cause unacceptable neurobehavioral impairment modulate the physiological activity of their ion channel targets in subtle ways and the specific details of the interactions, although not well defined, are critical. For example, sodium channel blocking AEDs like phenytoin bind with relatively low affinity to sodium channels. Indeed, a very large number of drug substances that do not have clinically useful anticonvulsant activity—including adrenoceptor, histamine and serotonin receptor ligands; neuroleptics; and tricyclic antidepressants—actually modulate voltage-activated sodium channels more potently than do sodium channel blocking AEDs (McNeal et al., 1985). Optimizing binding affinity is of no use in identifying molecules that possess useful anticonvulsant properties. A critical property of the sodium channel-blocking AEDs is that binding occurs in a state-dependent fashion, mainly to the inactivated conformation of the channel. Another key feature of sodium channel-blocking AEDs is that onset of block (binding) occurs slowly and recovery from block is also slow. Slow binding is crucial inasmuch as it allows fast action potentials evoked by synaptic depolarizations of ordinary length to remain unaffected by the drug while action potential bursts associated with long depolarizations, like those that occur with epileptic seizures, are inhibited. These features explain the ability of sodium channel-blocking AEDs to selectively inhibit high frequency action potential firing during pathological depolarizing events without interfering with normal firing. Thus, a useful AED that binds to the appropriate site on the target molecule must have just the right biophysical properties with respect to multiple kinetic, steric and energetic characteristics. It must also not interact with other critical biomolecules which would cause side effects or interfere with the desired activity. These characteristics constitute a multidimensional parameter space; neither the full set of relevant parameters nor the subset of the parameter space that specifies when a drug has useful anticonvulsant properties is known. Even if we knew which parameters were particularly important (for example, binding rate to the inactivated state), simply optimizing one or another parameter would not likely be of much utility. The optimization would not necessarily cause the parameter set to remain within the parameter space subset that confers clinically useful anticonvulsant activity inasmuch as there are likely complex interactions between parameters. Many AEDs act on more than one molecular target. This further complicates the challenge of optimization since more preferred interactions at one target would have unpredictable effects on other targets.

3.2. A second reason that screening against protein targets is not likely to lead to clinically useful AEDs is that such a screening approach does not assess bioavailability, brain accessibility, and local delivery to the target. That these factors are critical is obvious, but an example is nonetheless instructive. Gabapentin was discovered by screening in animal models. (Actually, the program that led to the discovery of gabapentin represented an attempt at rational drug design: GABA analogs were synthesized with the objective of creating a blood–brain barrier permeable GABA agonist, but gabapentin—although it did exhibit anticonvulsant activity—was later found not to interact with GABA receptors.) Recently, it has been learned that the system L transporter plays a critical role in conveying gabapentin and its analog pregabalin, which are zwitterionic molecules, into the systemic circulation following oral dosing and across the blood–brain barrier (Schwarz et al., 2005). Thus, analogs of gabapentin and pregabalin that are not substrates for this transporter do not have systemic anticonvulsant activity, even though they may protect against seizures when administered intraventricularly. Interestingly, the structure–activity relationships for $\alpha 2\delta$ and system L transporter activity are distinct. Optimizing $\alpha 2\delta$ modulatory activity to produce an intrinsically superior anticonvulsant substance would not necessarily result in a clinically useful AED since system L transporter substrate activity must be retained (Bellotti et al., 2005). In contrast to in vitro screening

approaches, animal test systems only select compounds that are inherently anticonvulsant and are able to access the relevant brain targets. In sum, the use of in vitro test systems for early AED identification is highly problematic and even optimization of a validated lead must be approached with caution. Moreover, limiting the screening to recognized targets eliminates the possibility of identifying drugs that act on as yet unknown targets.

4. Conclusions

Animal AED screening models are efficient and proven tools to identify new anticonvulsant compounds with clinical potential. Indeed, none of the currently approved AEDs—with their diverse and often distinctive clinical activities (Table 1)—would have been identified if they had not exhibited activity in such animal models. The models also represent powerful systems for detecting novel molecular targets for epilepsy therapy. Animal test systems have the important characteristic that they are unbiased with respect to assumptions about the specific nature of the target: any biomolecule could, in principle, be detected as a target. Having defined a novel target on the basis of studies in animal models, it should theoretically be possible to use in vitro systems to optimize the activity of a lead compound and detect related chemical structures with anticonvulsant properties. However, as noted, in vitro systems have only limited utility in AED discovery and it is always necessary to validate activity using animal models.

There is considerable interest in including models of refractory pharmacoresistant seizures and chronic epilepsy in the panel of tests used in the early identification of anticonvulsant compounds. Perhaps these newer models will provide the key to identifying drugs that will allow a substantial proportion of intractable epilepsy patients to achieve seizure freedom. However, at present there are no validated models of refractory epilepsy and chronic epilepsy models are technically difficult and not suited to routine screening. For the foreseeable future, acute animal screening models will continue to be essential tools in AED discovery. We can expect that these models will from time-to-time uncover novel AEDs with unanticipated clinical activities and unique mechanisms of action. Now that powerful biochemical tools are available to aid in the identification of the protein targets of drugs, the models have become platforms for the discovery of novel molecular targets. In the broad perspective, AED screening models have the powerful capability to detect drug substances with central nervous system activity that have acceptable neurobehavioral toxicity characteristics. Such substances have potential utility in a wide range of neurological and psychiatric conditions apart from epilepsy (Rogawski and Löscher, 2004b).

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Table 1**Selected Therapeutic Activities of Marketed Antiepileptic Drugs**

Antiepileptic Drug	Therapeutic Activities	Non-Epilepsy Conditions
	Epilepsy	
Predominant sodium (and calcium) channel activity		
Phenytoin	Partial seizures, GTC seizures <i>INACTIVE: absence, myoclonus</i>	Trigeminal neuralgia
Carbamazepine	Partial seizures, GTC seizures <i>INACTIVE: absence, myoclonus</i>	Trigeminal neuralgia, neuropathic pain, bipolar mania
Oxcarbazepine	Partial seizures, GTC seizures <i>INACTIVE: absence, myoclonus</i>	Neuropathic pain, bipolar mania
Lamotrigine	Partial seizures, GTC seizures, absence , myoclonus (JME), Lennox-Gastaut	Neuropathic pain, trigeminal neuralgia, bipolar depression
Zonisamide	Partial seizures, GTC seizures, myoclonus (JME), absence , infantile spasms, Lennox-Gastaut	Bipolar mania, bipolar depression (open label studies only)
Mixed, complex or novel actions		
Valproate	Partial seizures, GTC seizures, myoclonus (JME), absence	Migraine prophylaxis , bipolar mania
Felbamate	Partial seizures, Lennox-Gastaut	
Topiramate	Partial seizures, GTC seizures, myoclonus (JME), absence	Migraine prophylaxis , essential tremor
Gabapentin	Partial seizures, GTC <i>INACTIVE: absence, myoclonus</i>	Neuropathic pain , anxiety, migraine prophylaxis
Pregabalin	Partial seizures	Neuropathic pain , anxiety
Levetiracetam	Partial seizures, myoclonus (JME), absence	
Phenobarbital	Partial seizures, GTC seizures <i>INACTIVE: absence</i>	
GABA-mediated mechanisms		
Benzodiazepines	Partial seizures, GTC seizures, absence, myoclonus	Anxiety, essential tremor
Vigabatrin	Partial seizures, infantile spasms <i>INACTIVE: absence, myoclonus</i>	
Tiagabine	Partial seizures <i>INACTIVE: absence</i>	Anxiety

Approved or recommended indications that differentiate the drug from others in its class are shown in bold; other differentiating indications are also listed. The labeled neuropathic pain indication for gabapentin is postherpetic neuralgia, and for pregabalin is diabetic peripheral neuropathy and postherpetic neuralgia. GTC, generalized tonic-clonic seizures; JME, juvenile myoclonic epilepsy.