Epilepsy Research, 12 (1992) 87–110
0920-1211/92/$05.00

EPIRES 00482

Chemical models of epilepsy with some reference to their applicability in the development of anticonvulsants

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(Accepted 10 April 1992)

Key words: Epilepsy; Chemical model; Convulsant; Anticonvulsant; Drug screening

This paper reviews chemical models of epilepsy and their relevance in the identification and characterization of anticonvulsants. For each convulsant we discuss possible modes of administration, clinical type(s) of seizures induced, proposed mechanism(s) of epileptogenesis and, where available, responsiveness of the induced seizures to anticonvulsants.

The following compounds are reviewed: pentylenetetrazol, bicuculline, penicillin, picrotoxin, β-carbolines, 3-mercaptopropionic acid, hydrazides, allylglycine; the glycine antagonist strychnine; γ-hydroxybutyrate; excitatory amino acids (glutamate, aspartate, *N*-methyl-D-aspartate, quisqualate, kainate, quinolinic acid); monosubstituted guanidino compounds, metals (alumina, cobalt, zinc, iron); neuropeptides (opioid peptides, corticotropin releasing factor, somatostatin, vasopressin); cholinergic agents (acetylcholine, acetylcholinesterase inhibitors, pilocarpine); tetanus toxin; flurothyl; folates; homocysteine and colchicine.

Although there are a multitude of chemical models of epilepsy, only a limited number are applied in the routine screening of potential anticonvulsants. Some chemical models have a predictive value with regard to the clinical profile of efficacy of the tested anticonvulsants. Some chemical models may contribute to a better understanding of possible mechanisms of epileptogenesis.

Introduction

A distinction is made between genetic, electrophysiological, physical and chemical models of epilepsy. A chemical model of epilepsy is based on the application of, or withdrawal from, chemical substances with consequent appearance of epileptic symptomatology. A convulsant can thus be defined as a substance with demonstrated convulsive effects in vivo. Next, chemical models can differ according to the preparations used and their applications. These can include in vivo or in vitro systems. The latter including brain slices, monosynaptic systems or neuronal cultures. While in vivo preparations aim to mimic some or all of the pathophysiological, behavioral, electrophysiological and neurochemical alterations of the spontaneous human epileptic syndrome, this is less the case for in vitro models. Indeed, some of the simplified or restrictive in vitro preparations allow only the study of specific epileptogenic mechanisms or epileptic phenomena, e.g., spontaneous repetitive firing, paroxysmal depolarizing shifts, post-tetanic and long-term potentiation or inhibition of GABA and glycine responses.

Different chemical models of epilepsy, which

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mimic different clinical seizure types and acute versus chronic epileptic phenomena, are reviewed and discussed with regard to their applicability in anticonvulsant identification. It is not the aim of this paper to report on the contribution of chemical models of epilepsy to our understanding of the basic mechanisms of the epilepsies.

Specific chemical convulsants

Pentylenetetrazol (PTZ)

PTZ, a tetrazol derivative, is the prototype agent in the class of 'systemic' convulsants. PTZ administered parenterally has consistent convulsant actions in mice, rats, cats and primates. PTZ initially produces myoclonic jerks which subsequently become sustained and may lead to a generalized tonic-clonic seizure. The EEG shows spike-waves or polyspikes. With intravenous (i.v.) administration of PTZ (1% solution) to mice, the threshold dose for clonic seizures is about 50 mg/kg and for tonic-clonic seizures 90 mg/kg[115]. PTZ CD_{97} (convulsive dose in 97% of the animals) for subcutaneous (s.c.) administration is 85 mg/kg in the mouse and 70 mg/kg in the rat. The timed i.v. infusion test of PTZ as described by Orlof et al.[162] measures the minimal PTZ seizure threshold as well as the maximal PTZ seizure threshold; this model therefore allows the identification of anticonvulsants as well as proconvulsants. Chemical kindling occurs for pentylenetetrazol, as first demonstrated in rats by Mason and Cooper in 1972[133], and later in mice[99].

At a synaptic level, PTZ has been shown to interact with the $GABA_A$ receptor complex, possibly at the picrotoxin binding site[122,160].

PTZ was introduced as a screening test for anticonvulsants in part because the antiabsence drug, ethosuximide, which is effective against PTZ-induced seizures, fails to alter maximal electroshock seizure (MES) thresholds. In contrast, some drugs effective against MES seizures, such as phenytoin and carbamazepine, are ineffective against PTZ seizures. Therefore, it became common practice to presume that drugs effective against PTZ seizures would be potential antiabsence therapies, while drugs effective against MES would be potential therapies for tonic-clonic or partial seizures.

Sodium valproate, diazepam, clonazepam, trimethadione, phenobarbital and ethosuximide suppress PTZ-induced seizures in a dose-dependent manner in rodents while carbamazepine and diphenylhydantoin are ineffective or proconvulsant[102,115,174,189]. Santucci and co-workers[189] attempted to classify 13 standard antiepileptic drugs according to their effects on EEG modifications induced by low-speed i.v. infusion of PTZ in anesthetized rats. Only those drugs which are active against petit-mal epilepsies in humans were active in their model: valproate, dipropylacetamide, ethosuximide, trimethadione, diazepam, clonazepam, phenobarbital, barbital, carbamazepine, meprobamate and acetazolamide have clear-cut anti-PTZ EEG effects while phenacemide is completely ineffective and phenytoin enhances the convulsant effects of PTZ. Carbamazepine and diazepam completely suppress PTZ-induced burst discharges in CA3 region of mouse hippocampus in vitro, whereas phenobarbital has only a moderate effect and valproate does not display any protective effect at all[173].

Bemegride is a glutarimide derivative with activities similar to those of PTZ[83,183]. It produces clonic or tonic-clonic seizures and activates focal epilepsy[205].

Bicuculline

Bicuculline has been applied focally and systemically. It has been used to induce acute simple focal epilepsy after topical application in the sensorimotor cortex in rats[28]. Another model using bicuculline with induction of chronic simple partial seizures was developed by Remler and co-workers. This model mixes features of focal and generalize epilepsy and is referred to as 'systemic focal epileptogenesis'[179]. In this model, rats receive radiation to a limited volume (0.25 ml) of cerebrum. Three to six months later, when the blood-brain barrier is locally disrupted, bicuculline methiodide (2 mg/kg), which does not cross the intact blood-brain barrier, is injected systemically, inducing an epileptic focus with recurrent EEG spikes and focal seizures enduring for several weeks after a single injection. The spikes are suppressed by phenytoin, phenobarbital, chlordiazepoxide and valproic acid[180]. A region in the deep prepyriform cortex is exceptionally sensitive to the convulsant action of bicu-

culline, so that threshold doses of bicuculline induce limbic or forebrain seizures[173]. When given systemically in sufficiently high doses, bicuculline and picrotoxin produce generalized tonic-clonic seizures.

Bicuculline is believed to exert its epileptogenic effect through blocking GABAergic neurotransmission by competing with GABA for its binding site[44,155].

Clinically useful anticonvulsants are similarly effective against picrotoxin- and bicuculline-induced seizures, with the exception of carbamazepine which has some benefit against picrotoxin, but not bicuculline[174]. Phenytoin is ineffective against both types of seizures (in contrast with carbamazepine), while diazepam is highly effective against both types. Phenobarbital has intermediate effectiveness, and the antiabsence agents valproate and ethosuximide have low effectiveness.

Penicillin

One of the most popular models to study simple partial seizures has been the application of 'topical' convulsants such as the antibiotic penicillin. However, penicillin has also been applied in models of 'systemic focal epileptogenesis' and models of generalized tonic-clonic and absence seizures. The convulsive properties of penicillin were first observed by Walker and Johnson[230].

When penicillin is applied topically to exposed rat or cat cortex through a cottonoid pledget (soaked in 1.7–3.4 mM penicillin), acute focal seizures develop. Regionally placed electrodes record recurring interictal spikes that resemble human interictal spikes within a few minutes of application. Initial descriptions of the 'paroxysmal depolarization shift', considered to be a hallmark of epilepsy, were based upon intracellular recordings from penicillin foci in rat neocortex[134,176]. This model has also been considered suitable for study of spread of seizure activity.

Parenteral administration of penicillin produces generalized seizures in cats[177] and rats[68]. Seizures induced by parenteral penicillin in rats bear little resemblance to clinical absences. However, parenteral penicillin G administration ($\geqslant$300,000 units intramuscular (i.m.)/kg) in cats results in recurrent episodes of arrested activity, staring, myoclonus, facial-oral twitching and occasional progression to generalized tonic-clonic seizures. Epileptic activity begins about 1 h after injection of the drug and continues, intermittently, for 6–8 h. The EEG shows spike-wave activities emerging from a relatively normal background as in clinical absence seizures[70]. While absence epilepsy is hypothesized to originate subcortically, with involvement of the brainstem reticular formation and the thalamus, in this model spike-wave discharges appear in the cortex before they appear in mesial thalamus or reticular formation[7,70]. While discharges originate cortically, they are maintained and elaborated by recurrent thalamo-cortical circuitry[6]. Penicillin's ability to produce epileptiform discharges in the CA3 region of hippocampus has also been demonstrated in rat hippocampal slices[210].

Chen et al.[32] established the effective dose of penicillin given i.v. or intraperitoneally (i.p.) in rats and cats to induce experimental seizures. In rats, i.p. injection of penicillin, 2.5–5.0 MU/kg, induces spikes after about 45 min, and seizures after approximately 70 min. In cats, i.v. administration of penicillin, 0.5–1.0 MU/kg, induces spikes in on the average 10 min and seizures in 32 min, while i.p. administration of penicillin, 1–2 MU/kg, causes spikes in 24 min and seizures in 72 min.

It was suggested that the convulsive action of penicillin is based on its competition for GABA at the GABA receptor[121].

Response of feline generalized penicillin-induced epilepsy to anticonvulsant agents parallels the clinical response in absence epilepsy, valproate and ethosuximide being more effective than phenytoin[82,169].

Picrotoxin

When given systemically in mammals, picrotoxin induces minimal and maximal seizures in a dose-dependent manner. In rat, doses of 8 mg/kg produce hyperactivity, body tremors and forelimb clonus followed by tonic extension of the hind limbs and generalized tonic-clonic seizures.

Picrotoxin is known to be a GABA antagonist exerting its effect by binding to the 'picrotoxin binding site' which is closely related to the chloride ionophore in the $GABA_A$ receptor complex[161].

The protective effect of classical anticonvulsants against picrotoxin-induced seizures has been

studied[174,216]. While diazepam, carbamazepine and phenytoin have a high protective efficacy, phenobarbital has intermediate effectiveness; the antiabsence agents, valproate and ethosuximide, display a low effectiveness.

β-Carbolines

Various β-carboline derivatives bind with high affinity to benzodiazepine receptors. Some of these derivatives are true convulsants causing electrographic seizures as well as generalized tonic-clonic convulsions, while others display anticonvulsant properties. Although clonic convulsions induced by the β-carbolines harmine and harmaline were first described in 1895, these compounds became of broad interest following the identification of endogenous β-carbolines[3]. Braestrup et al.[20] isolated a β-carboline-3-carboxylic acid derivative from urine and brain tissue from rat, pig and human, and suggested that β-carbolines might act as endogenous benzodiazepine receptor ligands under physiological and pathophysiological conditions. Convulsant and proconvulsant actions of β-carbolines such as ethyl-β-carboline-3-carboxylate (β-CCE), methyl-β-carboline-3-carboxylate (β-CCM), and methyl-6,7-dimethoxyl-4-ethyl-β-carboline-3-carboxylate (DMCM) have been well documented.

β-CCE enhances convulsions in rodent and baboon epilepsy models[30,39]. Turski et al.[217] recently showed that β-CCE potentiated kainate- but not NMDA- or quisqualate-induced seizures. The potent convulsant β-CCM produced seizures after i.v. injections of 0.03 mg/kg in epileptic chickens but not in their heterozygote non-epileptic nest mates, and inhibited [^{3}H]diazepam binding in synaptosomes from chicken brain[97]. In mice, s.c. injections of β-CCM solutions induced dose-related generalized clonic and tonic-clonic convulsions and epileptiform EEG spiking several minutes after injection, and a CD_{50} was calculated of about 5 mg/kg[175]. The compound was also shown to potentiate the effects of PTZ, nicotine, picrotoxin and caffeine, and enhances tonic extension after maximal electroshock[157]. Convulsions and other forms of pathological behavior induced by β-CCM in mice were inhibited by the benzodiazepine agonist diazepam and the partial agonist Ro 15-1788[175], but also by the anticonvulsant β-carboline, propyl-β-carboline-3-carboxylate (β-CCP)[157]. Petersen[170] reported potent convulsant actions of DMCM. In fact, DMCM is a more potent convulsant than β-CCM in both NMRI and DBA/2 mice[21,94].

The convulsant action of the β-carbolines is often attributed to their interactions with the benzodiazepine receptor, and thus with the $GABA_A$ system[20]. For example, it was suggested that attenuation of GABA-mediated inhibition might be the leading cause of DMCM-induced convulsions[95]. DMCM reversibly reduces GABA responses of cultured mouse neurons and this effect was antagonized by diazepam and two experimental antiepileptic drugs (CGS 9896 and ZK 91296) but not by ethosuximide, dimethadione or sodium valproate[49]. However, there is some evidence for the involvement of excitatory amino acid systems as well. As Kerwin and Meldrum[103] pointed out, DMCM enhances aspartate release from rat cortex. Also, the NMDA receptor antagonist 2-amino-7-phosphonoheptanoic acid (AP7) protects DBA/2 mice but not photosensitive baboons against convulsions induced by β-CCM, and both species against those induced by DMCM[41].

3-Mercaptopropionic acid, hydrazides, allylglycine and others

These substances are mentioned under the same heading since they share a common action, namely antagonism of GABA synthesis through inhibition of glutamic acid decarboxylase (GAD). Three distinct mechanisms can be distinguished. 3-Mercaptopropionic acid, malic acid and glutaric acid inhibit GABA synthesis by substrate competition while several hydrazides, isoniazid, thiosemicarbazide[242], methoxypyridoxine, 4-deoxypyridoxine and methyl-dithiocarbazinate inhibit it by co-factor antagonism through impairment of the synthesis or coenzyme action of pyridoxal phosphate (for review see Meldrum[137]). L-Allylglycine produces convulsions through an irreversible inhibition of GAD by a metabolite (2-keto-4-pentenoic acid)[163]. Allylglycine-induced status epilepticus in baboon has been described by Meldrum et al.[140] and the L-allylglycine-induced amino acid alterations in brain were studied in rat[142]. A model for testing anticonvulsant drugs in primates employing allylglycine was described by Meldrum et al.[143].

Strychnine and related alkaloids

Strychnine and related alkaloids such as brucine and thebaine induce generalized convulsions after systemic administration. These substances block the physiological inhibitory action of glycine by a non-competitive action. This effect might explain their epileptogenic nature[42,132]. Strychnine-induced seizures are different from those produced by primary GABA antagonists since they are mainly extensor tonic, with little cortical EEG activity[205]. These seizures are not fully relieved by acceptable doses of any of the classical anticonvulsants including benzodiazepines[43].

γ-Hydroxybutyrate (GHB)

GHB is a naturally occurring metabolite of GABA that is shown to produce epileptic phenomena in a variety of species including man[198]. Winters and his collaborators were the first to specifically point out that the central effects of GHB were epileptiform rather than depressant, at all dose levels[126,238,239].

The hypersynchronous electrocortigram induced by i.p. administration of GHB, 100–150 mg/kg, in rats was characterized by 4–6/s spike-wave activities. Large doses of GHB (about 1500 mg/kg) cause convulsions, with corresponding EEG effects, in rabbits[26,192] and chicks[164], and cause convulsions in mice[11]. In cats, GHB prolongs the evoked seizure activity in hippocampus and amygdala[65].

The GHB-induced seizure has been characterized as 'petit-mal-like' because (1) concomitant with the paroxysmal electroencephalographic activity produced by GHB, the animal becomes immobile, stares, and in the case of non-human primates shows automatisms seen in the human condition; (2) in the monkey the paroxysms resemble those seen in children with absence seizures, and furthermore the seizure is a developmental phenomenon with prepubescent monkeys being more sensitive to the effects of the drug; (3) some antiabsence drugs selectively abort GHB-induced seizures.

The epileptogenic mechanisms of action of GHB remain unknown. A possible inhibition of GABAergic neurotransmission and interactions with other neurotransmitter systems have been suggested but remain to be proven as yet[92,187].

The GHB (200 mg/kg i.p.)-induced intermittent hypersynchronous electrocorticogram pattern without convulsive movements in rats was antagonized by the specific anti-petit-mal agents trimethadione, sodium-*n*-dipropylacetate and ethosuximide[80]. Sodium valproate and ethosuximide inhibited the depolarization-evoked release of GHB induced by 40 mM K^+ in rat hippocampal and striatal slices[228]. Thus the GHB model is a reliable indicator of antiabsence activity of the investigational compounds.

Excitatory amino acids

The acidic amino acid L-glutamate is now commonly believed to act as the main excitatory neurotransmitter substance in the mammalian central nervous system. At least three receptor subclasses are thought to exist for glutamate and they were named after their prototypical agonists: *N*-methyl-D-aspartate (NMDA), quisqualate or α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate[37]. NMDA receptors have received a great deal of attention and have been proposed to play a crucial role in cognitive functions as well as in many pathological processes[37,38,139]. NMDA receptors are functionally coupled to a cation channel that is modulated by magnesium ions in a voltage-dependent manner. Thus, persistent membrane depolarization is needed to open the channel[156]. Influx of calcium ions through the NMDA receptor ionophore can trigger intracellular processes that ultimately lead to an increase in efficacy of transmission at that synapse[232]. Several sites control the ionic permeability of the NMDA receptor complex. These include the NMDA binding site, the strychnine-insensitive glycine binding site, and a number of binding sites located within the ion channel[37,232].

NMDA receptor density is the highest in hippocampus, followed by neocortex, striatum and thalamus[188]. Specific roles for NMDA receptors in epilepsy have been proposed[61,237]. For example, the intensive neuronal firing that accompanies epileptic seizures depolarizes neurons sensitive to excitatory amino acids for a sufficiently long time to allow NMDA receptor activation. Enhanced synaptic efficacy resulting from NMDA receptor activation could be one of the epileptogenic mechanisms at an epileptic focus. Indeed, although many chemi-

cally induced seizures are only weakly attenuated or unaffected by competitive and non-competitive NMDA receptor antagonists, these compounds often display anticonvulsive properties in experimental models of epilepsy[37,138,184]. Sound-induced seizures in DBA/2 mice and threshold pentylenetetrazol seizures in Swiss mice are prevented by excitatory amino acid antagonists[40]. Interestingly, non-competitive NMDA antagonists like MK 801 are only weakly active against seizures in fully kindled rats, but potently inhibit seizure development by kindling[136].

Glutamate, aspartate and glycine. Excitatory neurotransmitters cause convulsions when administered into the brain. As early as 1952, Hayashi applied glutamate to the motor cortex of dogs and monkeys, resulting in clonic convulsions[84]. Curtis et al.[45] reported the excitatory effects of acidic amino acids like L-glutamate and L-aspartate upon cat spinal motor neurons. Repeated intracerebroventricular (i.c.v.) injections of 3 μmol glutamate or aspartate in rats caused hyperventilation, wet-dog shakes, tremor, running fits and generalized clonic and tonic-clonic convulsions, and approximately half of the animals died during status epilepticus[196]. Glycine facilitates the action of NMDA (as does its analogue D-serine), but also activates the inhibitory strychnine receptor, and so its role in epileptogenesis is still unclear. High doses of glycine (200 μg i.c.v.) do induce convulsions[113], but glycine in doses of 0.5–12.5 μg i.c.v. blocked seizures induced by i.c.v. kynurenine[204].

NMDA, quisqualate, kainate and related compounds. NMDA, quisqualate and kainate as well as other exogenous amino acids like ibotenate, AMPA and domoate (acting at same respective receptors) are powerful neuronal excitants[37]. Following i.c.v. administration to mice, NMDA, kainate and quisqualate induce running fits, generalized clonic and tonic-clonic convulsions[217]. Convulsant potency decreases in the order: NMDA > kainate > quisqualate.

Dingledine et al. induced epileptiform bursting in rat hippocampus in vitro which clearly involved NMDA receptors[60]. Turski et al.[217] reported a CD_{50} for NMDA after i.c.v. administration to mice of 0.28 nmol. These authors also found that antiepileptic drugs such as phenytoin, carbamazepine, trimethadione and ethosuximide did not protect the animals against the convulsions induced by either NMDA, quisqualate or kainate. NMDA-induced seizures were, however, inhibited by diazepam and valproate and by several NMDA receptor antagonists such as 2-amino-5-phosphonopentanoic acid (AP5) or MK-801 but not by midazolam or clonazepam. Also strychnine-insensitive glycine binding site antagonists like kynurenate and 7-chlorokynurenate effectively blocked NMDA-induced convulsions[33].

Quisqualate and the highly selective quisqualate receptor agonist AMPA induce epileptiform EEG changes as well as behavioral convulsions. Quisqualate and AMPA produce clonic seizures in epileptic chickens and also, but less potently, in non-epileptic heterozygotes[166]. AMPA is about 10 times more potent than quisqualate. Fukuda et al.[76] proposed a model of temporal lobe epilepsy using intrahippocampal injections of quisqualate in unanesthetized cats. In one animal 14 μg quisqualate induced limbic seizures, but most needed approximately 40 μg to trigger hippocampal spike discharges and behavioral changes. In mice, i.c.v. injections of quisqualate solutions result in clonic and tonic-clonic convulsions with a CD_{50} for minimal clonic seizures of 28 nmol[217]. The antiepileptics diazepam, valproate and phenobarbital, and the quisqualate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) block these quisqualate-induced seizures but midazolam and clonazepam do not.

Much research has been directed towards the excitatory and neurotoxic effects of kainate. Intra-amygdaloid kainate application in unanesthetized rats was proposed as a model of focal status epilepticus[16]. Such focal application of kainate produced behavioral convulsions and epileptiform EEG discharges. Severity of the seizures was related to the dose injected: a low dose of 0.4 μg induced partial amygdaloid focal seizures whereas a high dose of 1.6 μg induced secondary generalized status epilepticus leading to death of the animals, unless they were treated with diazepam. Systemic administration of kainate to rats (9–12 mg/kg i.p.) also induced convulsions and progressively developing status epilepticus, coupled to epileptiform discharges originating in limbic structures and propagated to other brain areas[17]. Threshold for

EEG seizures in limbic areas of the rat was 4 mg kainate/kg i.v., and seizure-induced neuronal damage appeared with a threshold of 7 mg/kg i.v.[119]. Following i.p. kainate injections rats display convulsive behavior starting with wet-dog shakes, staring, searching and gnawing, leading to hyperactivity, forelimb clonus and tonic-clonic convulsions[191]. Turski et al.[117] reported similar behavioral reactions in mice following i.c.v. injections of kainate and calculated a CD_{50} of 0.3 nmol. A single intra-amygdaloid injection of 1 μg kainate in nanesthetized cats initially results in focal status epilepticus, leading to secondary generalized seizures[213]. Several antiepileptic drugs give some protection against these kainate-induced convulsions in mice: phenobarbital, midazolam, clonazepam and valproate[217]. Kainate-induced seizures were proposed by Ben-Ari[15] as a model with particular relevance to human temporal lobe epilepsy.

A number of other excitant amino acids have been identified with presumed affinity for excitatory amino acid receptors[37]. These compounds often cause convulsions when injected intracerebrally or systemically. Ibotenate was first found to enhance discharges of spinal cord neurons by Johnston et al.[98], and to induce convulsions and brain damage when administered to rats[194]. Another example is domoate which causes epileptic spike-wave EEG discharges and clonic convulsions in a dose-related manner (threshold around 100 pmol) when injected into rat hippocampus in vivo[47]. In mice, domoate, injected i.p. in a dose of 4 mg/kg, produces hyperactivity, tremor and generalized clonic convulsions in some animals, and hippocampal lesions[207].

Quinolinic acid and related compounds. Quinolinic acid, kynurenine and other endogenous tryptophan metabolites induce clonic seizures after i.c.v. injection in mice[113]. Quinolinic acid was isolated in small amounts from rat and man[241]. As is also the case for other kynurenines, such as nicotine, administration of quinolinic acid to mice produces behavioral convulsions, epileptiform electrographic changes and brain damage (for a review, see Stone and Connick[204]). Quinolinic acid-induced convulsions are preceded by a phase of hyperactivity leading to generalized epileptic phenomena. Seizure threshold with i.c.v. administration in mice is 5 μg. Intrahippocampal injections of 120 nmol quinolinic acid in unanesthetized rats induce high-voltage EEG spiking associated with seizure episodes characterized by frozen posture with intermittent wet-dog shakes[193]. Quinolinic acid acts on NMDA receptors the role for endogenous quinolinic acid remaining obscure[37,204].

Guanidino compounds

Many biologically active molecules contain one or more basic guanidino groups (carbon atoms surrounded by three amino moieties). They are known as guanidino compounds and more than 120 of these guanidino compounds have been isolated from plant and animal tissues, and at least 14 different guanidino compounds have been identified in the animal brain[182]. The convulsive action and toxicity of guanidino compounds were reported as early as 1886, with the discovery in spoiled horse meat of methylguanidine, which causes dyspnea, muscle fibrillation and generalized convulsions[150]. Since then, many guanidino compounds were found to induce behavioral convulsions and epileptiform EEG spiking in mice, rats and rabbits (for a review see Mori[150]).

Some guanidino compounds are found to accumulate in biological fluids of hyperargininemic patients[129]. These guanidino compounds inhibit responses of cultured spinal cord neurons to GABA and glycine[51,53]. They also induce behavioral convulsions and EEG spiking after topical administration on the cortex of rats and rabbits[127,150].

Endogenous guanidino compounds are also generated as a result of normal protein and amino acid metabolism, and depend upon renal function for their excretion. In patients with renal insufficiency or uremia, creatinine, guanidine, guanidinosuccinic acid, and methylguanidine accumulate in serum and cerebrospinal fluid[55,130]. Dialysis removes these toxins but fails to normalize fully the guanidino compound levels[54–57]. As uremic patients often suffer from a wide range of neurological complications including epilepsy, the convulsant action and toxicity of guanidino compounds could hypothetically be one of the underlying causes of these complications. In support of this idea, De Deyn and Macdonald[50] showed that creatinine, guanidine, guanidinosuccinic acid and methylguanidine ap-

plied to mouse spinal cord neurons in primary dissociated cell culture inhibited GABA and glycine responses of these neurons. Jinnai et al.[96] applied creatinine intracisternally to rabbits and found that a dose of 13 mg/kg induced clonic and tonic convulsions. Systemic administration of high creatinine doses did not, however, produce very severe clonic or tonic convulsions in mice (unpublished observations). Marescau et al.[128] found this compound to be one of the three most abundant guanidino compounds in mouse, rat, rabbit and human brain, second to arginine and the metabolically related creatine. After systemic administration, guanidinosuccinic acid and methylguanidine induce convulsions which gradually increase in severity and which last for many hours (unpublished observations). Depending on the dose, i.p. injections of guanidinosuccinic acid suspensions in adult Swiss-Webster mice lead to myoclonic jerking, running fits, generalized clonic or clonic-tonic convulsions, often causing death of the animals. Phenobarbital attenuates these seizures while phenytoin only blocks the occurrence of tonic extension. Minimal generalized clonic seizures appear with a CD_{50} of 2.1 mmol/kg. The potency order of creatinine, guanidine, guanidinosuccinic acid and methylguanidine in the behavioral induction of epilepsy parallels the potency order of these compounds in the inhibition of GABA responses. Moreover, other guanidino compounds like δ-guanidinovaleric acid and guanidinoethane sulfonic acid are thought to induce convulsions through direct action on the $GABA_A$ receptor[86,150]. In the case of creatinine, guanidine, guanidinosuccinic acid and methylguanidine, it was suggested that the inhibition of GABA and glycine responses by these guanidino compounds is due to blocking of the chloride channel associated with inhibitory amino acid receptors[50]. Neurotransmitter systems other than the GABAergic system could also be involved. For example, methylguanidine was shown to inhibit acetylcholinesterase[150], and guanidinosuccinic acid decreases excitatory amino acid-mediated neurotransmission in rat hippocampal slices[59]. However, the effect of many guanidino compounds upon neurotransmitter systems remains to be investigated[150].

A number of exogenous guanidino compounds also induces convulsions. For example, dibenzoylguanidine induced generalized clonic and tonic-clonic convulsions associated with EEG spiking after systemic administration to mice, rabbits and cats[152]. Phenobarbital blocks the seizures induced by this compound but phenytoin, dipropylacetate and carbamazepine only slightly attenuate them[88].

Metals

Alumina hydroxide. The alumina cream model is an effective and commonly used chronic model of 'spontaneously' recurrent simple focal epilepsy. Kopeloff found that the application of alumina cream on the precentral cortex of monkeys and some rabbits resulted in the experimental induction of recurrent seizures[106]. The alumina cream technique creates foci which remain active for several months to several years and produces seizures which may increase in frequency[107,108,231]. The alumina cream model has been successfully applied in a wide variety of mammalian (e.g., rhesus monkeys, cats), reptilian and amphibian species. However, limited success was obtained in attempts to produce epileptic foci in the cebus monkey and rat. While the majority of studies using alumina cream have been restricted to the sensorimotor cortex, models including the amygdala, hippocampus, pyriform cortex and acoustic area have been developed as well (for a review Louis et al.[120]). However, several authors demonstrated the need of bilateral application of alumina when dealing with areas outside the sensorimotor cortex. For example, Soper et al. found that large amounts of bilaterally administered alumina cream were necessary to produce temporal lobe seizures in monkeys[200]. One might question whether a model that requires large bilateral lesions is a good representation of the human disease process.

In a typical preparation, 4% alumina hydroxide is injected into surgically exposed monkey or cat neocortex at a few adjacent sites. Spontaneous and recurrent seizures usually begin 1–2 months after the injection and may persist for as long as several years. After application to sensorimotor cortex, seizures develop that are similar to simple partial seizures in humans, with rhythmic jerking of an extremity or face contralateral to the inflicted lesion with occasional progression to generalization. Neuropathological examination of established alu-

mina foci reveals gliosis and distortion of dendritic neuronal trees as observed in human neocortical foci[234]. Standard anticonvulsants effective against focal epilepsy are protective in this chemical model of epilepsy in monkeys[116,117].

The alumina cream model can be criticized as crude since it creates lesions which are quite large and difficult to reproduce precisely. While several investigators state that the alumina lesions stabilize quickly and that diffusion of the active substance is limited, this is contradicted by others[12,78,234] and a progressive increase in lesion size over time has been described[203]. Another drawback of the alumina model is the long and somewhat unpredictable latency period prior to clinical and electrographic onset of spontaneous seizures (4–8 weeks in cats; 6–12 weeks in rhesus monkeys). On the other hand, a close correspondence to partial seizures in man and a comparable pharmacological profile of anticonvulsants are advantageous.

Cobalt. Cobalt powder was first implanted into the cortex of mouse and rat brain to produce chronic simple partial seizures in the early 1960s[63,105]. Biochemical and neuropathological changes induced by cobalt implantation have been studied and the effects of some anticonvulsants have been evaluated in this model[64]. Diphenylhydantoin and ethosuximide suppress spike discharges in cobalt-treated rats but do not prevent the development of the epileptic foci[64]. Although the recurrent seizures usually last for one month, as in all metal implantation models, the procedure is laborious and there is a long latency before the onset of seizures. However, Willmore et al.[236] showed that ionized cobalt could cause epileptiform discharges in the rat when applied by pial iontophoresis and Brown et al.[25] observed epileptic discharges after injection of cobaltous chloride into cat hippocampus. Moreover, Zhao et al.[245] developed a new application of the cobalt-induced epilepsy in the rat. These authors microinjected cobaltous chloride in the lateral cerebral ventricle of the rat with a CD_{50} of 0.45 μM/10 μl. The seizures are spontaneously recurrent for about 1 week and are similar clinically to kainate-induced seizures and amygdala kindling. All animals demonstrate increasing episodes of staring 10–30 min after cobalt injection. Approximately 1 h after injection all animals present wet-dog shakes; and with a latency of approximately 6 h, 56% of the animals exhibit a generalized convulsion characterized by clonus of facial muscles, salivation, rearing on hindlegs and forelimb clonus. Sodium phenobarbital and nitrazepam completely antagonize the seizures while carbamazepine antagonizes them in 60% of the rats only. Neither sodium valproate nor phenytoin antagonized the cobaltous chloride-induced seizures in Zhao et al.'s study.

The pathophysiology of cobalt-induced seizures remains unclear. Pathological changes of neurons around the implanted area and disorders in metabolism of amino acids in whole brain have been discovered[25,224]. In an in vitro study, Wu et al.[244] found that divalent cations including Co^{2+} were very potent inhibitors of L-glutamate decarboxylase, the GABA synthetic enzyme. Donaldson et al.[62] reported that inhibition of Na^+,K^+-ATPase could be responsible for the seizure-producing ability of divalent ions. Co^{2+}-induced seizures may be related to these or other biochemical alterations.

Zinc sulfate. Intracortical injection of zinc in rats or intracerebral injection of zinc in rabbits can produce partial seizures or generalized clonic seizures[165,168]. Pei and Koyama[167] developed a new chronic model of experimental epilepsy in rabbits by intrahippocampal injection of 10 μl of zinc sulfate solution ($ZnSO_4 \cdot 7H_2O$, 200 μg/kg). In this model, seizures occur over several weeks and are expressed clinically and electrophysiologically not only as partial seizures, resembling complex partial seizures, but also as secondary generalized seizures. Phenobarbital and phenytoin afford protection against the secondary generalized seizures but not against the complex partial symptomatology. The latter compounds do not antagonize the epileptic discharges. Nitrazepam, however, protects against the partial and secondary generalized seizures with a transient restoration of electrical alterations.

Other trace metals. Other metal models with some chronic characteristics such as the tungstic acid[19] and iron[112,178] models have been described but have not been documented extensively. Willmore et al.[235] suggested that the epileptogenic effects of hippocampal injection of iron salts might be related to the induction of peroxidation of neural lipids within the injection site.

Neuropeptides

Although neuropeptides are not commonly in use as chemoconvulsants in epileptic drug screening and development, they are increasingly considered as a promising group of substances in modern epilepsy research and several neuropeptides are now thought to play a role in in vivo epileptogenesis (see, e.g., Bajorek et al.[10]). However, literature dealing with the convulsant properties of neuropeptides often contains conflicting results; many neuropeptides show anticonvulsant effects in one study but convulsant effects in another. Peptides related to the opiate alkaloid family will be discussed first and separately from the other neuropeptides for the putative role of opioid peptides in epileptic mechanisms is without doubt the most extensively documented[10,72,195].

Opioid peptides. Historically, Cogswell[135] was the first to report convulsions in frogs after morphine administration. Many years have passed since this first study but the subject is still surrounded by much controversy, and reports concerning the convulsant properties of morphine and the opioid peptides have often been contradictory. As Frenk[72] already pointed out, both pro- and anticonvulsant actions of these compounds have been shown; opioid-induced seizures were termed non-convulsive by some authors, but behavioral convulsions were reported by others; some have reported the involvement of specific opiate receptors in opioid-induced seizures, while others have mentioned non-specific involvement of other receptors such as the GABA receptor; and finally, a limbic origin of opioid-induced seizures was proposed, but other CNS areas have been implicated as well.

Morphine and related opioid peptides have been shown to produce convulsive behavior in grasshoppers, frogs, pigeons, mice, rats, guinea pigs, rabbits, cats, dogs, monkeys and man[72]. When administered in large doses many narcotic analgesics induce behavioral convulsions as well as epileptiform EEG activity characterized by high-voltage spikes, polyspikes and waves[131]. Some opioids like heroin, propoxyphene and meperidine induce clonic convulsions, while large doses of naloxone, noremeperide and thebaine induce tonic-clonic seizures[131].

Under some circumstances small doses of opioid peptides are able to produce epileptic phenomena also. The i.c.v. administration of 100 μg morphine and between 10 and 100 μg [Leu]- and [Met]enkephalin induced EEG seizures in rats, often accompanied by myoclonus and wet-dog shakes, which were blocked by systemic administration of naloxone (10 mg/kg) prior to the i.c.v. injections[74,223]. These doses of enkephalin did not produce analgesia. Interestingly, Frenk et al.[73] found that 120 μg [Met]enkephalin injected in the vicinity of the dorsomedial nucleus of the thalamus induces EEG seizures while the same dose injected near the periaqueductal gray area causes analgesia without seizures. Apparently, [Met]enkephalin induces analgesia and seizures through pharmacologically different opiate receptors located in different brain areas[73].

Another endogenous opioid, β-endorphin, induces non-convulsive epileptiform EEG changes in rats after i.c.v. injection in doses which do not produce analgesia or abnormal behavior[85]. Cain and Corcoran[27] were able to kindle generalized convulsions by repeated injections of small 10-μg doses of [Met]enkephalin and β-endorphin into limbic structures of rats. In some animals in the latter model, the seizures were characterized by epileptiform EEG spiking and strong behavioral responses, with rearing and forelimb clonus eventually leading to loss of the righting reflex. Antiepileptic drugs such as diazepam and phenytoin do not abolish these β-endorphin-induced seizures, only attenuating the seizure episodes. Snead and Bearden[199] showed that [Leu]enkephalin-induced cortical seizures are antagonized by antiabsence drugs such as ethosuximide, trimethadione and sodium valproate but not by clonazepam, phenobarbital or phenytoin.

Miscellaneous convulsant neuropeptides. Tartara et al.[214] reported the occurrence of convulsions in rabbits following intracerebral administration of certain amino terminal fragments of adrenocorticotropin (ACTH), while Bajorek et al.[8] demonstrated slight anticonvulsant properties in gerbils for one of these fragments. Bajorek et al.[10] thoroughly reviewed the literature and found no report of epileptiform behavior or EEG following administration of the ACTH peptide. On the contrary, the ACTH peptide displayed slight anticonvulsant effects in some rodent models such as

kindled rats and gerbils[8,185]. In contrast, the ACTH releasing factor (CRF) displayed straightforward and potent convulsant actions. Several hours after its intracerebral administration to rats this peptide produces behavioral convulsions coupled to an epileptiform EEG[67].

Several observations are indicative of a proconvulsant or weak convulsant activity of somatostatin[34,36,87]. It was proposed that somatostatin may play some role in focal epilepsy since it enhances seizures in experimental models of this disorder which is manifested by a decrease in seizure threshold in kindled rats, and since it was found to be increased in rat amygdala after seizure kindling[101]. Also, somatostatin levels appear to be altered in some epileptic patients[104].

Intracerebral and even systemic administration of thyrotropin releasing hormone (TRH) has an excitatory effect in birds and rodents[9]. For example, Wei et al.[233] reported TRH-induced wet-dog shakes in rats. In seizure-prone gerbils TRH enhances the severity of the seizures of these rodents[9]. TRH levels increase in brain areas of kindled rats[148]. However, the observations of Sato et al.[190] point in another direction, illustrating the controversial involvement of neuropeptides in seizure mechanism. The latter authors reported antiepileptic effects of TRH and its derivative DN-1417 in kindled cats.

The pituitary peptide vasopressin has proconvulsant and convulsant actions in rats[1,111]. When administered i.c.v. in low doses, vasopressin induces convulsions in rats, but has no effect upon spontaneous seizures in gerbils[114]. A role was suggested for vasopressin in the mechanism of febrile convulsions due to the observation of a decrease in the threshold for febrile convulsions in vasopressin-treated rats[100].

Pilocarpine and other cholinergic agents

Acetylcholine or its analogues, acetylcholinesterase inhibitors and acetylcholine precursors, when administered into the brains of experimental animals, result in pronounced seizure activity[14,81,125]. Intraamygdaloid[159,219], intrahippocampal[218,219] or systemic[219,220] injections of large doses of muscarinic cholinergic agonists in rats produced electroencephalographic and behavioral limbic seizures. Pilocarpine, a muscarinic cholinergic agent with high potency, administered i.p. to mice (300–350 mg/kg) induced a sequence of behavioral alterations including staring spells, limbic gustatory automatisms and motor limbic seizures that built up progressively into limbic status epilepticus that lasted for several hours[221,222]. Interictal and ictal epileptiform activity was recorded on EEG. Diazepam was found to be protective in this model in mice[221] and γ-vinyl-GABA administration bilaterally in the substantia nigra in rats suppressed the appearance of electrographic and behavioral seizures[222]. Scopolamine 10 mg/kg prevented the pilocarpine-induced syndrome[221]. While antimuscarinic drugs are effective against epilepsy induced by cholinergic agents or inhibitors of acetylcholinesterase, they do not possess significant anticonvulsant properties in any spontaneous model of epilepsy.

Tetanus toxin

A model of recurrent chronic partial seizures can be produced by tetanus toxin injection in several brain regions and species. Despite the fact that tetanus toxin was first used 25 years ago as a model of epilepsy, it is still utilized in few laboratories. Tetanus toxin was first used to create chronic epileptiform events in 1962 by Carrea and Lanari who applied it to the cerebral cortex of dogs[29]. Tetanus toxin has successfully been applied to the hippocampus (rat, cat)[79,145,146], substantia nigra (rat)[135], caudate nucleus (rat), thalamus (rat) and cerebral cortex, including motor cortex (dog and cat)[24,29].

The most frequent application of this model has been hippocampal injections in rat and, to a lesser extent, in cat. The resulting complex partial seizure model probably results more from this injection site in limbic structures than from the properties of the toxin itself. After hippocampal injection in the mouse, seizures may occur within a day after injection and then on a chronically recurrent basis over weeks[147]. In rat, a seizure begins typically with arrest of activity, followed by myoclonic jerks of the forelimbs, and in some animals generalized tonic-clonic seizures[147]. The EEG shows concurrent 3–20-Hz spiking or spike-wave activity. For approximately 1 month, the animals have an average of about 100 seizures per day. The mechanisms of tetanus-induced seizures are not known yet. However, tetanus toxin seems to act by blocking the pre-

synaptic release of inhibitory neurotransmitters such as GABA and glycine[225].

Flurothyl

Flurothyl is a hexafluorinated non-flammable ether which is known to induce convulsions in mice[18]. The flurothyl test has some advantages: it is easy to perform the test and there is a clear endpoint of clonic-tonic seizure with loss of righting reflex and a threshold independent of body weight[48]. A typical experiment is conducted as follows: 1.5 ml of flurothyl is injected into a 2-gallon sealed and stirred jar containing the mouse; the measured parameter is latency to clonic-tonic seizure after injection of the convulsant.

Folates

Folate and some of its derivatives have been shown to induce seizures in experimental animals[90,91,154,158]. After topical injection into the hindlimb area of the neocortex, all these compounds produce contralateral myoclonic jerks yielding a model of simple partial seizures. Van Rijn et al. investigated the possible mechanisms of action of these substances by performing radioligand binding studies at the $GABA_A$ receptor complex[226,227]. Folates were found in this study to reverse the inhibiting effect of GABA on the binding of the cage convulsant [^{3}H]TBOB, an effect that was shown to correlate with the in vivo epileptogenicity in rat (see also Van Rijn, in this issue).

Homocysteine

Homocysteine induces convulsions after systemic application. Mice or rats given approximately 1 g/kg homocysteine thiolactone i.p. develop generalized clonic and clonic-tonic convulsions with latencies ranging from 10 to 60 min[4,71,201,202]. Its mechanism of action remains unknown. Although homocysteine is a pyridoxal phosphate antagonist, which can thus impair GABA synthesis, pyridoxal phosphate or pyridoxamine exacerbate homocysteine-induced seizures while hydrazine alleviates them[93]. It is suggested that homocysteine influences multiple neurotransmitter systems at several sites in the brain. Homocysteine accumulation in the brain may be responsible for the seizure sometimes observed in homocystinuria.

Colchicine

In high doses, colchicine administered intracranially causes generalized convulsions and consequent death[240]. Injections into rat hippocampus cause selective multiple population EEG spikes[209]. Reynolds and Oakley[181] described an experimental model of focal epilepsy in rats and cats in which colchicine in agar (10^{-3} M to 1 M) was focally applied to neocortex resulting in epileptiform discharges. The protective effect of anticonvulsants in the colchicine epilepsy model has to our knowledge not been investigated.

Withdrawal seizures

Long-term administration of drugs which depress brain function in one way or another (ethanol, barbiturates, benzodiazepines, etc.) may produce enhanced brain excitability and seizures during the withdrawal phase. Such drugs are often abuse substances and the modeling of seizures associated with the withdrawal syndrome can give insight into the mechanism of action of such withdrawal seizures and thus in the alleviation of withdrawal distress. Equally dramatic are withdrawal seizures that complicate the discontinuation of drug treatment in epileptic patients.

Chronic exposure of rats to ethanol is thought to decrease neuronal inhibition leading to a hyperexcitable withdrawal phase lasting more than 3 weeks after treatment[66]. Rats show a peak in hyperexcitability 8 h after alcohol withdrawal which is characterized by convulsions, tremors, and other signs of increased CNS excitability[13]. Chronic as well as acute administration of ethanol may have many direct neurotoxic effects on the nervous system, but there is clear evidence for close involvement of the GABA system[58,144]. Chronic alcohol treatment depressed the rate of GABA synthesis[208] and decreased the density of GABA receptors[215] in mouse brain.

The opiate abstinence syndrome has also been characterized by changes in excitability of the CNS[72]. Withdrawal seizures were observed in rats and in infants of drug-addicted mothers[89,186]. Rats have a decreased threshold to flurothyl-induced convulsions 40 h after withdrawal from morphine[2]. It was suggested that the changes in

excitability and the occurrence of withdrawal seizures following opiate dependence are caused by kindling of a non-specific proconvulsant system prior to withdrawal in conjunction with tolerance to a non-specific endogenous anticonvulsant system[72,171].

A particular model of drug withdrawal associated with epileptic manifestations is represented by the GABA withdrawal syndrome. The model was developed in rats and baboons by Brailowsky and coworkers[22,23,77] and it is referred to as an acute model for simple partial seizures[69]. When normal rats were chronically infused for 3 h to 14 days with 100 μg/100 μl GABA solutions into the motor cortex, epileptic phenomena were observed on withdrawal, with a seizure duration inversely related to the infusion time[23]. During the rebound period, epileptogenic EEG activity (spikes, polyspikes and high-voltage bursts) was recorded in the infused area, and sharp waves correlated with myoclonic jerks in hindlimb and body contralateral to the infused area. Brailowsky et al.[23] compared this model to the periodic lateralized epileptiform discharges in human epilepsy[31], and called it a model of a true partial status epilepticus with short latency after GABA withdrawal, long duration (up to 168 h) and spontaneous termination. Some important advantages of this model include the use of a well characterized endogenous neurotransmitter, the possibility of control of latency and duration of the epileptic phenomena, and the occurrence of stereotyped behavioral and electrographic patterns.

Contribution of chemical models to the evaluation of anticonvulsants

An ideal screening, quantification and evaluation test program should identify all anticonvulsants without false positives or negatives, perfectly predict the convulsant's clinical efficacy profile and predict toxicity, safety and tolerance in man.

While there is an impressive number of chemical convulsants, not all models have been validated satisfactorily with regard to their specificity in the identification of drugs, with or without particular clinical efficacy patterns. Nevertheless, suitable chemical test systems in animals or in vitro for the identification, quantification, differentiation and further preclinical evaluation of anticonvulsants are numerous. Screening tests allow relatively economical preliminary identification of anticonvulsants and provide some predictive information regarding clinical efficacy. Ideally, efficacy profiles in preclinical testing should indicate whether initial trials in man should study protective efficacy against, for example, primary generalized seizures or focal epilepsy. However, no model duplicates the multitude of human clinical epileptic conditions. Moreover, knowledge of the underlying causes of various types of convulsive disorders is incomplete and the development of animal models based on etiology is therefore not yet possible.

The specificity of models of epilepsy relates to the intensity and the mode of application of the stimulus, as well as the nature of stimulus. For example, minimal threshold tests (clonic response; minimal stimulus intensity) identify substances that raise the seizure threshold, whereas supramaximal tests (tonic extension of the hindlimbs; high stimulus intensity) identify substances that prevent seizure spread. On the other hand, maximal threshold tests (tonic extension of the hindlimbs; minimal stimulus intensity) are nondiscriminatory between substances that increase seizure threshold and those that prevent seizure spread.

The most commonly employed screening tests are the electrical 'maximal electroshock' model and the chemical pentylenetetrazol minimal seizure threshold test. The potencies of clinically established anticonvulsant drugs given i.p. against maximal electroshock and minimal PTZ seizures (after administration of a dose causing clonic seizures in 97% of the animals) in mice have been presented by Krall et al.[109]. The maximal electroshock (MES) test is assumed to identify anticonvulsants protective against partial seizures and generalized tonic-clonic seizures. Thus, phenytoin and carbamazepine are active in this experimental paradigm. Benzodiazepines are less active in this test than in the threshold PTZ seizure test. Some drugs, such as γ-vinyl-GABA, that are effective against tonic-clonic seizures are unfortunately not unequivocally identified by the MES test. Ethosuximide, a paradigmatic antiabsence drug, in agreement with the predictive profile of the MES test, is inactive in this model. Moreover, the MES test gives some false posi-

tives among centrally active drugs, including some antidepressants and neuroleptics[109]. In the minimal PTZ seizure model, benzodiazepines are the most potent of the established anticonvulsants with clonazepam being the most efficient. Benzodiazepines are a hundred to ten thousand times more potent against minimal PTZ seizures than against MES seizures. It is commonly stated that the threshold PTZ test is specific for drugs active against absence seizures. The inefficacy of phenytoin, carbamazepine and mexiletine (drugs that are clinically ineffective against absence seizures) and the highly protective effect of ethosuximide, trimethadione and valproate (classical antiabsence drugs) are in agreement with the proposed antiabsence specificity of the PTZ threshold test. Nevertheless, there are false positive (phenobarbital, progabide) as well as false negative findings (imipramine)[75,109].

In addition, many chemical convulsants other than pentylenetetrazol have been administered systemically to rodents as part of anticonvulsant screening programs (see Table I). Of the chemical convulsants listed in Table I, strychnine has probably been used most widely, despite the fact that it still remains to be shown whether it indicates a potentially specific clinical efficacy profile. Many chemical convulsants included in Table I have been shown to antagonize GABA-mediated inhibition, either by inhibiting its synthesis (allylglycine, isoniazid, 3-mercaptopropionate) or by interacting with the different components of the GABA/benzodiazepine/Cl^- ionophore complex (bicuculline, picrotoxin, penicillin and DMCM). Nevertheless, these tests do not systematically identify anticonvulsants that are thought to act through enhancement of GABA-mediated inhibition (GABA transaminase inhibitors, GABA agonists and benzodiazepines). Although kainate and pilocarpine given systemically trigger limbic seizures, they have not been shown to predict anticonvulsant activity for human limbic seizures.

The recent description of potent and specific antagonists of excitatory amino acids and their efficacy in the protection against excitatory amino acid-induced convulsions (as discussed in this issue by Meldrum) creates new possibilities in the further development of anticonvulsants. Indeed, in genetic models of epilepsy, excitatory amino acid antagonists have also been found to display anticonvulsant effects. When administered i.c.v. to DBA/2 mice (a model of sound-induced epilepsy) several antagonists of excitation like γ- glutamylglycine, 2-amino-5-phosphonopentanoic acid (AP5) and 2-amino-7-phosphonoheptanoic acid (AP7) were found to block seizure occurrence[40]. An activity of these agents against sound-induced seizures in rats and photically induced seizures in baboons has also been observed[141].

The Anticonvulsant Drug Development Program sponsored by the National Institute of Neurological Disorders and Stroke includes in its basic screening program the MES test, the PTZ thresh-

TABLE I

Chemical convulsants given systemically as additional tests in anticonvulsant testing

Convulsant	Dose (mg/kg)	Presumed mechanism of epileptogenesis	References
Allylglycine	160	inhibition of GABA synthesis	5
Bicuculline	2.7 (s.c.)	GABA antagonism	243,211,212
DMCM	15 (i.p.)	benzodiazepine antagonism	170
γ-Hydroxybutyrate	200	GABA antagonism	80
Isoniazid	450	inhibition of GABA synthesis	151
Kainate	10–30	direct excitation	206
3-Mercaptopropionate	40–60	inhibition of GABA synthesis	118
N-Methyl-D-aspartate	150–300	direct excitation	46
Penicillin	3–6 × 10^5 IU	GABA antagonism	82,169
Picrotoxin	3–4	Cl^- channel block	118
Pilocarpine	380 (i.p.)	cholinergic antagonism	221,110
Strychnine	1.2	glycine antagonism	118

old test as well as the strychnine-, bicuculline- and picrotoxin-induced seizure models[212]. In addition, selected substances are further characterized in additional preparations. $GABA_A$ receptor binding studies and adenosine uptake studies are performed in the extended program to differentiate between possible mechanisms of action. During the last 15 years more than 14,000 agents have been screened and more than 2000 of these have been subjected to anticonvulsant quantification and evaluation. Approximately 17 are at present in various phases of clinical trial. This rather clearly indicates that the yield is relatively small in the development of new, highly efficacious, less toxic and more specifically active anticonvulsants. However, the development of one compound fulfilling these criteria justifies the effort.

There are several confounding factors in the screening, quantification and evaluation of potential clinically efficacious anticonvulsants.

(1) Models reproducing the full clinical syndrome and pathophysiology are not available. Besides the etiological differences one has to consider species differences, ontogenetic differences as well as other shortcomings of the respective models. The recurrent nature of epileptic symptomatology for example, one of the hallmarks of the human epileptic condition, is a relatively rare feature of chemical models of epilepsy. Models failing to induce recurrent epileptic seizures should therefore preferentially be referred to as models of epileptic seizures and not models of epilepsy.

The unsatisfactory search for models reproducing absence seizures which select for antiabsence drugs illustrates this limitation. While PTZ seizures are still commonly used as an acute model to test potential antiabsence drugs, PTZ also induces a spike-and-wave EEG pattern and a convulsive syndrome that are not observed in absence seizures. Moreover, activity against PTZ is not essential for, nor constantly predictive of efficacy in the treatment of absence epilepsy. For example, the antiabsence drug trimethadione is less effective against PTZ than phenobarbital, generally used in convulsive epilepsy[149]. Another proposed chemical model of absence seizures is the GHB-induced epileptic syndrome. The GHB-induced EEG hypersynchrony is not only another possible functional model of absence seizures (i.e., useful in the evaluation of drugs), but also a phenomenological model of this type of seizures, imitating some characteristic behavioral phenomena of absences. Still another chemical model in which classical antiabsence agents are effective is the parenteral penicillin G-induced syndrome that consists of absence-like behavior such as arrested activity and staring but also of myoclonus, facial-oral twitching and occasional progression to generalized tonic-clonic seizures. In this model, the EEG shows spike-wave activities that have been found to emerge cortically and not subcortically as is hypothesized for clinical petit-mal seizures[7,70]. Moreover, in the laboratory animal, the frequency of the elicited spike-and-wave discharges varies with the technique and with the species and does not really mimic the human absence electrophysiological criteria: for instance, 3–4.5 cycles/s are observed during penicillin-induced seizures in the cat and 2.5–3 cycles/s in the GHB-induced seizures in the rhesus monkey[197] while 3–6 cycles/s or more are observed in the genetic model of the photosensitive baboon *Papio papio*[153] and still higher frequencies are observed in another model of generalized non-convulsive epilepsy in the Wistar rat[229]. However, one should realize that screening models should not necessarily fully mimic all the clinical and/or electrophysiological features of any particular syndrome. For example, the frequency of spike-wave discharges could depend on the anatomy and physiology of the species, and different frequencies could therefore still be equivalent. Another drawback of the non-convulsive models of epilepsy is that implantation of recording electrodes is required for verification of seizure occurrence, thus rendering these models less suitable for initial screening programs. As no single model seems to provide a decisive answer concerning the antiabsence efficacy of a potential drug, it seems necessary to use a combination of tests for screening new drugs.

(2) Simplified models have advantages in terms of feasibility but are more suitable for mechanistic studies than for the prediction of clinical efficacy. Some of these models aim at identifying anticonvulsants and/or their mechanisms of action, based on certain hypothesized epileptogenetic mechanisms. An example of this type of model is an ex-

perimental paradigm in which the influence of (potential) anticonvulsants is studied on GABA responses or the convulsant-induced reduction of GABA responses on neurons in cell culture[49,123,124]. This simplified model's rationale is based on the GABA hypothesis of epilepsy (for review see De Deyn et al.[52]).

(3) Efficacy against observed epileptic phenomena in certain epilepsy models does not always imply an anticonvulsant effect. Indeed, one cannot state that a compound is a potential antiepileptic drug simply because it antagonizes the behavioral component of seizures, since this could be due to spinal or peripheral effects such as neuromuscular blockade. Thus it is essential to demonstrate antagonism of both the behavioral and the electroencephalographic component of seizures.

(4) The efficacy of certain compounds in a given model might rest upon the characteristics of the model rather than on anticonvulsant effects, e.g., metal chelators in metal-induced focal acute or chronic epilepsy and scopolamine in the pilocarpine-induced seizures.

(5) Anticonvulsants with novel structures or models of action might not be identified since the anticonvulsant potential is usually assessed by comparing their effects with those of prototype anticonvulsants.

Proving the experimental efficacy of an anticonvulsant drug does not necessarily indicate clinical efficacy: the drug might not be superior to the anticonvulsants in clinical use; the drug might be more toxic and/or the drug might not display its experimentally suggested specificity in the treatment of certain seizures in clinical setting. An important step between the identification and quantification of new anticonvulsants in models and their evaluation in the clinic is the study of its chronic efficacy, safety and toxicity. Toxicity and safety studies performed in vivo in animals are usually conducted in parallel with the anticonvulsant efficacy testing. Again, there are problems in predicting toxic effects (dose- and/or time-related ones and idiosyncratic adverse effects) in man by extrapolation from animal experiments. Limitations rest among others on differences in pharmacokinetics. idiosyncratic reactions, physiology or pathophysiology, tolerance and enzyme induction. Nevertheless, protective indices (TD_{50}/ED_{50}) and safety ratios (TD_3/ED_{97}) obtained from animal experiments remain significant predictors of clinical safety and toxicity[212].

Acknowledgements

The authors thank Born-Bunge Foundation, University of Antwerp, OCMW Medical Research Foundation Antwerp and National Fund for Scientific Research Belgium (NFWO grant 3.0044.92 and CD F57) for financial support. The authors also thank Mrs. F. Franck for editing the manuscript.