New antiepileptic agents

Harold Kohn and Judith D. Conley

Epilepsy, known since ancient times as the 'falling sickness', is characterised by convulsive seizures and momentary lapses of awareness or body function. The generic term comes from the Greek *Epilepsia* which means 'to take hold of or seize'. Recent research suggests that 2 m people in the US and 500 000 people in this country suffer with epilepsy. But, despite this, there has been little in the way of new drugs over the past decade to help epileptics lead a more normal life. This situation is now changing. The shortcomings of conventional drug therapy have spurred chemists to seek drugs that are safer, more potent and more effective than the existing medication. Functionalised amino acids are the latest in a series of potent new agents for the treatment of epilepsy.

Epilepsy was recognised as a unique and terrible affliction as far back as the Babylonian times. Full clinical descriptions were first given around 400 BC in the Hippocratic monograph *On the sacred disease* and later in the *New Testament (Mark 9:17)*. In the 19th century, major and minor seizures became known as *grand mal* and *petit mal* respectively.[1] The signs and symptoms depend on the motor, sensory autonomic and mental functions of those regions in the brain in which a neuronal discharge arises or to which it spreads. If the discharge is violent, prolonged and ubiquitous, the maximal seizure of *grand mal* develops characterised by a sudden fall with a tonic spasm (rigidity and stiffening of the body) followed by clonic spasms (generalised jerking) with the victim's skin sometimes becoming blue. If the discharge is limited in strength, various sorts of minor seizures occur. An essential feature of *grand mal* is the abnormal ease by which neuronal discharges spread from the origin of excessive discharge to other areas of the brain. *Petit mal* is restricted to brief, frequent attacks (5–100 per day) of impaired consciousness associated with staring and eyelid movements, and occasionally, loss of posture and arm jerks. No specific cause is known for *petit mal* and this disorder occurs most commonly before puberty.[2]

Experimental models

In the past, drug research was hindered by the lack of experimental models for epileptic seizures. Not surprisingly, therefore, the early history of treatment is paved with failure. In fact some of the remedies tried, for example ashes of asses' hooves, stork's dung and the and phenobarbital (*1*)—were discovered by Locock in 1857 and by Hauptmann in 1912 respectively, by serendipity.

The situation changed in 1926 with the discovery of the convulsive action of pentylenetetrazole and then in 1937 Putman and Merritt developed an electroshock model for epilepsy whereby they screened around 70 compounds against electrically induced convulsions in cats. As a result of their work the anticonvulsant activity of 5,5-diphenylhydantoin (phenytoin, Dilantin) (*2*) was established.[3] Phenytoin is still the most widely used anticonvulsant despite its serious side effects. In 1948 Goodmann and his coworkers standardised the maximal electroshock seizure (MES) test and later introduced the subcutaneous pentylenetetrazole (Metrazol) (sc Met) seizure threshold test.

In the MES seizure test, animals are stimulated by using an alternating current applied through corneal electrodes for a short duration. Drugs with marked activity by this test are thought to prevent seizure spread and are likely to be effective in *grand mal* seizures. The sc Met seizure test measures the ability of the anticonvulsant drug to protect against seizures induced by a subcutaneous injection of pentylenetetrazole. The dose used is the amount of convulsant required to cause seizures in 97 per cent of the animals tested. Drugs with marked activity by this test are thought to elevate the seizure threshold and are likely to be effective against *petit mal* seizures.[4] These two tests are the most useful laboratory tests for screening large numbers of chemical compounds. In recent years, additional models for epileptic seizures have been advanced. These stances to the experimental animals.[5]

Development of antiepileptic agents

With the discovery of new laboratory procedures to screen anticonvulsant compounds, an intense era of antiepileptic drug development was initiated during the 1940s and 1950s. Thousands of candidate agents were evaluated and a cluster of new antiepileptic drugs were introduced—two hydantoins (mephenytoin (*3*) and ethotoin (*4*)), a barbiturate (metharbital (*5*)), and a deoxybarbiturate (primidone (*6*)) were developed and marketed for the treatment of *grand mal* seizures and two oxazolinediones (paramethadione (*7*) and trimethadione (*8*)) were introduced for the control of *petit mal* seizures.[3] Similarly, several phenylsuccinimides (*9*) were found to prevent pentylenetetrazole-induced seizures and control *petit mal* seizures.[6]

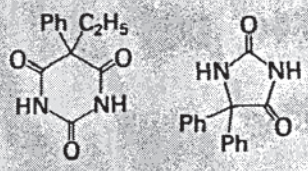
Progress in antiepileptic drug research slowed down in the early 1960s. By this time it was realised that most of the drugs in use contained a modified ureide moiety (*10*) embedded within their chemical framework. This structural theme had been extensively developed, prompting some researchers to suggest that further elaboration would probably not lead to an improved drug. Moreover, the search for new antiepileptic agents had failed to produce significant numbers of new lead compounds possessing novel structures.

In the late 1960s research in anticonvulsant drug development was stimulated by the creation of the Epilepsy Branch and the Epilepsy Advisory Committee within the United States National Institute of Neurological and Communicative Disorders and Stroke (NINCDS). Programmes were established to collate and review the neuroscience literature pertinent to epilepsy, to support controlled laboratory and clinical trials of drugs which needed proof of efficacy for marketing approval, and to screen large numbers of potentially new anticonvulsant agents. These programmes helped pave the way for the eventual introduction of three drugs, containing a novel structural moiety, for seizure management in the US. They were carbamazepine (*11*), an iminostilbene; clonazepam (*12*), a benzodiazepine; and

diazepine, clorazepate dipotassium (*14*), was also approved as an adjunctive drug for the treatment of epilepsy.[7]

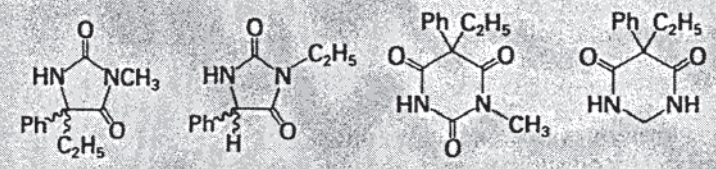
To date the most widely used anticonvulsants are phenytoin (*2*), phenobarbital (*1*), primidone (*6*) and carbamazepine (*11*). However, they all promote adverse side-effects such as drowsiness, dizziness, nausea, swelling of the gums and liver damage. So the search began in the late 1970s for new drugs that are safer, more potent and more widely effective than the existing medications. As a result, many new chemical classes have been identified that possess anticonvulsant activity but are less toxic and therefore potentially safer than those currently in use. Notable examples[8,9] include 2,3:4,5-bis-*O*-(1-methyl-ethylidene)-β-D-fructopyranose (*15*), 3-[3-(trifluoromethyl)phenoxy]-1-azetidinecarboxamide (*16*), 10,11-dihydro-5-methyl-5*H*-dibenzo[a,d]cycloheptene-5,10-imine (*17*), *N*-(2,6-dimethylphenyl)-*p*-aminobenzamide (*18*) and more recently functionalised amino acid derivatives (*19*).

Early anticonvulsant drugs.

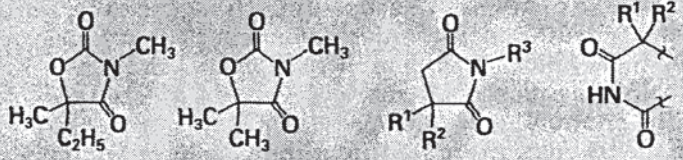


(*1*) (*2*)

Examples of antiepileptic drugs developed in the 1940s and 1950s.

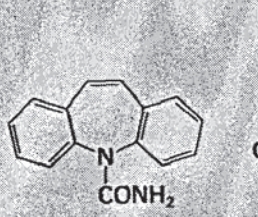


(*3*) (*4*) (*5*) (*6*)

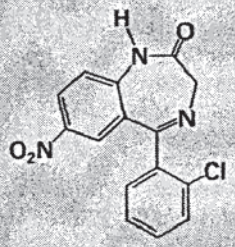


(*7*) (*8*) (*9*) (*10*)

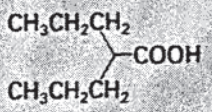
Antiepileptic drugs approved in the past 20 years.



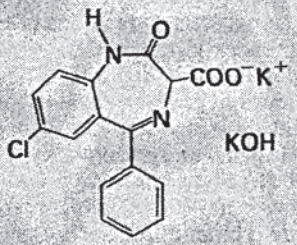
(*11*)



(*12*)



(*13*)



(*14*)

Amino acids

In the past amino acids and their derivatives were not considered as potential antiepileptic agents, probably because these polar compounds do not readily penetrate the blood–brain barrier.[10] Despite this, several amino acid derivatives have recently been shown to be effective in preventing seizure. These include derivatives of alicyclic and aromatic amino acids,[11] phosphono derivatives of aliphatic amino acids,[12] *N*-benzoyl- and *N*-phenylacetylglycine amides,[13] and structural analogues of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA).[14] The endogenous neuropeptides, Met- and Leu-enkephalin, have also exhibited anticonvulsant activity in a variety of test animals and may play an important role in the prevention of a static convulsive state or in the maintenance of normal brain function.[15]

Our investigation of chemotherapeutic agents possessing CNS depressant and anticonvulsant activity suggested a common structural motif. Three functionalities were prevalent in many of these compounds:

- a vicinal diamine linkage;
- an oxygen atom on the ethene chain bridging the two amino groups; and
- an aromatic ring one carbon removed from an amino residue.[16]

This empirical blueprint suggested to us that functionalised amino acids and peptides (*19*) could provide a rich source of future antiepileptic agents and this in fact led to our initial discovery that *N*-acetyl-D,L-alanine-*N*-benzylamide (*20*) was an anticonvulsant in mice.[17] To elucidate the scope of the anticonvulsant activity of (*20*), we did systematic structural variations, initially, at three sites in (*19*): the α-carbon [$R^2(R^3)$], the amide initial pharmacological studies were done on mice through the Epilepsy Branch of the NINCDS and later at the Eli Lilly Research Laboratories.

Our results for analogues of (*20*) revealed several significant factors.

- The principal biological activity of these compounds resided in their ability to prevent seizures in the MES test.
- Stringent steric and electronic requirements existed in (*19*) at both the α-carbon [$R^2(R^3)$] and the amide substituents (R^4) for maximal activity.
- Homologation of the α-carbon by one carbon atom to generate a 1,3-diamine linkage led to reduced CNS activity.
- Pronounced activity was observed for *N*-acetyl-D,L-alanine-*N*-*m*-fluorobenzyl- aromatic rings, both of which were one carbon removed from an amino residue.[18]

We then went on to show that compounds (*20–22*) were comparable to phenobarbital (*1*) in the prevention of electroshock-induced seizures in mice and perhaps more significantly were less toxic after injection and oral administration than phenytoin or phenobarbital. Moreover, these compounds displayed significantly different activity profiles compared to the conventional antiepileptic agents when tested against several chemical convulsants, *ie* pentylenetetrazole, bicuculline, picrotoxin, and strychnine, suggesting that these compounds represented a new important class of anticonvulsant agents.[18]

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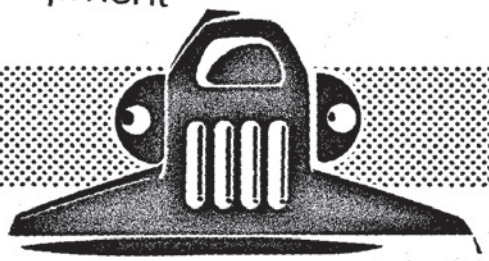

Acetylenedicarboxylic acid salts and esters

Adenine-9-β-D-Arabinofuranoside (Vidarabine)

2-Amino-6-Chloropurine

5-Bromo-2'-deoxyuridine (Broxuridine)

Calcium Folinate (Leucovorin Calcium), pure

Dacarbazine USP 21

2-Deoxycytidine

2-Deoxy-d-ribose

2-Deoxyuridine

5-Fluorocytosine (Flucytosine) USP 21

5-Fluoro-2'-Deoxyuridine (Floxuridine) USP 21

5-Iodo-2'-Deoxycytidine

5-Iodo-2'-Deoxyuridine (Idoxuridine) USP 21

Methotrexate USP 21

Thioacetic acid

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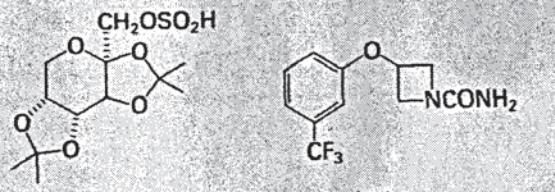
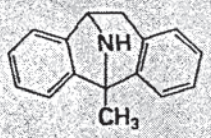


(15)

(16)



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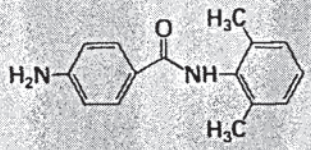
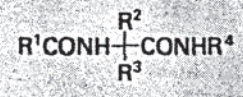


(18)

Amino acid antiepileptic drug candidates.



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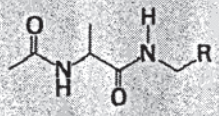
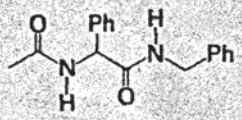


(20) (R = Ph)
(21) (R = *m*-F-Ph)



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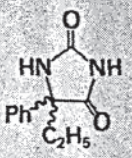

(23)

based substrates prompted us to examine whether the observed activity resided in a single enantiomer. Surprisingly, previous pharmacological differentiation of enantiomeric antiepileptic agents had proved to be largely unsuccessful. Most currently used drugs are not chiral, *eg* phenytoin (*2*), valproic acid (*13*) and phenobarbital (*1*). But in those instances where a single chiral centre exists, anticonvulsant activity is typically observed for both stereoisomers, with one of the two enantiomers exhibiting only marginally enhanced activity. For example, Nirvanol (*23*) displays the largest pharmacological differentiation in activity in animal studies for its two enantiomers. The (*R*)-stereoisomer is 3.8 times more potent than the (*S*)-isomer against seizures induced by electroshock in mice.[19] In other cases, enantiomeric discrimination is considerably less.[20]

However, in our most potent amino acid derivatives, (*20*) and (*22*), we found that the D-stereoisomers were over 10 times more effective in the MES test than the corresponding L-enantiomers when injected in mice. Differentiation between the two stereoisomers of (*20*) was further magnified (13.1 times) when the drugs were given orally. All compounds tested were less toxic after oral administration than after intraperitoneal injection. Significantly, the low toxicity of the D-isomers as well as the racemates contributed to their large protective indices* (PI), which approached that of phenytoin.[21]

Conclusions

The pharmacological profiles exhibited by functionalised DL-amino acid derivatives establishes that this class of compounds is worthy of further detailed inspection. The specific activities of these compounds in the MES, sc Met, and toxicity tests can be independently modulated by altering the substitution pattern at the α-carbon atom, the *N*-acyl, and the *N*-amido moieties. The pharmacological evaluation of the individual enantiomers of (*20*) and (*22*) showed that the anticonvulsant activity observed resided primarily in the D-stereoisomers and represents the greatest pharmacological stereochemical differentiation reported to date among antiepileptic agents possessing a single chiral centre. These observations may provide the first important step for the identification of a potential receptor site for this series of amino acid anticonvulsants. Investigations currently in progress are aimed at elucidating the generality of these observations as well as determining the

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